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BIOCHEMICAL RESEARCHES

COLLECTED REPRINTS OF PUBLICATIONS

FROM THE

LABORATORY OF PHYSIOLOGICAL CHEMISTRY
OF COLUMBIA UNIVERSITY

TOGETHER WITH

CONTRIBUTIONS FROM SIMILAR LABORATORIES
IN OTHER INSTITUTIONS

BY

WILLIAM J. GIES
AND COLLABORATORS

VOLUME I

EDITED AND ISSUED BY WILLIAM J. GIES
COLUMBIA UNIVERSITY
JULY 31, 1903

GIFT
Mr. H. S. Huntington
1/7/13

To the Men of

1901, 1902, 1903, 1904 AND 1905

AT

THE COLLEGE OF PHYSICIANS AND SURGEONS

THIS VOLUME,

TO WHICH SOME OF THEM HAVE MATERIALLY CONTRIBUTED,

IS INSCRIBED

IN LIVELY REMEMBRANCE OF THEIR UNFAILING
COURTESY AND MANY KINDNESSES,

AND WITH THE

REGARDS AND BEST WISHES

OF

THE AUTHOR

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PREFACE.

This volume contains reprints of all the research papers, and of all published abstracts of preliminary reports of investigations, that have been issued, to date, from this laboratory since the establishment of the department in the academic year of 1898–'99. The volume also contains reprints of the several research papers by the writer, and by the writer in collaboration with his teachers, which have been published from other laboratories.

It has been my purpose to bring together the results of all the investigations in which I have personally engaged. This volume is also intended as the first of a series of biochemical studies to be issued from this laboratory, from time to time, as the results of our work may determine.

The investigations thus far concluded in this laboratory should be judged in the light of the special conditions under which they were conducted. During the first two years of the department's history the routine work connected with its equipment, and with the organization of laboratory instruction of large classes of medical students, made it impossible for us to give more than occasional attention to research. At the beginning of the second year the writer started, in addition, two laboratory courses in advanced physiological chemistry. In the fourth year a laboratory course in the physiological chemistry of plants was added to those offered in this department. During the past year the writer has also coöperated with Professor F. S. Lee in giving an undergraduate course in physiology, and has given laboratory instruction and assisted in directing chemical research at the New York Botanical Garden.

The exactions of our routine work and administrative affairs may be estimated from the figures on the next page for the total number of students at this University who have received *laboratory* instruction in physiological chemistry for not less than six hours weekly, during a period of *at least* one half-year, under the writer's constant oversight and personal direction :

Academic Year.	Number of Students.
1898–1899	159
1899–1900	177
1900–1901	231
1901–1902	212
1902–1903	223
Total,	1,002

The courses lately offered in this department of the University, and which will be given in 1903–1904, are indicated in the following abbreviated statements taken from the "Announcement of the Division of Biology," issued May 23:

1. *General Physiological Chemistry.* — This course is given twice during the year and is *required* in the *second* year of all candidates for the degree of M.D. The student is required to attend each week one lecture (1 hour), one conference and recitation (1 hour), and three laboratory exercises, including frequent demonstrations (2 hours each).

Lecture. Weekly (entire class) 1 hour. Professor Gies.

Conference and recitation. Weekly (each section) 1 hour. Professor Gies.

Laboratory exercises, including frequent demonstrations. Three per week (each section) 2 hours each. Professor Gies and Drs. Richards and Hawk.*

2. *Laboratory Course in Advanced Physiological Chemistry, Including a Study of Clinical Methods.* — This course is a continuation of Course 1, but gives more detailed instruction in the various subjects belonging to physiological chemistry than the time for Course 1 will allow. 6 hours. Professor Gies and Dr. Richards.

3. *Laboratory Course in Special Physiological Chemistry.* — This course is arranged for students who wish to make a very thorough study of the science. 12 hours. Professor Gies.

4. *Physiological Chemistry of Plants, Including a Study of Laboratory Methods.* — This course is arranged for the benefit of students of botany and of materia medica. The course may be taken in whole or in part at the New York Botanical Garden, where Professor Gies is Consulting Chemist. 6 hours. Professor Gies.

Courses in Physiology given with the coöperation of this department, quoting from the same "Announcement:"

4. *Laboratory Course in Special Physiology.* — Given with the coöperation of the Department of Physiological Chemistry. 3 hours. Professors Curtis, Lee and Gies, and assistants.

6. *Elementary Physiology.* — Given at Columbia College with the coöperation of the Department of Physiological Chemistry. One hour lecture, and two hours laboratory work. Professors Lee and Gies and Dr. Burton-Opitz.

Much of the work of investigation in this department has been conducted by the writer with the aid of students of medicine who have been particularly interested in physiological chemistry, but who have had little time for *special* work in the subject. The character and extent of these researches in collaboration has been determined largely by the little time remaining from that given to class

* Since the above announcement was first published Dr. Hawk resigned his position in this department to accept that of Demonstrator of Physiological Chemistry at the University of Pennsylvania.

instruction and has depended, also, on the limited biochemical training and preparation of the students referred to. The writer has given much of his time and energy to the encouragement of the spirit of research among these men, but only such relatively simple investigations as it was possible to conduct to advantage during short periods, at irregular intervals and also at night, could be undertaken with them.

My name always follows those of my associates under the titles of the papers and reports which have been published by me, from this laboratory, in collaboration with medical students and assistants. This has occurred so regularly that it may be easily misinterpreted. My chief purpose in following this course, instead of doing as present customs permit, has been to specially emphasize the large share of credit due to those who assisted me in the practical work of analysis and experiment. Although I have encouraged my associates to engage in these researches with me, I have not suggested to them, at the conclusion of our work, that they agree to a plan of publication which might possibly magnify at their expense my own share in the investigations. My name is associated with another under the titles of only such of the papers from this laboratory as resulted from researches which were strictly coöperative and in which I myself did a large share of the actual labor of experiment. As far as the composition of the papers is concerned — I alone am responsible for their defects.

During the first five years of the department's history, Professor R. H. Chittenden, Director of the Sheffield Scientific School, Professor of Physiological Chemistry in the Sheffield Scientific School and Professor of Physiology in the Yale Medical School, was its official, non-resident Director. Professor Chittenden visited the department for several hours once a week, from October to May, and on those occasions gave a lecture in physiological chemistry to the class of second-year medical students. The department was organized, for the laboratory instruction of these students, under Professor Chittenden's supervision, and with the advice and guidance of Professor John G. Curtis of the Department of Physiology.

The direction and stimulation of the *research* work in this department has devolved upon the writer from the beginning. With

the few exceptions referred to below, all investigations published from the laboratory were carried out by the writer himself or were conducted by him in collaboration with others.

The following researches in this laboratory were carried out as indicated below:

I. Preliminary Reports.

Under Professor R. H. Chittenden's direction.

ii.* The proportion of basic nitrogen yielded by elastin on decomposition with hydrochloric acid. By R. H. Chittenden (for Allan C. Eustis).

At the suggestion and with the coöperation of Dr. Eugene Hodenpyl.

ff. Report of a chemical examination of a knife-grinder's lung. By Eugene Hodenpyl, assisted by Allan C. Eustis and A. N. Richards.

Independently by Dr. A. N. Richards, Research Scholar of the Rockefeller Institute, and Mr. Charles H. Vosburgh.

ee. A modified Eck fistula, with a note on adrenalin glycaemia.

II. Papers.

Independently by Dr. P. A. Levene.

12. On the nucleoproteid of the brain (cerebronucleoproteid).†

i, 15. Embryochemical studies. I. Some chemical changes in the developing egg.

Under the direction of, or done chiefly by, Dr. S. J. Meltzer.

17. On the influence of the contents of the large intestine upon strychnine. By William Salant, Fellow of the Rockefeller Institute.

18. A further study of the influence of the contents of the large intestine upon strychnine. By William Salant, Fellow of the Rockefeller Institute.

24. Studies on the influence of artificial respiration upon strychnine spasms and respiratory movements. By William J. Gies and S. J. Meltzer.

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* The letters and numerals before this and the succeeding titles correspond with those before the same titles on pages 25–28.

† At the conclusion of the paper, Dr. Levene acknowledges his indebtedness to ofessor Chittenden for suggestions while the work was in progress.

Under the direction of, or at the suggestion and with the counsel of, Professor C. A. Herter.

23. Note on the glycosuria following experimental injections of adrenalin. By C. A. Herter and A. N. Richards, Research Scholar of the Rockefeller Institute.

25. An experimental study of the sugar content and extravascular coagulation of the blood after administration of adrenalin. By Charles H. Vosburgh and A. N. Richards, Research Scholar of the Rockefeller Institute.

The researches which were conducted in other laboratories by the writer, under the guidance of or in collaboration with his teachers, are listed in Section III of the Bibliography, page 20. The paper by Dr. Meltzer and myself (24), also belongs to this group in the bibliographical list, even though it was issued from this laboratory. On the opening page of the paper issued from this laboratory by Lesem and Gies (11), acknowledgment is made of the fact that the research was begun by the writer, at Yale, at Professor Chittenden's suggestion.

The general results of all the investigations may be quickly ascertained from the index, pages 733–746, where only the data of our own researches are classified.

The statements already made here, on a few matters of departmental history relating to our researches, would be very incomplete without additional reference to the important parts taken in the work of this department, from its beginning, by my colleague Dr. A. N. Richards and by our worthy laboratory helper, Mr. Christian Seifert. I have had help from each of them in connection with many of the details of organization, laboratory instruction and research, whenever aid was needed or desired. Whatever success may have been attained in the work of this department of the University has been due, in large part, to the enthusiastic, painstaking and efficient coöperation given at all times by Dr. Richards and Mr. Seifert. It is a pleasure to make permanent record of the fact.

WILLIAM J. GIES.

DEPARTMENT OF PHYSIOLOGICAL CHEMISTRY OF COLUMBIA UNIVERSITY,
COLLEGE OF PHYSICIANS AND SURGEONS,
July 31, 1903.

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II. Reports and papers on investigations in which all or nearly all of the *chemical* work was done in the Laboratory of Physiological Chemistry, of Columbia University, at the College of Physicians and Surgeons: 1899–1903 (XLIX–LXVI)..... 18

III. Reports and papers on researches carried out in various laboratories by William J. Gies under the guidance of, or in collaboration with, his teachers: 1896–1903 (LXVII–LXXXII)..... 20

BIBLIOGRAPHY.

[The titles of publications under each head are arranged in chronological order.]

1. Publications from the Laboratory of Physiological Chemistry, of Columbia University, at the College of Physicians and Surgeons.*

1900–1903. I–XLVIII.

1900.

ABSTRACTS OF PRELIMINARY REPORTS OF RESEARCHES.

I. **William J. Gies.** The preparation of a mucin-like substance from bone. *Proceedings of the American Physiological Society, New Haven*, December, 1899. *American Journal of Physiology*, March; iii, p. vii.

II. **A. N. Richards and William J. Gies.**† A preliminary study of the coagulable proteids of connective tissues. *Ibid.*, p. v.

III. **William D. Cutter and William J. Gies.** The glucoproteids of white fibrous connective tissue. *Ibid.*, p. vi.

IV. **L. D. Mead and William J. Gies.** The physiological action of tellurium compounds. *Ibid.*, p. xx.

V. **R. H. Chittenden (for Allan C. Eustis).** The proportion of basic nitrogen yielded by elastin on decomposition with hydrochloric acid. *Ibid.*, p. xxxi.

VI. **William J. Gies.** Notes on the constituents of ligament and tendon. *Proceedings of the American Association for the Advancement of Science, New York*, June, 1900. *Permanent Secretary's Report of the Proceedings*. December, p. 123.

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* The department was established in 1898–1899. Research could not be effectively started until 1899. The first reports of researches were made late in 1899, but no abstracts of these reports were published before 1900. The first publications of *completed* researches appeared in 1901. A list of publications of investigations carried out in part in this laboratory, in part in other laboratories, is given on page 18.

† The names of the authors of this and the two succeeding abstracts were transposed under the titles in the "Proceedings," by the Editor of the Journal. This was done in conformity with the custom of the Journal of giving precedence to the name of the author presenting the report. The names are here placed in the order in which the writer preferred them. See preface, page 7.

VII.* **William J. Gies.** New methods for the separation of some constituents of ossein. *Ibid.*, p. 131.

1901.

A. ABSTRACTS OF PRELIMINARY REPORTS OF RESEARCHES.

VIII. **G. A. Fried and William J. Gies.** Does muscle contain mucin? *Proceedings of the American Physiological Society, Baltimore*, December, 1900. *American Journal of Physiology*, March; v, p. x.

IX. **A. N. Richards and William J. Gies.** Methods of preparing elastin, with some facts regarding ligament mucin. *Ibid.*, p. xi.

X. **P. B. Hawk and William J. Gies.**† A further study of the glucoproteid in bone. *Ibid.*, p. xv.

B. PAPERS ON OUR OWN INVESTIGATIONS.

XI. **L. D. Mead and William J. Gies.** Physiological and toxicological effects of tellurium compounds, with a special study of their influence on nutrition. *American Journal of Physiology*, March; v, p. 104.

XII. **A. F. Chace and William J. Gies.** Some facts regarding "ureine." *Medical Record*, March 2; lix, p. 329.

XIII. **William J. Gies.** The toxicology of tellurium compounds, with some notes on the therapeutic value of tellurates. *Philadelphia Medical Journal*, March 23; vii, p. 566.

XIV. **William J. Gies.** A note on the excretion of kynurenic acid. *American Journal of Physiology*, April; v, p. 191.

XV. **William J. Gies.** An improved method of preparing and preserving meat for use in metabolism experiments. *American Journal of Physiology*, May; v, p. 235.

XVI. **G. W. Vandegrift and William J. Gies.** The composition of yellow fibrous connective tissue. *American Journal of Physiology*, June; v, p. 287.

XVII. **P. B. Hawk and William J. Gies.**† Chemical studies of osseomucoid, with determinations of the heat of combustion of some connective tissue glucoproteids. *American Journal of Physiology*, July; v, p. 387.

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* Reported in person by the author, but, by an oversight, the abstract was omitted by the Secretary and only the title given in the Proceedings. See page 32.

† Most of the elementary analysis and the determination of the heat of combustion, comprising Dr. Hawk's share of the work, was done during the summer vacation of 1900, in Professor Atwater's laboratory at Wesleyan University.

XVIII. **W. D. Cutter and William J. Gies.** The composition of tendon mucoid. *American Journal of Physiology*, November ; vi, p. 155.

XIX. **William J. Gies.** A new constituent of bone. *American Medicine*, November 23 ; ii, p. 820.

XX. **Leo Buerger and William J. Gies.** The chemical constituents of tendinous tissue. *American Journal of Physiology*, December ; vi, p. 219.

C. MISCELLANEOUS PUBLICATIONS.

XXI.* **A. N. Richards.** Report of the Proceedings of the American Physiological Society, Baltimore meeting, December, 1900. *Boston Medical and Surgical Journal*, cxliv. First paper, January 24 ; p. 91. Second paper, January 31 ; p. 116.

XXII.* **J. E. Kirkwood and William J. Gies.** Composition of the body (plant). *MacDougal's Practical Text-Book of Plant Physiology*, Longmans, Green & Co. Composing Chapter ix, pp. 147–174.

XXIII. **William J. Gies.** Animal coloring matters. *Buck's Reference Handbook of the Medical Sciences*, William Wood & Co. Vol. iii, pp. 223–225.

1902.

A. ABSTRACTS OF PRELIMINARY REPORTS OF RESEARCHES.

XXIV. **P. B. Hawk and William J. Gies.** The composition and chemical qualities of the albumoid in bone. *Proceedings of the American Physiological Society, Chicago*, December, 1901. *American Journal of Physiology*, March ; vi, p. xxvii.

XXV. **L. D. Mead and William J. Gies.** A comparative study of the reactions of various mucoids. *Ibid.*, p. xxviii.

XXVI. **E. R. Posner and William J. Gies.** Are proteids which are prepared by the usual methods combined with fat or fatty acid? *Ibid.*, p. xxix.

XXVII. **I. O. Woodruff and William J. Gies.** On the toxicology of selenium and its compounds. *Ibid.*, p. xxix.

B. PAPERS ON OUR OWN INVESTIGATIONS.

XXVIII. **H. O. Mosenthal and William J. Gies.** Proteosuria. *American Medicine*, March 8 ; iii, p. 387.

* Not reprinted.

XXIX. **A. N. Richards and William J. Gies.** Chemical studies of elastin, mucoid and other proteids in elastic tissue, with some notes on ligament extractives. *American Journal of Physiology*, April; vii, p. 93.

XXX. **William J. Gies.** Chemical changes in the body in which the methyl group may be involved. *Therapeutic Monthly*, April; ii, p. 144.

XXXI. **E. R. Posner and William J. Gies.** Experiments to determine the possible admixture or combination of fat or fatty acid with various proteid products. *American Journal of Physiology*, July; vii, p. 331.

XXXII. **P. B. Hawk and William J. Gies.** On the composition and chemical properties of osseoalbumoid, with a comparative study of the albumoid of cartilage. *American Journal of Physiology*, July; vii, p. 340.

XXXIII. **P. B. Hawk and William J. Gies.** On the quantitative determination of acidalbumin in digestive mixtures. *American Journal of Physiology*, September; vii, p. 460.

1903. January–July.

A. Abstracts of Preliminary Reports of Researches.

XXXIV. **William J. Gies.** Further mucoid studies. *Proceedings of the American Physiological Society, Washington*, December, 1902. *American Journal of Physiology*, February; viii, p. xiii.

XXXV. **William J. Gies.** A proteid reaction involving the use of chromate. *Ibid.*, p. xv.

XXXVI. **William J. Gies.** The influence of the H ion in peptic proteolysis. *Ibid.*, p. xxxiv.

XXXVII. ***S. J. Meltzer and William J. Gies.** Studies on the influence of artificial respiration upon strychnine spasms and respiratory movements. *Ibid.*, p. xlii.

XXXVIII. **William J. Gies.** An improved cage for metabolism experiments. *Proceedings of the Society for Experimental Biology and Medicine*, February. *Science*, March 20; xvii, p. 469. *American Medicine*, May 2; v, p. 708.

XXXIX. **William J. Gies.** Properties of "Bence Jones' body." *Ibid.*

* The report was not abstracted. The paper was published in the succeeding number of the Journal. See first footnote on page 17.

XL. **A. N. Richards.** A modified Eck fistula, with a note on adrenalin glycæmia. *American Medicine*, May 2; v, p. 709. *Science*, May 8; xvii, p. 742.

XLI. **W. A. Taltavall and William J. Gies.** The influence of chinic acid on the elimination of uric acid. *Proceedings of the American Physiological Society, Washington*, May, 1903. *American Journal of Physiology*, July; ix, p. xvi.

XLII. **William J. Gies.** Peptic proteolysis in acid solutions of equal conductivity. *Ibid.*, p. xvii.

B. PAPERS ON OUR OWN INVESTIGATIONS.

XLIII. **Gordon Lindsay and William J. Gies.** Some notes on Pollacci's new method of detecting albumin in the urine. *American Medicine*, January 31; v, p. 175.

XLIV. **William J. Gies.** Chemical studies of the pitcher plant, *Sarracenia purpurea*. *Journal of the New York Botanical Garden*, February; iv, p. 37.

XLV.* **William J. Gies and S. J. Meltzer.** Studies on the influence of artificial respiration upon strychnine spasms and respiratory movements. *American Journal of Physiology*, March; ix, p. 1.

XLVI. **Charles H. Vosburgh and A. N. Richards.** An experimental study of the sugar content and extravascular coagulation of the blood after administration of adrenalin. *American Journal of Physiology*, March; ix, p. 35.

C. MISCELLANEOUS PUBLICATIONS.

XLVII.† **William J. Gies.** On the normal occurrence of arsenic in organisms. *Letter to the President of the Medico-Legal Society*, New York, February 17. *Medico-Legal Journal* March; xx, p. 541.

XLVIII. † **William J. Gies.** Proceedings of the Society for Experimental Biology and Medicine. *Secretary's Reports. Science*, March 20; xvii, p. 468: May 8; xvii, p. 741. Also *American Medicine*, May 2; v, p. 707.

* This paper is also included with those listed in Section III. See page 22.

† Not reprinted.

II. Reports and papers on investigations in which all or nearly all of the chemical work was done in the Laboratory of Physiological Chemistry, of Columbia University, at the College of Physicians and Surgeons. 1899–1903. XLIX–LXVI.

1899.

A. ABSTRACT OF A PRELIMINARY REPORT OF RESEARCH.

XLIX. **Eugene Hodenpyl, assisted by Allan C. Eustis and A. N. Richards.** Report of a chemical examination of a knife-grinder's lung. *Proceedings of the New York Pathological Society*, November 8. *Medical Record*, December 23; lvi, p. 942.

B. PAPERS ON OUR OWN INVESTIGATIONS.

L. **P. A. Levene.** On the nucleoproteid of the brain (cerebronucleoproteid). *Archives of Neurology and Psychopathology*, ii, p. 1.

LI. **P. A. Levene.** Embryochemical Studies. I. Some chemical changes in the developing egg. *Archives of Neurology and Psychopathology*, ii, p. 557.

1900.

ABSTRACTS OF PRELIMINARY REPORTS OF RESEARCHES.

LII.* **P. A. Levene.** Some chemical changes in the developing egg. *Proceedings of the American Physiological Society*, New Haven, December, 1899. *American Journal of Physiology*, March; iii, p. xii.

LIII. **J. E. Kirkwood and William J. Gies.** Some chemical notes on the composition of the cocoanut. *Proceedings of the New York Academy of Sciences*, May. *Science*, June 15; xi, p. 951. Also, *Annals of the New York Academy of Sciences*, 1900–1901, xiii, p. 489.

LIV. **J. E. Kirkwood and William J. Gies.** The composition of the endosperm and milk of the cocoanut. *Proceedings of the American Association for the Advancement of Science*, New York, June. *Science*, October 19; xii, p. 585. Also *Permanent Secretary's Report of the Proceedings*, December, p. 275.

* This report was made shortly after the publication of the preceding paper.

1901.

A. ABSTRACT OF A PRELIMINARY REPORT OF RESEARCH.

LV. **J. E. Kirkwood and William J. Gies.** Changes in the composition of the cocoanut during germination. *Proceedings of the American Physiological Society*, Baltimore, December, 1900. *American Journal of Physiology*, March; v, p. xiv.

B. PAPER ON OUR OWN INVESTIGATIONS.

LVI. **Rolfe Floyd and William J. Gies.** An extreme case of simple anæmia. *Medical Record*, April 27; lix, p. 650.

1902.

PAPERS ON OUR OWN INVESTIGATIONS.

LVII. **C. A. Herter and A. N. Richards.** Note on the glycosuria following experimental injections of adrenalin. *Medical News*, February 1; lxxx, p. 201.

LVIII. **J. E. Kirkwood and William J. Gies.** Chemical studies of the cocoanut, with some notes on the changes during germination. *Bulletin of the Torrey Botanical Club*, June 20; xxix, p. 321.

LIX. **Francis W. Murray and William J. Gies.** A case of pancreatic fistula of three years' duration, with a chemical study of the fluid eliminated. *American Medicine*, July 26; iv, p. 133.

LX. **William Salant.** On the influence of the contents of the large intestine upon strychnine. *American Medicine*, August 23; iv, p. 293.

LXI. **William J. Gies.** On the nutritive value and some of the economic uses of the cocoanut. *Journal of the New York Botanical Garden*, September; iii, p. 169.

LXII.* **W. W. Lesem and William J. Gies.** Notes on the "protagon" of the brain. *American Journal of Physiology*. December; viii, p. 183.

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* This research was begun by the writer in the Sheffield Biological Laboratory at the suggestion of Professor Chittenden.

1903. January–July.

A. TITLES OF PRELIMINARY REPORTS OF RESEARCHES.

LXIII.* **D. T. MacDougal for William J. Gies.** "Alkaverdin," a hitherto unknown pigment found in leaves of *Sarracenia purpurea*. *Proceedings of the Botanical Society of America*, December, 1902. *Secretary's Report. Science*, February 27; xvii, p. 338.

LXIV.* **D. T. MacDougal for William J. Gies.** The digestive action ensuing in the pitchers of *Sarracenia purpurea*. *Ibid.*

LXV.* **D. T. MacDougal for B. C. Gruenberg and William J. Gies.** Chemical studies of various kinds of logwood. *Ibid.*, p. 339.

B. PAPER ON OUR OWN INVESTIGATIONS.

LXVI. **William Salant.** A further study of the influence of the contents of the large intestine upon strychnine. *American Medicine*, June 27; v, p. 1027.

III. Reports and papers on researches carried out in various laboratories by William J. Gies under the guidance of, or in collaboration with, his teachers. 1896–1903. LXVII–LXXXII.

A. ABSTRACTS OF PRELIMINARY REPORTS OF RESEARCHES.

1899.

LXVII. **William J. Gies.** On stimulation and excitability of the anæmic brain. *Report of the meeting of the British Association for the Advancement of Science*, Dover. September, p. 897.

1900.

LXVIII. **Leon Asher and William J. Gies.**† The influence of protoplasmic poisons on the formation of lymph (with a note on lymph formation after death). *Proceedings of the American Physiological Society*, New Haven, December, 1899. *American Journal of Physiology*, March; iii, p. xix.

LXIX. **William J. Gies.** The influence of protoplasmic poisons on the formation of lymph. *Second report. Proceedings of the New York Academy of Sciences*, January. *Science*, February 16; xi, p. 269. Also, *Annals of the New York Academy of Sciences*, 1900–1901, xiii, p. 434.

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* Report not abstracted. See paper No. 40 (LXIII–LXIV).

† The statement in the second footnote on page 13 applies here also.

1903. January–July.

LXX. * **R. H. True and William J. Gies.** The physiological action of heavy metals in mixed solutions. *Proceedings of the Botanical Society of America*, December, 1902. *Secretary's Report. Science*, February 27; xvii, p. 339.

LXXI. **Jacques Loeb and William J. Gies.** Further studies of the toxic and antitoxic effects of ions. *Proceedings of the American Physiological Society, Washington*, December, 1902. *American Journal of Physiology*, February; viii, p. xiv.

(XXXVII.) † **S. J. Meltzer and William J. Gies.** Studies on the influence of artificial respiration upon strychnine spasms and respiratory movements. *Ibid.*, p. xlii.

B. PAPERS ON OUR OWN INVESTIGATIONS.

1896.

LXXII. **R. H. Chittenden and William J. Gies.** The mucin of white fibrous connective tissue. *Journal of Experimental Medicine*, i, p. 186.

1898.

LXXIII. **R. H. Chittenden and William J. Gies.** The influence of borax and boric acid upon nutrition, with special reference to proteid metabolism. *American Journal of Physiology*, January; i, p. 1.

1900.

LXXIV. **Leon Asher and William J. Gies.** Untersuchungen über die Eigenschaften und die Entstehung der Lymphe. IV. Ueber den Einfluss von Protoplasma-Giften auf die Lymphbildung. V. Einiges über Lymphbildung nach dem Tode. *Zeitschrift für Biologie*, November; xl, p. 180.

1901.

LXXV. **William J. Gies.** Do spermatozoa contain enzyme having the power of causing development of mature ova? *American Journal of Physiology*, October; vi, p. 53.

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* The report was not abstracted. The paper was published shortly afterward. (LXXIX.)

† The report was not abstracted. The paper was published in the succeeding number of the Journal. See footnote on page 16.

LXXVI.* **William J. Gies.** On the nature of the process of fertilization. *Medical News*, November 16; lxxix, p. 767.

1902.

LXXVII. **Jacques Loeb and William J. Gies.** Weitere Untersuchungen über die entgiftenden Ionenwirkungen und die Rolle der Werthigkeit der Kationen bei diesen Vorgängen. *Archiv für die gesammte Physiologie*, December; xciii, p. 246.

1903. January–July.

(XLV.) † **William J. Gies and S. J. Meltzer.** Studies on the influence of artificial respiration upon strychnine spasms and respiratory movements. *American Journal of Physiology*, March; ix, p. 1.

LXXVIII. **William J. Gies.** On the irritability of the brain during anæmia. *American Journal of Physiology*, May; ix, p. 131.

LXXIX. **Rodney H. True and William J. Gies.** On the physiological action of some of the heavy metals in mixed solutions. *Bulletin of the Torrey Botanical Club*, July; xxx, p. 390.

C. Miscellaneous Publications.

1895.

LXXX.‡ **William J. Gies.** Preparation of a new beverage from milk. *Dietetic and Hygienic Gazette*, April; xi, p. 212.

1896.

LXXXI.‡ **William J. Gies.** On the normal occurrence of iodine in the animal body. *Dietetic and Hygienic Gazette. First paper*, March; xii, p. 158. *Second paper*, June; xii, p. 352.

1898.

LXXXII. **William J. Gies.** On the decomposition and synthesis of proteids in living plants. *Yale Scientific Monthly*, February; iv, p. 204.

* In conducting the researches referred to in this and the preceding paper, the author enjoyed the use of the investigator's room, at Wood's Holl, reserved for the Department of Physiology of Columbia University.

† See footnote on page 17.

‡ A translation. Not reprinted.

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† The page numerals of the volume are *printed* on reprints Nos. 12–17 and 38–40. See preceding footnote.

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4.	Chemical studies of elastin, mucoid and other proteids in elastic tissue, with some notes on ligament extractives (XXIX.). (d, e, f)	93–134	121
5.	The composition of tendon mucoid (XVIII.). (d, g)	155–172	163
6.	On the composition and chemical properties of osseoalbumoid, with a comparative study of the albumoid of cartilage. (XXXII.). (b, h)	340–358	181
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40.	Chemical studies of the pitcher plant, *Sarracenia purpurea* (XLIV.)	729–731	729

The following miscellaneous publications have not been reprinted, as was indicated in the Bibliography, pp. 15–22: XXI, XXII, XXXVII, XLVII, XLVIII, LXIII, LXIV, LXV, LXX, LXXX, LXXXI.

ABSTRACTS

OF PRELIMINARY REPORTS OF RESEARCHES, PUBLISHED IN VARIOUS JOURNALS (see BIBLIOGRAPHY) AND MADE BEFORE THE FOLLOWING SOCIETIES:

British Association for the Advancement of Science, 1899—m.*

New York Pathological Society, 1899—ff.

American Physiological Society, 1899–1903—a, c, e, f, g, h, i, j, k, n, o, r, aa, bb, cc, gg, hh, ii, jj, kk, mm.

American Association for the Advancement of Science, 1900—b, d, q.

New York Academy of Sciences, 1900—l, p.

Society for Experimental Biology and Medicine, 1903—dd, ee, ll.

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* The letters following the names of the societies correspond with those before the titles listed on pages 25 and 26.

A. ABSTRACTS OF REPORTS OF RESEARCHES WHICH HAVE BEEN FOLLOWED BY PUBLICATIONS GIVING THE RESULTS IN DETAIL, pages 31–51 ; a–r.

Reprinted from the American Journal of Physiology, 1900, iii ; Proceedings of the American Physiological Society, p. vii.

a.* THE PREPARATION OF A MUCIN-LIKE SUBSTANCE FROM BONE.

By William J. Gies.

Young, in 1892, working under Halliburton's direction, was unable to separate mucin from bone. This negative result has gained general acceptance in spite of the fact that the method employed by Young could hardly have been expected to yield any other. The bone powder and shavings, in quantities ranging from 2.5 to 11 grams, were extracted with 100 to 500 c.c. of lime or baryta water, and, after several days, the filtered solution was treated with acetic acid. Failure to obtain precipitates under these conditions led to the conclusion that bone does not contain mucin. Calling attention to the main defect in this procedure, it is sufficient to suggest that the inorganic substances in bone must necessarily impose a mechanical obstacle to the action of the dilute alkali, and that their removal ought to be the first step in any attempt to get at whatever glucoproteid might be contained in the tissue.

I have prepared a mucin-like substance from the rib and femur of the ox by the following method : The perfectly clean bones were kept in 0.2 per cent. to 0.5 per cent. HCl. As the inorganic matter dissolved out, the bones were shaved and the shavings accumulated in 0.2 per cent. HCl. These were finally run through a meat chopper, then washed free from acid, and extracted in half-saturated lime water. The filtered extract gave a heavy precipitate with 0.2 per cent. HCl in excess. 1,700 grams of femur shavings yielded 7 grams of this material : 875 grams of rib shavings gave 3.5 grams. This substance appears to be mucin, though

* The letters preceding the titles correspond with those in the list on pages 25–26.

it may be chondromucoid or a relative of each. It dissolves easily in 0.1 per cent. Na_2CO_3. It is acid to litmus. It gives the proteid color reactions, yields a reducing substance, and contains ethereal sulphuric acid. The nitrogen and sulphur content of the unpurified substance approximates that of tendon mucin and chondromucoid. The filtrate from the mucin precipitate contains a substance which has many of the qualities of chondroitin sulphuric acid. A careful investigation of the composition and character of the mucin-like substance, and the body supposed to be chondroitin sulphuric acid, is now being made. The general method employed for their detection and separation promises, also, to yield material well suited for the studies we shall make of bone gelatin and the various organic bone constituents.

It is evident from these results that ordinary compact bone, just like the other forms of connective tissue, does contain mucin substance, and, further, that in the process of ossification the connective tissue matrix is not completely removed.

Reported in person by the author before the American Association for the Advancement of Science at the June meeting in 1900, but, by an oversight, the abstract was omitted by the Secretary from the Proceedings of the Society and only the title there given; on p. 131.* The abstract below is the one presented to the Secretary for publication.

b. NEW METHODS FOR THE SEPARATION OF SOME CONSTITUENTS OF OSSEIN.

BY WILLIAM J. GIES.

The author improved the method of preparing ossein by placing the compact bone in 0.2 to 0.5 per cent. HCl for three or four hours, and then scraping off with a scalpel the thin, softened layer of tissue in somewhat elastic, translucent shavings. The dilute acid has little or no effect on the bulk of the organic constituents and the latter may be separated easily from the shavings, especially after the latter have been put through a meat chopper. The method was demonstrated.

The new constituents of bone, prepared by the author from the ossein obtained in this way, are a chondromucoid-like substance

*This abstract and that on page 34 are the only ones in which additions have been made to the original form of publication.

and another glucoproteid having some of the qualities of paramucin. The filtrate from these precipitates also contains a substance apparently identical with chondroitin-sulphuric acid. Chemical analysis of these bodies is now in progress.

Ossein prepared in this manner may be kept in artificial pancreatic juice at 40°C. for several days without perceptible decrease in quantity. This method suffices, therefore, to remove glucoproteid and nucleoproteid and elastin in the preparation of bone collagen. The gelatin obtained from the latter is especially pure as a result of this preliminary treatment. An elastin-like albuminoid remains after the finely minced ossein has been extracted with dilute alkali for several days and has been boiled, also, in water for a week or more. Both the gelatin and the elastin prepared by these methods are about to be carefully studied.

The author concludes that this general method of preparing ossein will be favorable, also, to studies of the fat, the bone pigment and such nucleoproteid as may be assumed to exist in osseous tissue.

Reprinted from the American Journal of Physiology, 1901, v; Proceedings of the American Physiological Society, p. xv.

c. A FURTHER STUDY OF THE GLUCOPROTEID IN BONE.

BY P. B. HAWK AND WILLIAM J. GIES.

Five different preparations from the femur of the ox have been analyzed since the figures for the first two (from rib and femur of the ox) were reported to this society a year ago. The elementary composition of the seven varies between the extremes here given in percentage figures:

C.	H.	N.	S.	Ash.
45.75–48.08	6.66–7.29	11.97–14.15	1.36–2.21	0.33–2.72

The ash-free substance does not contain phosphorus. The amount of sulphur that could be split off in the form of ethereal sulphate varied from 0.49 to 1.10 per cent.

The following figures show the average percentage composition of the preparation of osseomucoid which we have good reason to think is the purest, and also of chondromucoid, as determined by Mörner:

	C.	H.	N.	S.	O.	S (as eth sulph)
Osseomucoid.....	46.41	6.76	12.08	2.04	32.71	1.08
Chondromucoid	47.30	6.42	12.58	2.42	31.28	1.72

Compared with the glucoproteid of cartilage, osseomucoid appears to contain more hydrogen and oxygen and correspondingly less of the other elements. In its reactions it is practically the same.

This abstract was abbreviated unsatisfactorily in the Proceedings of the American Association for the Advancement of Science, 1900, p. 123, and is here given in the form in which it was presented to the Secretary for publication.

d. NOTES ON THE CONSTITUENTS OF LIGAMENT AND TENDON.*

BY WILLIAM J. GIES.

Qualitative.—Ligament consists mostly of elastin. The author and his assistants find that it contains considerable glucoproteid; also, coagulable proteid in appreciable quantity. These facts have not been duly considered, heretofore, in the preparation of elastin, and may account for variations reported in the composition of elastin as also in the character of its decomposition products.

There appears to be more than one glucoproteid in tendon. Various products separated by differential methods show nitrogen content varying from 11.5 to 14.7 per cent.; sulphur content, from 1.3 to 2.8 per cent. These facts seem to explain the discrepancies in former analyses of tendon mucin. A large proportion of the sulphur of the molecule is in the form of ethereal sulphate and it is quite probable that mucin and chondromucoid are more intimately related than had been supposed.

Both ligament and tendon contain crystalline nitrogenous extractives. Thus far creatin has been separated from each.

These points are being worked out in detail with the help of Messrs. A. N. Richards and William D. Cutter.

Quantitative.—The author presented the following figures for averages of many analyses of ligament and tendon, the first to be reported for these tissues. They show relative general composition.

* This abstract and that on page 32 are the only ones to which additions have been made.

	Fresh Ligamentum Nuchæ (Ox). Per cent.	Fresh Tendo Achillis (Ox). Per cent.
Water,	57.57	62.87
Solids,	42.43	37.13
Inorganic matter,	0.47	0.47
Organic matter,	41.96	36.66
Collagen,	7.23	31.58
Elastin,	31.67	1.63
Coagulable proteid,	0.62	0.22
SO_3 in the ash,	5.64	6.65

The high content of SO_3 in the ash is noteworthy. In all probability much of it arises on incineration from the ethereal sulphate in the glucoproteids of each tissue. The results for elastin and collagen are particularly instructive.

The data in this connection have been obtained in work in which Messrs. Leo Buerger and G. W. Vandegrift are coöperating.

Reprinted from the American Journal of Physiology, 1900, iii; Proceedings of the American Physiological Society, p. v.

e. A PRELIMINARY STUDY OF THE COAGULABLE PROTEIDS OF CONNECTIVE TISSUES.

BY A. N. RICHARDS AND WILLIAM J. GIES.*

This investigation was prompted by the belief that there is, perhaps, more metabolic activity in the connective tissues than their "passive mechanical functions" suggest, and, therefore, that an increase of our knowledge of their chemical units will be of some value. Ligament, tendon and hyaline cartilage are the representative forms of connective tissue we have studied thus far in a preliminary way. Aqueous and magnesium sulphate extracts of the thoroughly clean tissues were made, examination with the spectroscope showing the absence of haemoglobin. Cartilage has thus far given entirely negative results. Tendon seems to contain two coagulable proteids; one separates at 54°–57° C., the other at 73°.

Ligament contains much more coagulable proteid than the other forms. Quantitative determinations with the ligamentum nuchæ of the ox show that that particular form of ligament contains 0.65 per cent. of coagulable proteid in the fresh moist tissue and 1.98 per cent. in the dry. Proteid is precipitated regularly in the

* See second footnote, page 13.

various extracts at 42°–50°, 54°–58°, 66°–70°, 74–76° and 83°–85° C. We do not have sufficient faith in the heat coagulation method to conclude from these results alone that there are as many coagulable proteids in ligament as these temperatures may indicate. We think these results are suggestive rather than conclusive, and expect, by fractional precipitation methods and chemical analysis, to determine definitely the number present. Upon extraction with half saturated lime-water, ligament yields mucin-like material, which later investigation may show is closely related to the glucoproteids in tendon.

These results with the ligament suggest that, in the preparation of elastin, due regard must be paid to the fact that the tissue contains a fairly large proportion of soluble and coagulable proteid. Possibly some of the variations in the figures reported for the composition of elastin, and in the nature of its decomposition products, may be due to proteid which had not been removed in its preparation.

Along with this research a study of connective tissue extractives is being made. Ligament has been found to contain an unexpectedly large quantity of creatin, and the concentrated extract yields a fairly heavy, brownish precipitate with silver nitrate in the presence of ammonia. Future results in this connection, also, may bear directly on the question of metabolism in the connective tissues.

Reprinted from the American Journal of Physiology, 1901, v; Proceedings of the American Physiological Society, p. xi.

f. METHODS OF PREPARING ELASTIN, WITH SOME FACTS REGARDING LIGAMENT MUCIN.

BY A. N. RICHARDS AND WILLIAM J. GIES.

In continuation of the studies reported at the previous session of the Physiological Society, we find that the ligamentum nuchæ of the ox contains an appreciable quantity of mucin, having all the qualities of the glucoproteids separable from white fibrous connective tissue. The nitrogen of five different preparations varied from 12.90 per cent. to 13.86 per cent.; the sulphur from 1.32 per cent. to 2.05 per cent.

In order to insure removal of mucin and coagulable proteids from ligament in the preparation of elastin, we have extracted the finely divided tissue for several days in large excess of cold half saturated lime-water. This preliminary process makes extraction of the tissue with hot alkali unnecessary, and thereafter, when the usual method is continued, neither albumin nor globulin is present to be coagulated and there is no mucin to be decomposed.

By this improved method we have made three different preparations of elastin from the ligamentum nuchae of the ox. Each contains less sulphur than elastin obtained by the old method, the quantity varying from 0.13 to 0.17 per cent. (not deducting S of the ash, amounting to 0.11 per cent. of the purified substance). We have observed in two preliminary experiments that all the sulphur in the elastin prepared by our own method is firmly united in the elastin molecule and is not broken away on boiling with 1 per cent. KOH. This result is not obtained with elastin prepared by the older method, in which extraction with alkali is avoided.

Using Schultze's method, the distribution of nitrogen in the elastin prepared by the improved process as contrasted with that of the old was found to be as follows:

	Ammonia.	Bases.	Amido Acids.	Total Percentage.
A. Old method (1)	2.26	2.98	95.44	100.68
(2)	2.34	2.26	98.42	103.02
B. Improved method.	1.73	3.08	95.23	100.05

Our results in this connection seem to indicate that elastin does yield organic bases, as Kossel and Kutscher have contended in opposition to Bergh and Hedin.

Reprinted from the American Journal of Physiology, 1900, iii; Proceedings of the American Physiological Society, p. vi.

g. THE GLUCOPROTEIDS OF WHITE FIBROUS CONNECTIVE TISSUE.

BY WILLIAM D. CUTTER AND WILLIAM J. GIES. *

Thus far two series of continuous fractional extractions of ox tendon have been made with half saturated lime-water and the

* See second footnote No. 2, page 13.

mucins precipitated from each of the extracts analyzed. The semi-cartilaginous character of the sheath in which the divisions of the main shaft of the Achilles tendon move suggested, at the outset, that possibly the mucin from the sheath may be different from the mucin of the strictly tendinous portion. In the previous work no such discrimination was made, but both parts were extracted together. A comparison of the results for the nitrogen content of the mucins, of the first three extracts of both series from the tendon and its sheath, show that the nitrogen is lower in the second of each than in the first and third, and highest in the third. The figures range from 11.69 to 13.27 per cent. The sulphur content is highest in the first of each, the figures varying from 1.38 to 2.78 per cent. These results indicate that there are several mucins in white fibrous connective tissue; just how many our future work may determine.

Further experiments on the glucosazone-like substance obtainable from the reducing bodies gave products melting at 182° C. Thus far it has not been possible to entirely free the crystals from the brownish globules that occur with them, so that probably these figures are still too low.

Before these experiments were started the similarity in the percentage composition of Mörner's chondromucoid and the tendon mucin analyzed by Chittenden and Gies four years ago suggested to us that the two substances are perhaps closely related. This was further emphasized by the fact that the osazone crystals they obtained had the same general appearance as the crystals of glucosazone and, therefore, might have arisen from glucosamin, one of the decomposition products of chondromucoid. Our own results increase the probability that the two substances are nearly identical.

We believe that continued investigation will show that the differences among the mucins, mucoids and chondroproteids are not as great as their varying physical properties and behavior have suggested, but that each is a combination of proteid with a glucosulphonic acid, the characters of each compound, just as in the case of the nucleoproteids, being dependent largely on the proportions of proteid and acid radicals.

Reprinted from the American Journal of Physiology, 1902, vi; Proceedings of the American Physiological Society, p. xxvii.

h. THE COMPOSITION AND CHEMICAL QUALITIES OF THE ALBUMOID IN BONE.

BY P. B. HAWK AND WILLIAM J. GIES.

In the first report to this society of the discovery of osseomucoid attention was drawn to the fact that the method used for the preparation of the glucoproteid would also favor a study of the albuminoid constituents of osseous tissue. The collaginous residue remaining after extraction of osseomucoid from ossein yields an insoluble, elastin-like substance on boiling in water. This substance is neither the elastin of Smith nor the keratin of Broesicke, but appears to be almost or quite identical with Mörner's chondroalbumoid. Although our product is digestible in pepsin-hydrochloric acid, it appears to be somewhat more soluble in dilute acid and alkali than chondroalbumoid. Unlike the latter body, however, it does not contain loosely bound sulphur.

We have prepared a number of samples of osseoalbumoid from ossein by the method Mörner used for the preparation of the albumoid substance in cartilage. The chief difficulty in this work has been the removal of phosphates and the preparation of ash-free products. Our analyses thus far indicate the average elementary composition given in the summary below, where comparison is also made with keratin and elastin.

	C.	H.	N.	S.	O.
Osseoalbumoid . . .	50.03	6.85	15.93	0.55	26.64
Ligament elastin . .	54.08	7.20	16.85	0.30	21.57
Hair keratin	50.65	6.36	17.14	5.00	20.85

Osseoalbumoid does not contain phosphorus. Unfortunately, analytic comparisons with chondroalbumoid are not now possible, as Mörner made no analyses of that body, although he found that the nitrogen content (three determinations) of albuminates made from it varied between 15 and 16 per cent. We have obtained larger proportions of this residual substance from bone than from cartilage. It is our purpose to study chondroalbumoid in this connection also.

Reprinted from the American Journal of Physiology, 1900, iii; Proceedings of the American Physiological Society, p. xii.

i. SOME CHEMICAL CHANGES IN THE DEVELOPING EGG.

BY P. A. LEVENE.

This work gives the results of an attempt to elucidate the chemical process of construction of animal tissue. Thus far the investigation has been limited to the distribution of nitrogen in the different nitrogenous compounds of the developing egg of different ages. All the nitrogenous substances produced on decomposition of proteids may be classified in two distinct groups: Those of acid nature, like the monoamido-acid, and those of basic nature. The following table demonstrates to some extent the part the same substances play in tissue construction:

	Unfertilized Eggs. Per Cent.	24 Hours After Fertilization. Per Cent.	10 Days After Fertilization. Per Cent.	19 Days After Fertilization Per Cent.
Nitrogen in monoamido compounds.	21.10	21.37	22.72	0
Nitrogen in form of bases.	12.07	25.10	12.48	28.25
Nitrogen in form of proteids.	66.00	53.57	64.79	71.84

It has also been found that the quantity of the xanthin bases and of nucleo-compounds increases with the growth of the egg embryo. The importance of mineral salts for the formation of tissues was demonstrated by the increasing quantity of mineral substance in the egg in the course of its growth.

Reprinted from the American Journal of Physiology, 1900, iii; Proceedings of the American Physiological Society, p. xx.

j. THE PHYSIOLOGICAL ACTION OF TELLURIUM COMPOUNDS.

BY L. D. MEAD AND WILLIAM J. GIES.*

Our work with tellurium compounds was begun at the suggestion of Dr. Victor Lenher, who very kindly furnished us with an abundant supply of chemically pure tellurium preparations. In view of the use of potassium and sodium tellurates as antihydrotics, to reduce the night sweats of pulmonary consumption, we

* See second footnote, page 13.

have determined the influence of small quantities of tellurium compounds on the nutritional processes. We find that quantities of tellurious oxide, sodium tellurite, and tellurium tartrate, not exceeding 0.1 gram daily in two doses, do not materially alter proteid metabolism in dogs brought to a state of nitrogenous equilibrium, even when the dosage is continued for a week. After the administration of these non-toxic amounts the feces were fairly constant in elimination, quantity and character. There was no appreciable effect on the elimination of water. Digestion did not appear to be materially hindered. Tellurium was eliminated in the urine and the odor of methyl telluride in the expired air was very pronounced.

Larger doses, however, 0.2 to 0.5 gram at a time, cause violent vomiting and induce disintegration of the gastric mucous membrane. Our experiments on a dog with gastric fistula show that there is a very decided interference with the secretion of hydrochloric acid after the administration of tellurium in these quantities and, also, that regurgitation of bile is one of the consequences. The action of pepsin and trypsin outside the body is not materially influenced by quantities of tellurium tartrate and sodium tellurite under one per cent.

Tellurium is eliminated in the breath, urine and feces of the dog. Reduction to the metallic state occurs when tellurium compounds come in contact with the tissue cells, though tellurium itself is soluble in the body juices and is distributed to the various organs. Two days after subcutaneous injection of a little more than 1 gram of the tartrate, 38 milligrams of tellurium were recovered from the tissue about the point of injection, 12 from the liver, 9 from the kidneys, 7 from the bile, and 3 from the brain. Additional experiments will be made with sodium and potassium tellurates.

Reprinted from the American Journal of Physiology, 1900, iii; Proceedings of the American Physiological Society, p. xix.

k. THE INFLUENCE OF PROTOPLASMIC POISONS ON THE FORMATION OF LYMPH. (WITH A NOTE ON LYMPH FORMATION AFTER DEATH.)

BY LEON ASHER AND WILLIAM J. GIES.*

The work reported upon here very briefly was done in the Physiological Institute at Bern. An attempt was made in this investigation to ascertain, as far as possible, the changes which may occur in lymph after the administration of protoplasmic poisons, by studying the influence of such poisons on the phenomena usually produced by well-known lymphagogues. In this way we attempted to distinguish between the so-called physiological and the physical factors participating in the production of lymph. Our experiments were on dogs, and with quinine and arsenic. The usual methods of lymph collection and analysis afforded the data for our conclusions.

Quinine did not interfere with the usual influence of dextrose, although it did suppress the action of leech extract. Our results with dextrose, therefore, indicate that the increase in the quantity of lymph following its injection in large quantity is due mainly to physical factors. In the case of leech extract, on the other hand, we conclude there has been an interference with the action of the physiological factors that appear to be responsible for the changes usually brought about by this lymphagogue.

That the increase in the amount of lymph after large quantities of dextrose have been injected is not due specifically to increased capillary pressure, as is held by Cohnstein and Starling, was shown in one of our experiments in no uncertain way. After an injection of 1 gram of quinine, 25 grams of dextrose and 0.5 gram more of quinine followed ten minutes later, and 35 c.c. of blood was drawn off. Almost immediately the usual effect of dextrose became evident. In a few minutes, however, the dog died, yet, for more than three hours thereafter, the flow continued, and that, too, without artificial respiration or any mechanical assistance whatsoever. The rate of flow gradually increased for more than an hour,

* See second footnote on page 20.

when it slowly fell back to, and below, the rate of the first period. During the three and a half hours of the experiment the total flow of lymph was 140 c.c. During the first half hour, when the normal conditions prevailed, the flow was only 12.8 c.c. The amount of total solids at the start was 5.02 per cent., at the end 5.9 per cent. The sugar rose from 0.19 per cent. to 2.2 per cent. This experiment seems to emphasize Heidenhain's view that the increase of lymph following injections of large quantities of dextrose is due to changes of osmotic pressure in the tissue spaces.

Following injections of arsenic, which is said to very greatly increase the permeability of the blood vessels, especially those of the portal system, there was little in the flow and character of the lymph resembling the usual effects of lymphagogues. We conclude, therefore, that Starling's hypothesis does not fully account for the action of lymphagogues, and that the mechanical theory of lymph formation fails so long as it does not explain the most striking phenomena of the process — those following the injection of Heidenhain's lymphagogues or Asher's "liver stimulants." The physiological theories of Heidenhain and of Asher and Barbèra would explain them.

Reprinted from the Proceedings of the Section of Biology of the New York Academy of Sciences: Annals of the New York Academy of Sciences, 1900–1901; xiii, p. 434; also, Science, February 16, 1900, xi, p. 269.

1. THE INFLUENCE OF PROTOPLASMIC POISONS ON THE FORMATION OF LYMPH. (SECOND REPORT.)

BY WILLIAM J. GIES.

The author reported upon the changes which may occur in lymph after the administration of protoplasmic poisons. Quinine did not interfere with the usual influence of dextrose, although it did suppress the action of leech extract. The results with dextrose indicate, therefore, that the increase in the quantity of lymph following its injection in large quantity is due mainly to physical factors. In the case of leech extract, on the other hand, there is an interference with the action of the physiological factors that appear to be responsible for the changes usually brought about by this lymphagogue.

That the increase in the amount of lymph after large quantities of dextrose have been injected is not due primarily to increased capillary pressure, as is held by Cohnstein and Starling, was shown in one of the experiments in which quinin caused the death of the animal, and yet from which the lymph continued to flow for three hours. After injecting arsenic, which is said very greatly to increase the permeability of the blood vessels, especially those of the portal system, there was little in the flow and character of the lymph resembling the usual effects of lymphagogues.

It appears, therefore, that Starling's hypothesis of increased capillary permeability does not fully account for the action of lymphagogues and that the mechanical theory of lymph formation fails so long as it does not explain the most striking phenomena of the process — those following the injection of Heidenhain's lymphagogues or Asher's "liver stimulants." The physiological theories of Heidenhain and Asher would explain them.

Reprinted from the Report of the Meeting of the British Association for the Advancement of Science, 1900, p. 897.

m. ON STIMULATION AND EXCITABILITY OF THE ANÆMIC BRAIN.

By WILLIAM J. GIES.

[From the Physiological Institute of the University of Bern.]

The research indicated by this subject was conducted in the Physiological Institute at Bern, upon the suggestion and under the constant direction of Professor Kronecker. Our aim was to determine definitely the sequence of events during perfusion of various so-called indifferent solutions through the brain, the data thus obtained to afford a starting-point for future research with such liquids as may be found to exert specific and characteristic influences.

In this report I shall present only the briefest outline of the experiments and the results obtained.

The animals employed were toads, frogs, rabbits and dogs.

The solutions used were various strengths of pure sodium chloride, Ringer's solution and Howell's modification of it; Schücking's solution, both of calcium and sodium saccharate, and serum.

The perfusion in the cold-blooded animals was conducted with the least possible pressure through the abdominal vein. All of the various solutions already enumerated, except the serum, were used. We made thirteen experiments (seven with toads and six with frogs), each of which continued for a period of two to eight hours, with a total transfusate of 250 to 1,600 c.c.

During the period of perfusion the following functions gradually weakened and then usually disappeared in this order: (*a*) Respiration; (*b*) skin reflex; (*c*) lid reflex; (*d*) nose reflex; (*e*) heart beat.

The times of disappearance of these functions varied with the total length of the experiments, and apparently also with the amount of fluid transfused.

Convulsive extension of the limbs occurred in all of the experiments in the earlier stages, but toward the close of each, and before the reflex movements of the eyelids ceased, no such manifestations could be induced.

In passing it should be noted that:

(*a*) All of the animals became edematous; even those in which perfusion took place at the lowest possible pressures and for the shortest periods.

(*b*) Also, that it was impossible to entirely remove the blood corpuscles, even when the perfusion continued uninterruptedly for eight hours, and as much as 1,600 c.c. of fluid had slowly passed through the body. In all cases the fluid flowing from the cannula, and particularly that pressed from the heart and brain, contained quite an appreciable number of red and white corpuscles.

We carried out thirteen experiments with rabbits and two with dogs, all of the previously mentioned fluids having been used.

Considerable difficulty was encountered in the attempt to find a method which would prevent almost instant death of the animals, and yet which would speedily result in pronounced anæmia.

Ligaturing, either in the neck or in the chest, the arteries to the brain, before or simultaneously with the beginning of the perfusion, brought on convulsions immediately. Even when the perfusion had been begun shortly before the arterial blood was completely shut off, it remained impossible to prevent convulsions and quickly ensuing death.

Finally, instead of closing the arteries to the brain, the abdominal aorta, vena cava and vena porta were tied off and the heart's action utilized to pump the liquid through the brain, the perfused fluid going into the heart by one jugular and from the brain through the other. By this method anæmia could also be induced, convulsions entirely prevented, and life considerably prolonged.

As in the experiments with the cold-blooded animals, there was in these also a fairly regular disappearance of functions, the intervals appearing to vary with the total time of perfusion. With all of the solutions, including serum, both in the rabbits and in the dogs, the order of cessation usually was : (*a*) Respiration ; (*b*) lid reflex ; (*c*) nose reflex ; (*d*) heart beat.

In some of the experiments, it should be noted, the nose and lid reflexes ceased at practically the same instant. In a few, also, it was impossible to determine the sequence of termination of these two and respiration.

In a single special experiment with a small dog (5 kilos), 200 c.c. of blood was taken, and an equal quantity of horse serum immediately afterwards was transfused to take its place. This process was repeated three times at intervals of half an hour. After the fourth withdrawal of fluid, the dog ceased to breathe and did not recover when the serum was transfused. Aside from variations in heart action and respiration, there were no special functional changes until the end, when respiration suddenly ceased, and the other functions quickly disappeared in the order of the other experiments. Death was neither preceded nor accompanied by convulsions.

The more important conclusions of this preliminary research are :

1. When the brain is subjected to acute anæmia produced by the ligature of its arteries, or by the transfusion of indifferent solutions such as physiological saline, Ringer's, Schücking's and also serum, its functions are not maintained and convulsions ensue ; but these may be prevented by producing *gradual* instead of *acute* anæmia.

2. In *gradual* anæmia of the brain, as induced in these experiments, the following functions cease, usually in this order: (*a*) Respiration ; (*b*) lid reflex ; (*c*) nose reflex ; (*d*) heart beat.

Reprinted from the American Journal of Physiology, 1903, viii ; Proceedings of the American Physiological Society, p. xiv.

n. FURTHER STUDIES OF THE TOXIC AND ANTITOXIC EFFECTS OF IONS.

BY JACQUES LOEB AND WILLIAM J. GIES.

This research was conducted at Wood's Holl during the past summer. It confirmed Loeb's original observation that each electrolyte in solution at a certain concentration is able to prevent the development of the *Fundulus* egg after fertilization, and also to destroy the egg. Our experiments further confirmed the fact that this poisonous action can, in general, be wholly or partly inhibited by the addition of a proper amount of another electrolyte.

We also obtained results emphasizing the fact first observed by Loeb, and furnishing new evidence to show that the degree of antitoxic influence exerted by the second electrolyte increases with the valency of the cation. The antitoxic action of bivalent cations was found to be very much greater than that of univalent cations ; the antagonistic power of trivalent cations is considerably greater than that of the bivalent. This rule does not hold with all cations, however ; such cations as Cu, Hg and Cd are exceptions.

Our experiments made it very apparent that the antitoxic action of the salts employed was not due to slight amounts of H or OH ions in their dissociated solutions, since neither solutions of pure acids nor of pure alkalies were able to exert such antagonism.

It was found, finally, that solutions of non-electrolytes, *e. g.*, urea, cane-sugar, glycerin, alcohol, have no antitoxic influence except under conditions which favor the formation of less soluble or less dissociable compounds with the electrolyte (such as saccharate), whereby the concentration of the toxic ion is considerably reduced.

Koch's recent investigations on the influence of ions on lecithin solutions emphasize the possibility previously suggested by Loeb, that the observed antagonistic effects of ions may be referred, in part at least, to changes induced in the physical and perhaps chemical conditions of substances such as lecithin in the cell.

Reprinted from the American Journal of Physiology, 1902, vi; Proceedings of the American Physiological Society, p. xxix.

o. ARE PROTEIDS WHICH ARE PREPARED BY THE USUAL METHODS COMBINED WITH FAT OR FATTY ACID?

BY E. R. POSNER AND WILLIAM J. GIES.

Chemical analysis of the glucoproteids has resulted in wide variations in the figures for elementary composition, not only for bodies from different sources, but for products of similar origin. Such variation has been attributed to admixture of impurities, particularly of non-nitrogenous character. Nerking's recent experiments with mucins, ovomucoid and various simple animal and vegetable proteids indicate that possibly the mucin substances, and other proteids as they are commonly prepared, are admixed or combined with fat or fatty acid.

In order thoroughly to test this matter we have analyzed numerous samples of "chemically pure" connective tissue mucoids and albuminoids. Using Dormeyer's method on quantities of proteid from 2 to 13 grams in weight, and following Nerking's procedure, our extractive results were always entirely negative.

We are convinced, therefore, that the mucoids and albuminoids as they are prepared to-day are not "fat-proteid compounds."

Reprinted from the Proceedings of the Section of Biology of the New York Academy of Sciences: Annals of the New York Academy of Sciences, 1900–1901, xiii, p. 489; also Science, June 15, 1900, xi, p. 951.

p. SOME CHEMICAL NOTES ON THE COMPOSITION OF THE COCOANUT.

BY J. E. KIRKWOOD AND WILLIAM J. GIES.

The authors carried out qualitative work on the ungerminated nut, preparatory to a study of the digestive processes during germination. The chief constituents of the endosperm are cellulose and fat. Some soluble carbohydrate is present, besides globulin and proteose, but no albumin or pepton. Only amylolytic ferment has so far been found.

The milk of the nut is normally acid; probably due to acid phosphate. It contains earthy phosphate, reduces Fehling's solu-

tion, sours on standing and acquires much of the odor and physical appearance of soured cows' milk. It shows only small quantities of proteid and fat.

The "meat" of the average nut contains from 2 to 3 gms. of globulin, which may be obtained in crystalline form. We have made three preparations by the usual methods. The nitrogen averages for these were 17.91 per cent., 17.81 per cent., 17.68 per cent. The ash for the same was 0.13 per cent., 0.41 per cent., 1.05 per cent.

From the "meat" of 12 nuts it was possible to separate a little more than 3 gms. of proteose by the usual method. The average of three closely agreeing determinations of nitrogen was 18.57 per cent.; of the ash it was 1.71 per cent.

The quantitative relationships of these and other constituents will be subjects of continued investigation.

Dr. Custis drew attention to the irritation of the mucous membrane of the bladder and urethra caused by drinking too freely of cocoanut milk. Dr. Gies, in answer to a question, stated that the content of proteid food-stuff is small.*

Reprinted from the Proceedings (of the Section of Botany) of the American Association for the Advancement of Science, 1900, p. 275. Also Science, October 19, 1900, xii, p. 585.

q. THE COMPOSITION OF THE ENDOSPERM AND MILK OF THE COCOANUT.

BY J. E. KIRKWOOD AND WILLIAM J. GIES.

The analyses reported by the authors are intended to prepare the way for exact study of the nutritional changes in the germinated nut.

The milk of the fresh nut is acid to litmus (acid phosphates) and its specific gravity averages about 1022. It quickly sours on standing, acidity increasing as fermentation progresses. Its chief constituents are water, carbohydrates and saline matters. It contains only traces of proteid and fat. General analysis of the milk gave the following average data: Water, 95.3 per cent.; solids,

* In the abstracts in Science and in the "Annals" this answer was quoted incorrectly. See footnote No. 1, on page 324 of reprinted paper No. 36.

4.7 per cent. Of the latter, 88.5 per cent. is organic, 11.5 per cent. is inorganic.

The main bulk of the solid matter in the endosperm consists of fat and cellulose ("crude fiber"). There is some soluble carbohydrate; a small proportion of globulin and proteose; at most only a slight quantity of albumin; no pepton. The globulin has been separated in crystalline form (octahedra and hexagonal plates mostly), and in reactions and composition corresponds closely with edestin. Its coagulation temperature varies from 66° to 79° C., with different conditions. The nitrogen content of the purest preparation made was 17.91 per cent.; the ash, 0.13 per cent. The proteose we analyzed contained 18.57 per cent. of nitrogen and 1.71 per cent. ash. The fresh endosperm contains 0.75 per cent. of nitrogen, which, multiplied by the usual factor (6.25), would correspond to 4.7 per cent. "albuminoid." Some of this nitrogen, however, is undoubtedly closely associated with the non-proteid fibrous elements; much of it, probably, is in the form of nitrogenous extractive. Very active amylolytic ferment is contained in the endosperm; no others have yet been found. These and various other points are still under investigation. The following figures represent the average general composition of the endosperm: Water, 46 per cent.; solids, 54 per cent. Of the latter 98.1 per cent. is organic and 1.9 per cent. inorganic; 43.4 per cent. is fat and 4.3 per cent. is "crude fiber" (cellulose).*

While this work was in progress we accumulated considerable data on the gross relationships of the main parts. Three dozen determinations gave the following average weights and percentages:

Weight of whole nut,	610 grams.
Integument,	170 grams = 27.9 per cent.
Endosperm,	333 grams = 54.5 per cent.
Milk,	107 grams = 17.6 per cent.

The volume of the milk averaged 105 c.c.

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*The figures given for "crude fiber" in the original abstract were by mistake those we then had for "carbohydrate" — 12.9 per cent. By a typographical error this mistake was further emphasized by the figures "21.9 per cent."

Reprinted from the American Journal of Physiology, 1901, v; Proceedings of the American Physiological Society, p. xiv.

r. CHANGES IN THE COMPOSITION OF THE COCOANUT DURING GERMINATION.

BY J. E. KIRKWOOD AND WILLIAM J. GIES.

The fresh nuts in the husk were placed on earth kept constantly moist at a tropical temperature. After a period of about four months the shoots appeared through the husk. At the end of a year of germination chemical examination was begun. At this time the milk cavity of the ovule was completely filled with the fully developed cotyledon, which had almost entirely absorbed the endosperm at the "stem end," and considerably thinned it posteriorly.

The cotyledon, particularly the central, more vascular portion, contains considerable diastatic ferment, and apparently, also, a trace of proteolytic enzyme. Cellulose-dissolving and fat-splitting enzymes have, however, not yet been detected. The appended table presents a few of our analytic results in percentage figures, showing the distribution of water, solids, inorganic matter, and nitrogen, from which numerous deductions as to general growth may be readily drawn:

		Water. Per Cent.	Solids. Per Cent.	Inorganic Matter. Per Cent.	Nitrogen. Per Cent.
A. Roots.	Tips	89.89	10.11	1.33	
	Tips to husk.	86.41	13.59	1.57	
	Very near husk.	82.79	17.21	1.60	
	Inside of husk.	77.92	22.08	1.20	0.27
B. Stem.	"Root crown"	86.21	13.79	1.05	0.53
Petioles		83.63	16.37	1.43	0.29
Leaves.	Young	74.66	25.34	1.65	
	Old	71.99	28.01	1.90	0.45
C. Cotyledon.	"Neck"	78.98	21.02	1.42	
	Cortex	80.83	19.17	1.74	0.31
	"Heart"	88.99	11.01	0.78	0.14
D. Endosperm.	Anterior.	23.42	76.58	0.84	
	Posterior	46.08	53.92	0.98	0.65
E. Ungerminated nut.	Endosperm.	46.00	54.00	1.03	0.75
	Milk	95.30	4.70	0.50	

B. ABSTRACTS OF REPORTS OF RESEARCHES WHICH HAVE NOT YET BEEN PUBLISHED IN GREATER DETAIL, PAGES 52–63;

aa — mm.

Reprinted from the American Journal of Physiology, 1901, v; Proceedings of the American Physiological Society, p. x.

aa. DOES MUSCLE CONTAIN MUCIN?

By G. A. Fried and William J. Gies.

With a view of testing the work which led to disagreement between Schepilewsky and Goodman, the connective tissue residues from 3–5 lbs. of beef and veal, prepared by Schepilewsky's method, were extracted in the usual manner in half saturated lime- or baryta-water. (Muscle fibers could never be completely removed before the extraction.) Seven such extractions were made with as many samples of fresh muscle in appropriate quantities of dilute alkali. On neutralization, and weak acidification, with 0.2 per cent. HCl, a heavy precipitate was obtained in each extract, but the substance so precipitated quickly dissolved each time in slight excess of acid (alkali albuminate?). In this respect its behavior was very different from that of connective tissue glucoproteid. Only a faint turbidity suggested traces of mucin. In one experiment, in which Goodman's procedure was somewhat altered, the connective tissue residue obtained by Schepilewsky's method was treated first with half saturated lime-water, and later with 5 per cent. KOH. On rendering the extract only very faintly acid a proteid precipitate was obtained in each case. This was filtered off, purified and analyzed. With another portion of tissue half saturated baryta-water and subsequently 5 per cent. NaOH were used with the same result. The average nitrogen content of the ash-free substance obtained from each extract was as follows:

1. $Ca(OH)_2$, 16.39%. KOH, 15.12%.
2. $Ba(OH)_2$, 16.69%. NaOH, 14.84%.

None of these preparations yielded reducing substance on decomposition with acid. We are strongly inclined to the belief that these products are alkali albuminate, or at least are admixed with the same. They are neither the "stroma substance" of

Goodman nor the mucin of Schepilewsky. Schepilewsky's method will not detect very small quantities of mucin.

Reprinted from the American Journal of Physiology, 1902, vi; Proceedings of the American Physiological Society, p. xxviii.

bb. A COMPARATIVE STUDY OF THE REACTIONS OF VARIOUS MUCOIDS.

BY L. D. MEAD AND WILLIAM J. GIES.

Comparative studies of many of the precipitation reactions of osseomucoid, chondromucoid and tendomucoid have shown thus far a very striking sameness in result. Each of these glucoproteids also is digested in pepsin-hydrochloric acid, with a formation of proteoses and peptones, and the separation of nitrogen-containing substance rich in reducing material, probably chondroitin-sulphuric acid or essentially the same body in each case. The microscopic appearance of the phenylosazone bodies obtained from each is the same as that of dextrosazone, indicating glucosamine among the products of acid hydration.

All these compound proteids contain sulphur obtainable as sulphate and as sulphide. They are acid to litmus, neutralize alkali, have essentially the same elementary composition and yield practically the same amount of heat on combustion. In physical appearance the substances whether dry, freshly precipitated, or in solution, are practically identical. Attempts to obtain crystalline mucoid, by the methods which recently have given such fruitful results in other connections, have thus far been without success. When the electric current is passed through neutral or alkaline mucoid solutions (consisting of sodium or calcium salts of mucoids) turbidity results within a short time and flocks eventually form and can be filtered off.

Our studies in this general connection have not been completed. We are convinced, however, that the connective tissue mucoids are practically identical substances.

Reprinted from the American Journal of Physiology, 1903, viii; Proceedings of the American Physiological Society, p. xiii.

cc. FURTHER MUCOID STUDIES.

BY WILLIAM J. GIES.

I. Investigations into the distribution of osseomucoid indicate that glucoproteid is a normal constituent of all bones. It has thus far been found in the large bones of wild and domestic mammals and birds, and of reptiles.

II. Connective tissue mucoid shows a tendency to combine with other proteids. Thus, for example, an alkaline solution of potassio-mucoid and gelatin yields a precipitate with acid more promptly than a solution of the equivalent amount of the mucoid salt alone. Furthermore, the compound precipitate is different physically. In the case of the gelatin product the precipitate possesses semi-gelatinous qualities. The compound precipitates of mucoid obtained from proteid solutions weigh more than the control mucoid precipitates. This added weight rises, within certain limits, as the proportion of associated proteid in the solution increases.

III. Acidification of tissue extracts is not sufficient for complete precipitation of the mucoid. Even with a fifth alkaline extract of the same tendon pieces, the water-clear acid filtrate from the precipitated mucoid contains additional glucoproteid.

IV. Precipitated mucoid shows practically no combining power with acids. In the hydration of mucoid by pepsin-acid, however, acid combines with the dissolved proteid products formed in the process.

V. The blood serum of a rabbit, which had been treated with several subcutaneous and intraperitoneal injections of neutral solution of potassio-mucoid, produced precipitates in neutral and very slightly acid solutions of the latter proteid compound.

These researches are still in progress with the coöperation of Messrs. E. R. Posner, C. Seifert and H. G. Baumgard.

Reprinted from the Proceedings of the Society for Experimental Biology and Medicine: Science, 1903, xvii, p. 469; also American Medicine, 1903, v, p, 708.

dd. PROPERTIES OF "BENCE JONES' BODY."

BY WILLIAM J. GIES.

Through the kindness of Dr. Meltzer a patient's urine containing this substance had been placed at our disposal for chemical study. Some of the results of this investigation were presented and various properties of the body demonstrated. Special attention was drawn to a test of Boston's new method of detecting "Bence Jones' body" in the urine.

Reprinted from the Proceedings of the Society for Experimental Biology and Medicine: American Medicine, 1903, v, p. 709; also Science, 1903, xvii, p. 742.

ee. A MODIFIED ECK FISTULA, WITH A NOTE ON ADRENALIN GLYCÆMIA.

BY A. N. RICHARDS.

A method devised by Vosburgh and Richards for extablishing communication between the portal vein and the inferior vena cava of the dog was described and demonstrated. In this method two cannulas are employed. They are constructed on the same principle as the one used by Vosburgh and Richards in collecting blood from the hepatic and portal veins without interfering with the normal circulation in those vessels.* After suitable incision through the abdominal wall a cannula of that type, 1 cm. long, was inserted into the portal vein about 2 cm. below the entrance of the pancreatico-duodenalis. A second cannula of similar design was introduced into the vena cava at a corresponding point. By connecting the cannulas with a rubber tube, communication was established between the two vessels. On ligating the hepatic arteries and the portal vein at the hilum of the liver, circulation through the liver ceased and the gland was extirpated.

By the successful use of this method Vosburgh and Richards have found that the application of adrenalin to the surface of the pancreas brings about a slight rise in the sugar content of the

* American Journal of Physiology, 1903, ix, p. 43. See Reprint No. 25, p. 43.

blood even after extirpation of the liver. Their experiments thus far have covered periods of from two to three hours, no systematic attempts having yet been made to get the animals to survive the operation.

Reprinted from the Medical Record, 1899, lvi, p. 942. (Proceedings of the New York Pathological Society.)

ff. REPORT OF A CHEMICAL EXAMINATION OF A KNIFE-GRINDER'S LUNG.

BY EUGENE HODENPYL,

ASSISTED BY ALLAN C. EUSTIS AND A. N. RICHARDS.

The subject of this report was a knife-grinder, thirty-five years of age, who had died of pernicious anæmia. The history was that he had worked at his trade for fifteen years. For the first ten years he was employed as a grinder and worked in a large room with some forty others; for about five years previous to his death he had worked in a very small and ill-ventilated room at the same occupation with some seven others similarly employed. The lungs presented a maximum degree of pigmentation, and it had, therefore, occurred to the speaker that it might be instructive to determine the amount of carbon contained in the lungs and, if possible, the amount of emery and iron also. Such an investigation seemed especially desirable, since the speaker had been at the time studying the literature of "Staubinhalation" without finding a single case in which the amount of carbon had been determined in similar cases of anthracosis, and, moreover, upon inquiring among his colleagues, he had found that none had the slightest idea as to the amount of carbon which might reasonably be expected to be obtained from such a lung. There were many reports in literature, notably those by Arnold, in which gold and silver had been extracted from the lungs of artisans working with these metals, but no case had been observed in which the amount of carbon, emery and iron had been determined in the lungs of knife-grinders.

The technique employed was to digest the lung, which weighed 900 gms., and then obtain the charcoal, emery and iron by precipitation. The lung was cut into small pieces, placed

in a little water, to which was added two ounces of Johnson's preparation of papoid and enough hydrochloric acid to give a reaction of free acid in the solution. This mixture was kept at a temperature of 40° C. for ten days, when the lung became completely liquefied. It was then necessary, on account of the viscidity of the mass, to add large quantities of water, in order to secure precipitation. About sixty gallons of water was added, and this mixture was allowed to stand in a number of tall jars for many days until precipitation was complete. The precipitate was then repeatedly washed in water until it was believed that all of the substance soluble in water had been removed. It was then evaporated to dryness and powdered. At this juncture, Mr. Allan C. Eustis and Mr. A. N. Richards, assistants in the department of physiological chemistry of the Columbia University, kindly undertook the chemical examination, and the speaker took this opportunity of extending his thanks to these gentlemen for the very complete analysis which they had made.

Analysis of lung taken from the body of a knife-grinder:

Total weight of lung dried and powdered, 48.1009 gms. Total solids, 44.7986 gms.; water, 3.3023 gms.

Soluble in ether, 14.6017 gms.; insoluble in ether, 30.1969 gms.

Composition of the portion which was soluble in ether: Free fatty acids, 7.498 gms.; neutral fats, 4.044 gms.; cholesterin, 3.037 gms. (lecithins ?).

Composition of portion insoluble in ether: Proteids, melanins, etc. (total nitrogen × 6.25), 15.4759 gms.; charcoal (total carbon — proteid carbon), 7.1989 gms.; ash, 4.2909 gms.

Composition of ash: K_2O, 0.2167 gm.; Na_2O, 0.3523 gm.; CaO, 0.0965 gm.; Fe_2O_3, 0.0879 gm.; Al_2O_3, 1.4628 gm.; SO_3, 0.0704 gm.; P_2O_5, 0.9565 gm.; SiO_2, 1.20434 gm.

Dr. Hodenpyl said that, on first receiving this report, he had been somewhat disappointed that the amount of carbon was not greater, but since then he had made some simple experiments which demonstrated that, after all, 7 gm. + of this particular charcoal was really an enormous amount to be obtained in a lung. It is to be remembered that this charcoal was in an exceedingly fine state of subdivision. Thus, on mixing 0.1 gm. of very finely powdered animal charcoal in 500 c.c. of water, the fluid was only very slightly

darkened. One tenth of a gram of the precipitate from the lung, dissolved in 500 c.c. of water, made the fluid almost jet black in color, even though of this precipitate, 0.1 gm. represented only about $\frac{1}{40}$ gm. of carbon. Again, it will be seen that about one fourth of the ash was in the form of an oxide of iron. The amount of emery was represented by oxide of aluminium and oxide of silicon. These two together made up about 2.5 + gm., so that considerably over one-half of the ash was in the form of emery, and the emery and iron together made up more than three fourths of the total amount of the ash.

Dr. Prudden remarked that more than a barrel of water had been made as black as ink by the pigment contained in the lungs of this person. The investigation had an obvious and important bearing on infection through the lung, because it showed how many particles might pass all the safeguards which the air passages present.

Reprinted from the American Journal of Physiology, 1902, vi ; Proceedings of the American Physiological Society, p. xxix.

gg. ON THE TOXICOLOGY OF SELENIUM AND ITS COMPOUNDS.

BY I. O. WOODRUFF AND WILLIAM J. GIES.

The researches of Tunnicliffe and Rosenheim indicate that the numerous cases of "arsenical poisoning" in England recently may have been due in part to selenium. Through the kindness of Professor Victor Lenher our studies are being made with absolutely chemically pure preparations. Thus far our results on dogs confirm most of the general observations of Rabuteau, and of Czapek and Weil. We are unable, however, to discover Rabuteau's crystals in the blood of the heart after death, or to agree with him that death results from mechanical interference with the circulation.

Selenium is very much more toxic than tellurium, although its poisonous effects are qualitatively much the same. The expired methyl compound of selenium is produced in much less quantity than that of tellurium under similar conditions. Injection of four milligrams of selenite or selenate per kilo under the skin of dogs usually results in death in a few minutes. Speedy death follows

the introduction of like amounts per os or rectum. Four grams of the finely powdered metal, when taken into the stomach, manifested no toxicity whatever, and passed out in the fæces. The introduction of soluble salts is quickly followed by elimination of selenium in the urine and the breath. After subcutaneous injections, the distribution of selenium to the organs is similar to that found by us recently for tellurium. Selenium, although chemically related to sulphur, is very much like arsenic in its toxic properties.

Reprinted from the American Journal of Physiology, 1903, ix; Proceedings of the American Physiological Society, p. xvi.

hh. THE INFLUENCE OF CHINIC ACID ON THE ELIMINATION OF URIC ACID.

BY W. A. TALTAVALL AND WILLIAM J. GIES.

Our work thus far has shown that the uric acid output in the urine of dogs is not materially affected by the administration of chinic acid. We observed only a slight lowering of the small amounts of uric acid present in the urine to begin with. This result was obtained when the animal was in approximate nitrogenous equilibrium on a mixed diet consisting of hashed meat, cracker meal, lard, bone ash and water, and after daily doses, for ten days, of chinic acid in amounts varying from 1 to 20 grams. These results were obtained before the recent publication of the data of Hupfer's experiments on himself. They agree with this observer's conclusions that the therapeutic deductions of Weiss, Blumenthal and others, in this connection, are without foundation.

Reprinted from the American Journal of Physiology, 1900, iii; Proceedings of the American Physiological Society, p. xxxi.

ii. THE PROPORTION OF BASIC NITROGEN YIELDED BY ELASTIN ON DECOMPOSITION WITH HYDROCHLORIC ACID.

BY R. H. CHITTENDEN (FOR ALLAN C. EUSTIS).

The lack of agreement between Bergh and Hedin, and Kossel and Kutscher in their study of the basic cleavage products of elastin led us to a study of the proportion of basic nitrogen split

off from pure elastin by boiling for 100 hours with 20 per cent. HCl and stannous chloride. Following the method adopted by E. Schulze, and determining the total nitrogen in the solution, the nitrogen in the form of ammonia, and the nitrogen in the phosphotungstic acid-precipitate, we have obtained very divergent results. In all, five distinct experiments were tried with the following results:

Experiment.	Percentage of Nitrogen in form of organic bases.
I.	0.86
II.	17.69
III.	15.57
IV.	6.50
V.	15.14

Our results led us to the conclusion that the method now in use for the separation of the hexone bases by phosphotungstic acid, and determination of the nitrogen therein, is unreliable for quantitative purposes, and that consequently results hitherto obtained by this method must be accepted with caution.

Reprinted from the American Journal of Physiology, 1903, viii; Proceedings of the American Physiological Society, p. xv.

jj. A PROTEID REACTION INVOLVING THE USE OF CHROMATE.

BY WILLIAM J. GIES.

Several years ago, during a comparative study of the reactions of various gelatins, the results of which have not yet been published, it was observed by Dr. D. H. M. Gillespie and myself that dilute solutions of potassium chromate did not precipitate gelatin solutions, but that when such proteid chromate mixtures were further treated with acid, a fine yellow flocculent precipitate formed at once. Acids as "weak" as acetic, and also the common mineral acids, effected the result, the latter acids more promptly, however, even in smaller amount.

At intervals I have returned to this reaction, and lately have made a more careful study of it. Solutions of chromates of mono- and divalent cations (the only ones thus far employed) cause no precipitates in neutral or alkaline proteid fluids, but on further

treatment with small amounts of dilute acids — strongly dissociable ones particularly — flocculent precipitation of a proteid-chromate compound occurs in every case. The reaction is especially striking with such bodies as gelatin and proteose (the precipitates with these disappearing on warming and reappearing on cooling), and it seems to be more delicate than the acetic acid and potassium ferrocyanide test. Salts containing dichromion or trichromion behave differently.

Since bichromate is formed from chromate on the addition of acid, it might be supposed that such production is responsible for the precipitation observed. But bichromate solutions are as inert as the chromate. When, however, acid is added to a mixture of proteid and bichromate, precipitation occurs, as in the case with chromate. Hydroxidion prevents the reaction in all cases. Possibly the precipitation is due to the formation of dichromic acid, just as in the acetic acid and potassium ferrocyanide test it is dependent on the formation of hydroferrocyanic acid.

Further study is expected to determine exactly the character of the ions responsible for the reaction. The results thus far point to dichromanion in the presence of hydrion.

Reprinted from the American Journal of Physiology, 1903, viii; Proceedings of the American Physiological Society, p. xxxiv.

kk. THE INFLUENCE OF THE H ION IN PEPTIC PROTEOLYSIS.

BY WILLIAM J. GIES.

The fact that pepsin shows digestive power only when acid is present implies the dependence of the enzyme upon hydrion for its activity. It has frequently been observed that various acids are efficacious in this connection, though in different degrees.

In some recent experiments on the influence of acidity, I have used purified fibrin, edestin and elastin as the indicators. Undigested residue, neutralization precipitate and uncoagulable products were determined quantitatively in each digestive mixture. Various common mineral and organic acids were employed. Varying proportions of pepsin and acid were taken in uniform volumes (100 c.c.), with the same amount of proteid (1 gm.). In *equiper-*

centage solutions of acids whose anions have no precipitative effect on proteid, the relative proteolysis is very different, being greatest in "strong" acids such as HCl and least in "weak" acids, such as $CH_3.COOH$. *Equimolar* solutions of the same acids gave more concordant results in some respects, although the differences between the effects in such acids as HCl and $CH_3.COOH$ were still very wide. With *equihydric* solutions, the results showed greater harmony, though there were still striking divergences. H_3PO_4, HCl, HNO_3, $HClO_3$, H_3AsO_4 and $(COOH)_2$, in strengths equivalent to decinormal KOH (with 50 mgm. of pepsin preparation, in 100 c.c. at 40°C., four hours), showed practically the same ability to assist pepsin in the digestion of 1 gm. of fibrin.

Additional experiments, especially with *equidissociated* solutions of the acids referred to above, are expected to show the influence not only of hydrion, but also of the anions, if the influence of the latter in the acids referred to be appreciable. Similar experiments are about to be extended to other enzymes.

Reprinted from the Proceedings of the Society for Experimental Biology and Medicine: Science, 1903, xvii, p. 469; also, American Medicine, 1903, v, p. 708.

ll. AN IMPROVED CAGE FOR METABOLISM EXPERIMENTS.

By William J. Gies.

A cage specially designed for experiments on dogs was shown. The parts are so adjusted as to favor the collection and separation of feces, urine and hair. The improvements consist mainly of mechanical devices suggested by experimental experiences of the past few years in metabolism work, all of which are designed to ensure quantitative accuracy as well as comparative convenience in the collection of excreta.

Reprinted from the American Journal of Physiology, 1903, ix; Proceedings of the American Physiological Society, p. xvii.

mm. PEPTIC PROTEOLYSIS IN ACID SOLUTIONS OF EQUAL CONDUCTIVITY.

By William J. Gies.

Numerous digestive experiments with various equidissociated acids, and with fibrin as the indicator, have invariably given re-

sults lacking quantitative agreement. Undigested residue, neutralization precipitate, and uncoagulable products were determined gravimetrically. With all conditions exactly the same for each mixture in a series, except the *character* of the acid, the digestive products differed not only in the rate of their formation, but also in their amounts. The digestive results were particularly discordant in mixtures containing relatively small amounts of pepsin acting for comparatively short periods of time. That the anions greatly modified the action of the common cation seems certain, the SO_4 anion being especially antagonistic in its influence.

The temperature of the digestive mixtures in each experiment was kept steadily at 25°C. The acids used thus far were of the same conductivity as a 0.2 per cent. solution of hydrochloric acid.

I am much indebted to Mr. C. W. Kanolt, of the Department of Physical Chemistry of Columbia University, not only for the acid solutions already used, but for others about to be employed in additional experiments.

PAPERS

REPRINTED FROM THE FOLLOWING JOURNALS:

Journal of Experimental Medicine, 1896—13.*

American Journal of Physiology, 1898–1903—1, 3, 4, 5, 6, 7, 8, 9, 11, 16, 21, 24, 25, 26, 30, 34, 35.

Yale Scientific Monthly, 1898—39.

Archives of Neurology and Psychopathology, 1899—12, 15.

Zeitschrift für Biologie, 1900—19.

American Medicine, 1901–1903—2, 17, 18, 28, 32, 33.

Medical News, 1901–1902—10, 23.

Reference Handbook of the Medical Sciences, 1901—14.

Philadelphia Medical Journal, 1901—20.

Medical Record, 1901—27, 31.

Therapeutic Monthly, 1902—22.

Archiv für die gesammte Physiologie, 1902—29.

Bulletin of the Torrey Botanical Club, 1902–1903—36, 37.

Journal of the New York Botanical Garden, 1902–1903—38, 40.

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* The numerals following the titles of the journals correspond with those before the titles of the papers listed on pages 26, 27 and 28.

A. CHEMICAL INVESTIGATIONS OF ANIMAL TISSUES AND TISSUE CONSTITUENTS.

Reprints, Nos. 1–15.

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VOL. V. — MAY 1, 1901. — NO. IV.

1

AN IMPROVED METHOD OF PREPARING AND PRESERVING MEAT FOR USE IN METABOLISM EXPERIMENTS.

BY WILLIAM J. GIES.

[*From the Laboratory of Physiological Chemistry, of Columbia University, at the College of Physicians and Surgeons, New York.*]

THE chemical problems in metabolism experiments are as difficult as they are numerous. Not only must the excreta be analyzed in detail, but, in work of the highest value, the composition of the food must also be definitely ascertained. Usually, the purely analytic labor involved in studies of this character is so great that important phases of the experiments have to be ignored or left for subsequent special investigation. Methods of the greatest simplicity, which are easily carried out in the shortest time and with the highest degree of accuracy, are naturally the first to be selected. Consequently, any improvements of acceptable methods, which increase their adaptability in any one of these particulars, are to be welcomed.

The process the author has lately been employing to prepare proteid food in bulk for experiments on dogs includes a few improvements which make it perfectly adapted to metabolism work, and which, besides, lessen considerably the analytic and mechanical labor involved.

In the method referred to, the fresh lean beef, after all loose fat and connective tissue has been removed, and tendonous layers excised, is put through a meat-chopper. The hash thus obtained is then divided into portions of convenient bulk, and each portion is enclosed in cheese-cloth bags, and submitted to increasing pressure[1] as long as bloody fluid accumulates. Three to four hours are usually sufficient for getting rid of all fluid that can be separated. The compressed masses thus obtained may be kept under moistened cloth to prevent the surfaces from drying during the pressing of the remaining portions. Too much hash in the press makes thorough removal of surplus fluid impossible. In preparing about 50 kilos of the meat,

[1] The ordinary "meat press," employed for various purposes, such as the preparation of tinctures from herbs, etc., serves very well.

the author has found it convenient to press 6 to 10 kilos at a time. The size of the press in use would, however, naturally determine the amount of the hash to be pressed at one time.

The compact cakes are next broken in a large dish, intimately mixed by thorough kneading, and then very small quantities, picked out here and there all through the mass, are transferred directly to capacious tubes, weighed and analyzed.[1] Thus far we have not had occasion to make other than nitrogen determinations in the meat prepared in this manner. Excellent results were obtained with 2 to 3 grams for each analysis, although larger quantities may readily be utilized, perhaps with even greater accuracy.

Simultaneously with the sampling of the hash for analysis it should be quickly rolled between the hands into balls weighing about 50 to 100 grams. These are dropped lightly into wide-mouthed bottles of a capacity sufficient to hold five or six of the balls. The latter are not to be pressed together, but ought to rest very lightly on each other. The bottles are then promptly sealed and placed in a cold-storage room, where the temperature is maintained at or below 0° C. The meat-balls quickly solidify, and in the frozen condition can, of course, be kept indefinitely. After the balls are frozen there is usually a very light and delicate film of frost on the inside walls of the bottle, in places, indicating naturally that only a very slight quantity of water leaves the meat during the interval before the frozen state is reached. Under these conditions there is never sufficient movement of fluid to result in the formation of ice at the bottom. If, however, the frozen condition is not reached within a few hours, and maintained, bloody fluid is certain to trickle slowly to the bottom, in spite of the preliminary removal by pressure, thus changing the composition of the substance throughout the entire mass.

The hash prepared and kept in this way retains its normal appearance, odor, and taste for a very long time. If the bottles are small, containing little more than enough for one, or at most two days' feeding, practically no change can take place while material is being withdrawn, if this be done quickly. The globular form is of particular

[1] If the tubes are weighed after they have been thoroughly dried at room temperature, and before the hash is put into them, any interior condensation of water from the meat would be included, as it should be, with the weight [by difference] of prepared substance. This procedure would serve very well for nearly all of the analyses commonly made. The hash should, of course, be completely removed from the weighing tube in each determination.

advantage, in this connection, because it makes the removal of the meat, even in the frozen condition, very easy. When it is desired to take out meat for use, the bottles need to be kept at room temperature for only a few minutes before the delicate icy connections between the balls have thawed sufficiently to permit of easy withdrawal. Special thawing of the contents in bulk, in order to take out a sufficient supply of meat, is unnecessary. The balls remaining after each removal may be speedily returned to the cold-room without undergoing any change to speak of. The weighing, after removal, may be made very accurate by shaving off sufficient from an additional ball to give the desired quantity.

After the weighed meat has been placed in the feeding-dish, the hash soon softens and is ready for ingestion in a few minutes. Its treatment after removal from the bottle must naturally depend upon the requirements of the experiment in which it is to be used. In the researches in this laboratory on dogs in nitrogenous equilibrium, the meat has been weighed in a common glass crystallization dish,[1] in which were also placed definite quantities of cracker dust and lard, with subsequent addition of given proportions of water. On thoroughly stirring this mixture, the balls quickly fall apart, and, if the quantity of water is not excessive, the fluid finally has the consistency of thick soup. The odor of fresh meat is predominant when the cracker dust and lard are not too great in amount. Gentle warming suffices to raise the mixture to the ordinary temperature. It may be added that dogs eat this mixture very readily for weeks. Further, it is very digestible and nutritious.

To answer the question whether any important changes in the chemical composition of the meat take place during prolonged periods of preservation, the nitrogen content was determined in two samples of each of several preparations, at intervals of about ten days, for several weeks, with the results tabulated below.[2]

The analytic data obtained not only show the general uniformity in composition of meat preserved in this way, but demonstrate, likewise, that no important chemical alteration takes place at any time

[1] In shape the common glass crystallization dish is very well adapted to the licking up of last portions. Because of its transparency the operator can also easily bring together to the centre the fine particles which the animal missed at first, thus favoring final ingestion of the entire meal.

[2] The analyses were made by the Kjeldahl method. The quantities of hash used varied from 2.1362 to 3.3550 grams.

during the period of preservation, if the proper precautions are observed. The unimportant fluctuations in nitrogen percentages in the table are all within the limits of unavoidable error of analysis. The average percentages emphasize the fact of perfect uniformity throughout.

Percentages of Nitrogen.

Preparation. No.	Before freezing.	After freezing.		
	At time of preparation.	10 days.	21 days.	30 days.
1	3.58 3.49	3.56 3.51	3.57 3.45	3.58 3.57
2	3.60 3.55	3.58 3.46	3.69 3.59	....
3	3.58 3.67	3.60 3.59	3.64 3.58	3.59 3.67
4	3.69 3.73	....	3.70 3.75	3.64 3.68
Averages.				
1	3.53	3.53	3.51	3.57
2	3.57	3.52	3.64	....
3	3.62	3.59	3.61	3.63
4	3.71	....	3.72	3.66

It may be suggested that the use of this method is impracticable where special cold-storage facilities are lacking. It can be said, however, in anticipation of such a conclusion, that practically the same satisfactory preservative results could be obtained, although with less convenience, of course, if the bottles were placed in an ordinary refrigerator and surrounded each day with the common freezing mixture of crushed ice and salt. Melting of the ice would not be very rapid, under these conditions, and it could be renewed at little expense whenever necessary.

The chief advantages gained by the use of nitrogenous food material prepared by the method just described are:—

1. The perfect freshness of the food at the time of its consumption, even weeks after its preparation; therefore, its similarity in appearance, odor, and taste to ordinary fresh meat, and its superiority to forms of nitrogenous food to which the animal is unaccustomed, or for which it has no desire.

2. The constancy of composition of the food throughout even the longest experiments, by which circumstance the labor of analysis is reduced to a minimum.

This method is therefore especially useful in metabolism experiments on dogs.

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A NEW CONSTITUENT OF BONE.

BY

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of New York.

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Early in the last century (1838) Johannes Müller was the first to observe that when hyaline cartilage is boiled in water a product is formed which closely resembles gelatin, physically and chemically. Müller gave the name "chondrin" to the cartilage jelly formed in this way. Marchand, a few years later, applied the term "chondrigen" to the antecedent substance in the tissue which on boiling was transformed into "chondrin." For many years "chondrin" and "chondrigen" were looked upon as distinct and definite chemic substances, and numerous deductions regarding connective tissue relationships were based upon this assumption.

About a decade after Müller's discovery, Hoppe-Seyler, in a study of their decomposition products, showed that these proteid materials were not as nearly related to gelatin and collagen as had been inferred. Subsequently, Bödecker and others found that a reducing substance could be separated from "chondrin." Eichwald and Obolensky, about the same time, obtained similar reducing bodies from various mucoids.

This coincidence led von Mering in 1873, under Hoppe-Seyler's direction, to make a search for mucoid in cartilage. He identified it in aqueous extracts of the tissue by the acetic acid method. Three years later, Morochowetz, under Kühne's direction, made more extended experiments in this connection and demonstrated that "chondrin" is a mixture—containing gelatin, mucoid and inorganic matter. Mörner has lately shown that cartilage contains collagen, albumoid (elastin?), chondromucoid and chondroitin sulphuric acid, in considerable quantity, and that "chondrin" is a mixture of gelatin, chondromucoid, chondroitin sulphuric acid and soluble salts.

We now know that mucoids are normally present, in

small quantity at least, not only in cartilage, but all forms of connective tissue, although for a long time this fact was not appreciated. The author has lately shown the presence of mucoid in bone, thus establishing closer chemic relationship between mature bone and cartilage than had been supposed to exist, and demonstrating, further, that, as far as mucoid content is concerned, osseous tissue is not an exception among connective tissues, as previously it seemed to be.

In referring to Morochowetz's discovery that "chondrin" is a mixture containing mucoid, Drechsel, in 1883, wrote as follows: "If chondrin is in reality gelatin and mucin the transformation of cartilage into true bone is all the more easily comprehended, for in that case such development would consist essentially in only the elimination of the mucoid constituent." The deposition of inorganic matter in addition is, of course, to be understood.

For years it has been said that cartilage would yield "chondrin," but that true bone would not. The views of Hofmann, expressed in 1875, are representative of those held in this connection until very recently. He stated that "chondrin may be obtained from bone *before* ossification, but ossified bone yields only gelatin. . . . Embryonic bones contain no collagen, but do contain chondrigen, which is not transformed into the first-named, but before ossification is displaced by it. Completely calcified bone does not contain even a trace of chondrigen." Until the author's work was begun it had been generally accepted that osseous tissue does not contain glucoproteid. An examination of the statements in recent textbooks on the chemic qualities of bone shows that the pressure of mucoid is either denied or the question ignored.

The later and more prominent experimental results repeatedly given as authority for the statement that mature compact bone does not contain mucoid, have led to inaccurate conclusions. Von Ebner, in 1887, indicated that the decussating fibers of Sharpey are similar to those in fibrous connective tissue in general, and that they are not calcified, but that the calcareous deposit in bone is confined to the interfibrillar areas. These observations led Young[1] to investigate the question whether the matrix, in which the fibers of the bone structure are embedded, "is completely calcified or not." He con-

[1] Young: *The Journal of Physiology* (English), 1892, Vol. xiii, p. 803.

cluded that this question could be most readily solved by ascertaining whether mucin, "the most abundant constituent of the uncalcified matrix or ground substance of connective tissue, is present or absent." Working under Halliburton's superintendence, Young failed to extract from bone, with lime-water or dilute baryta-water, any substance that could be precipitated with acetic acid. He concluded, because of this seeming absence of glucoproteid from compact bone, that "in the process of ossification the connective tissue matrix is apparently completely calcified."

Unfortunately this important conclusion was brought about by three very obvious defects of procedure. In the first place, Young employed too much alkaline extractive fluid in proportion to the amount of bone taken in his experiments, thus making it exceedingly difficult to detect any existent mucoid. Again, the absolute quantities of bone extracted were so small that no positive result could reasonably have been expected.

The chief objection, however, to the method Young employed was the direct application of dilute lime or baryta-water to a dense, compact tissue, thoroughly impregnated with salts which for the most part are insoluble in such medium. It is not difficult to understand how, in the case of the femur, for example, the stone-like structure of the compact portion, composed as it is largely of tribasic earthy phosphates, imposed a serious obstacle to the usual action of lime-water on contained mucoid substance, and therefore it is natural to assume that for this reason, if for no other, no glucoproteid was detectable in Young's experiments. Certainly, removal of the salts from bone is the necessary preliminary to extraction in dilute alkali, if any hope is to be entertained of finding mucoid in that tissue.

The several difficulties just alluded to have been overcome by very ordinary means, and the author has succeeded in obtaining a surprisingly large yield of mucoid from both the femur and the rib of the ox by the following general method:

After the fresh bones had been thoroughly freed of adherent muscle and connective tissue, they were kept in 0.2 to 0.5% hydrochloric acid for the removal of inorganic matter. In the course of a few hours the dilute acid took out the salts from the surface of the bones just as satisfactorily, although not as rapidly as stronger acid would have removed it. After this treatment the bones were scraped twice daily with a stout, well-sharpened

scalpel. The superficial decalcified layer was thus easily removed in long, narrow, thin, elastic shavings, very soft and pliable. The dilute acid was completely renewed after each scraping. The ossein obtained in the first two scrapings was thrown away, for fear it was contaminated with minute particles of superficial connective tissue elements belonging to the periosteum, which might not have been completely removed in the preliminary treatment. While the shavings accumulated they were kept in dilute alcohol to prevent putrefactive changes. As much as six to seven kilos of moist shavings were used at one time. The shavings were next run through a meat-chopper, and the resultant hash thoroughly washed free of alcohol and acid by decantation in distilled water. Finally the bulky ossein hash was transferred to several large bottles and repeatedly shaken at intervals for about 48 hours, with moderate excess of half-saturated lime-water. On strongly acidifying the filtered extract with 0.2% hydrochloric acid, a bulky flocculent precipitate rapidly separated. This was purified by the process of washing, reprecipitating, etc., usually employed for final preparation of pure glucoproteids.

This newly-discovered substance, osseomucoid, is practically the same as the mucoid in tendon, cartilage and other connective tissues. It not only responds to the general proteid tests, but appears to have the same solubilities and precipitative reaction as the other connective tissue mucoids, and yields the same large proportion of reducing substance on decomposition with mineral acids. Furthermore, the combustion equivalents of osseomucoid, chondromucoid and tendomucoid, as shown in the table below, are practically identical, indicating close chemic relationship of these glucoproteid products.[1]

The average composition of four purified preparations of osseomucoid is given below, where comparison may also be made with the elementary composition of similar products:

	C.	H.	N.	S.	O.	Combustion equivalent.
Osseomucoid.....	47.07	6.69	11.98	2.41	31.85	4,992c.
Chondromucoid...	47.30	6.42	12.58	2.42	31.28	4,883c.
Tendomucoid.....	47.47	6.68	12.58	2.20	31.07	4,967c.
Average.....	47.28	6.60	12.38	2.34	31.40	4,947c.

These variations are quite within the limits of unavoidable errors of analysis. In the analytic work the author received the able assistance of his colleague, Mr. P. B. Hawk.

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[1] More detailed reference to the method of preparation and the chemic qualities of this substance was made in a recent number of the *American Journal of Physiology:* 1901, Vol. v, p. 387.

This discovery makes it evident that ordinary compact bone, like the other forms of connective tissue, contains mucin substance, and also, contrary to Young's deduction, that in the process of ossification, the connective tissue matrix is not completely removed. Further, it makes it easier to understand the accumulation of mucoid in various pathologic formations in osseous tissue which numerous observers, in recent years, have shown may often be considerable in amount.

The influence of disordered metabolism of this mucoid substance on the development of various bone tumors, particularly of the myxomatous type, can only be guessed, at present, but may prove to be more pronounced than the writer now supposes. Our knowledge of mucoid degeneration, not only in bone, but also in other tissues, will doubtless greatly advance as we learn more definitely the chemic phases of glucoproteid synthesis under normal conditions, and as we come to an understanding of the functions in the tissues of the various forms of these peculiar substances.

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CHEMICAL STUDIES OF OSSEOMUCOID, WITH DETERMINATIONS OF THE HEAT OF COMBUSTION OF SOME CONNECTIVE TISSUE GLUCOPROTEIDS.

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I. PREPARATION OF OSSEOMUCOID.[1]

HISTORICAL.

IT seems to have become generally accepted that osseous tissue does not contain glucoproteid. A study of the statements in the recent text-books, regarding the composition of bone, reveals the fact that either the existence of mucoid in bone structure proper is directly denied or else that nothing whatever is said as to its possible presence. The marrow of bone, however, has repeatedly been said to contain mucin, although reference to the sources of the information usually given in this connection shows that very little

[1] GIES: Proceedings of the American Physiological Society (New Haven meeting, December, 1899); This journal, 1900, iii, p. vii. Also, GIES: Proceedings of the American Association for the Advancement of Science (New York meeting, June, 1900), 1900, p. 131. See foot-note, p. 402, for reference to subsequent report.

work has been done to ascertain the facts, and that the results of that work are anything but conclusive.

Neumeister[1] states, in this connection, that "neither mucin, nor any body belonging to the glucoproteids, has ever been detected in osseous tissue, although fibrous connective tissue and cartilage do contain such substance." Referring to ossein, prepared in the usual manner, Gautier[2] writes: "It does not yield glucose (reducing substance) after prolonged boiling in dilute acid." "The absence of mucin in compact bone is noteworthy," says Halliburton,[3] "showing that the ground substance is entirely replaced by calcareous matter. Marrow, however, yields mucin." Hammarsten[4] gives considerable attention to the composition of bone, but ignores this phase of the subject altogether.[5]

Morochowetz,[6] in 1876, called attention to the fact that the so-called "chondrin" or "cartilage jelly" of the older writers was in reality a mixture of substances. Morochowetz stated that it consisted of gelatin and mucin. Drechsel,[7] referring a few years ago to Morochowetz's deductions in this regard, wrote as follows: "If chondrin is in reality gelatin + mucin, the transformation of cartilage into true bone is all the more easily comprehended, for in that case such development would consist essentially in only the elimination of the mucigenous constituent." The deposition of inorganic matter in addition is, of course, to be understood.

For years it was said that cartilage would yield chondrin, but that true bone would not. The views of Hofmann[8] are representative of those held for a long time. He stated that "chondrin may be obtained from bone *before* ossification, but ossified bone yields only gelatin." At another place Hofmann writes:[9] "Embryonic bones contain no collagen but do contain chondrigen, which is not transformed into the first-named, but before ossification is displaced by it. Completely calcified bone does not contain even a trace of

[1] NEUMEISTER: Lehrbuch der physiologischen Chemie, 1897, p. 453.

[2] GAUTIER: Leçons de chimie biologique normale et pathologique, 1897, p. 108.

[3] HALLIBURTON: Schäfer's Text-book of Physiology, 1898, i, p. 111.

[4] HAMMARSTEN: Lehrbuch der physiologischen Chemie, 1899, p. 326 *et seq.*

[5] See note, p. 400.

[6] MOROCHOWETZ: Jahresbericht über die Fortschritte der Thierchemie, 1877, p. 37.

[7] DRECHSEL: Hermann's Handbuch der Physiologie, 1883, Bd. v, Th. 1, p. 598.

[8] HOFMANN: Lehrbuch der Zoochemie, 1875–78, p. 25.

[9] HOFMANN: Ibid., p. 32.

chondrigen." Mörner[1] finally showed that cartilage contains chondromucoid ("mucin"), chondroitin sulphuric acid, collagen and albumoid (elastin?), and that chondrin is composed of the first two of these and gelatin.

Bone marrow. — Hoyer's[2] histological studies led him to assume that the ground substance of bone marrow is a loose, soft, mucous tissue. He did nothing in a chemical way to substantiate this view. Rustizky,[3] some time later, working with Rexlinghausen and under Hoppe-Seyler's direction, pointed out the incorrectness of this inference of Hoyer's, but, nevertheless, claimed to have shown the presence of a water-soluble mucin in the marrow of the bones of the rabbit. It was found to be absent from the marrow of the ox. Bone marrow from other animals was not examined.

It may reasonably be doubted, however, whether Rustizky's work is entirely reliable, for his deductions were based solely on the reduction test with alkaline copper solution after acid-decomposition of acetic acid precipitates, and no assurance was given that reducing substances were removed before the treatment with acid was begun, nor, indeed, that the precipitate itself had any proteid qualities other than precipitability with acetic acid. Further, the positive result with the rabbit tissue is referred by Rustizky and those who quote him, to marrow alone, although in Rustizky's experiments, after the adherent muscle had been removed, the whole bone, including the periosteum, was finely broken up in a mortar and the *mixture* extracted for mucin. It might with good reason, therefore, be assumed that any mucin really detected came from the periosteum, or the compact portion, instead of the marrow of the bones of the rabbit, and that a negative result was obtained with the ox marrow because the latter had been previously removed from the bone and, as Rustizky states, treated separately.

The question should still be regarded as an open one. Since Rustizky's time no results have been reported bearing on this subject. The author hopes to complete, in the near future, more definite experiments in this connection.

Compact Bone. — The experimental results repeatedly given as authority for the statement that mature, compact bone does not contain mucin have led to equally uncertain conclusions. No particularly

[1] C. Th. Mörner: Skandinavisches Archiv für Physiologie, 1889, i, p. 210.

[2] Hoyer: Centralblatt für die medicinischen Wissenschaften, 1869, p. 257.

[3] Rustizky: *Ibid.*, 1872, p. 561.

chemical investigations seem to have been made in this connection until a few years ago. Von Ebner[1] had shown that the decussating fibres of Sharpey are similar to those in fibrous connective tissue in general, and that they are not calcified, but that the calcareous deposit in bone is confined to the interfibrillar areas. These observations led Young[2] to investigate the question whether the matrix, in which the fibres of the bone structure are embedded, "is completely calcified or not." He concluded that this question could be most readily solved by ascertaining whether mucin, "the most abundant constituent of the uncalcified matrix or ground substance of connective tissue, is present or absent." Working under Halliburton's superintendence, Young failed to extract from bone with lime water or dilute baryta water any substance that could be precipitated with acetic acid. He concluded, because of this seeming absence of glucoproteid from compact bone, that, "in the process of ossification, the connective tissue matrix is apparently completely calcified." Young's results would imply the absence, from bone, not only of mucin but of chondromucoid as well, deductions which remained undisputed, so far as the author knows, until this work was begun.

Young's result and his general conclusion did not seem to harmonize with several well-known facts. Mörner's[3] researches, for example, on the proteids of cartilage, which were published in detail several years before Young's results were announced, showed that chondromucoid is present in relatively large quantity in that tissue, and of course suggested, further, that bone derived from cartilage contains a chondromucoid residue.

Practically all forms of uncalcified fibrous tissue from which the intercalated material has not entirely disappeared are known to contain mucin; yet bone, according to Young, would be regarded as an exception, although its large quantity of ground substance holds "bone corpuscles" in great number, and it contains circumferential, decussating and perforating fibres, as well as the fibrillar tissue of the Haversian canals and the fibrous structures among the "systems."

Since bone is formed in all cases by an ossification of connective tissue, and as collagen and other proteids are among the substances regularly contained in bone, it seems natural to suppose that during the developmental changes some of the connective tissue glucoproteid

[1] Von Ebner: Archiv für mikroskopische Anatomie, 1887, xxix, p. 213.

[2] Young: The journal of physiology (English), 1892, xiii, p. 803.

[3] C. Th. Mörner: *Loc. cit*

would remain with the other organic substances. Furthermore, if glucoproteid has any definite function to perform in the connective tissues, if its presence there signifies anything, there is certainly reason to believe that it plays some part, however obscure, in bone metabolism, also. The organic constituents already identified in bone, or, let us say, the usual connective tissue elements which remain in bone after ossification is complete, are, according to Halliburton, "collagen, small quantities of elastin from the lining of the lacunae and canaliculi, proteids and nuclein from the cells, and a small quantity of fat even after the removal of all the marrow."[1] Why not mucin or chondromucoid? Surely, unless the ground substance of the antecedent tissue is *entirely* removed as impregnation with inorganic matter proceeds and permanently replaced in the mature bone — and there is no histological evidence of any such fact — mucoid substance ought to be separable, in small proportion at least, from osseous tissue.

Upon referring to Young's paper the author was impressed with the inadequacy of the method which had led to only negative results and conclusions. Young treated hard, compact bone, either in the form of fine shavings or in powder, for from three to five days with a "*large excess* of lime water or dilute baryta water." Just what the "large excess" was intended to accomplish it is hard to surmise; for, on the assumption that probably at most only a very small proportion of mucin could be present in bone, subsequent precipitation would be favored if the extract were kept concentrated. Even finely divided tendon is usually treated with only 2 to 4 c.c. of half saturated lime water for every gram of tissue extracted, when easy separation of its glucoproteid is desired, and tendon probably contains relatively as much mucin as any other form of connective tissue. In Young's experiments as much as 100 c.c. of the dilute alkali was taken for each gram of substance extracted.

Another defect in Young's work that the author regrets to call attention to was the use of too small quantities of bone. In one experiment only 2.5 grams of bone powder were used; in the best of them only 11 grams were taken. According to Halliburton the normal adult connective tissues contain 0.5 to 0.8 per cent of mucin.[2]

[1] HALLIBURTON: *Loc. cit.* It is in connection with this statement that Halliburton accepts the results of the work of Rustizky and Young, with the comment already quoted.

[2] HALLIBURTON: Text-book of chemical physiology and pathology, 1891, p. 478.

The largest amount of mucin Halliburton and Stevenson obtained in their quantitative work was 1.02 per cent — from skin.[1] From the human Achilles tendon the largest amount obtained by them was 0.77 per cent. Now, if we assume for the moment that bone might contain as much mucin as was found in the skin analyzed by Halliburton and Stevenson — roughly 1 per cent — an assumption far too liberal, then the 2.5 grams of bone employed in one of Young's experiments might have yielded 0.025 gram of mucin in the 100 c.c. of dilute alkali used, or the 11 grams in the best of Young's experiments might have given 0.11 gram in 500 c.c. of solution. But these amounts are the greatest which could have been assumed to occur in bone and certainly it would have been extremely difficult, if not impossible, to precipitate smaller quantities than these from extracts purposely made so dilute. Solutions of pure mucin containing approximately these minute amounts of the proteid may yield flocculent precipitates with concentrated acetic acid after standing some time,[2] but tissue extracts, holding other dissolved proteids and saline matters, act differently.

As has just been indicated, the very small quantities of bone powder or shavings, used in Young's experiments, were treated for several days with a large excess of lime or baryta water. At the end of that time, varying amounts of acetic acid were added and, to use Young's own phrase, "no precipitate came down in any case." Nothing is said about turbidity, yet traces of mucin under these conditions certainly could hardly have caused more than cloudiness.

The chief objection, however, to the method Young employed was the direct application of dilute lime or baryta water to a dense compact tissue, thoroughly impregnated with salts which for the most part are insoluble in such medium. It is not difficult to understand how, in the case of the femur, for example, the stone-like structure of the compact portion, composed as it is largely of tribasic earthy phosphates, imposed a serious obstacle to the usual action of lime water on contained mucoid substance, and therefore it is natural to assume that for this reason, if for no other, no mucin was detectable in Young's experiments. Minute division of the dense tissue in this instance could hardly make the conditions more favorable for extrac-

[1] HALLIBURTON and STEVENSON: *Ibid.*, p. 478.

[2] This can occur only when the mucin has been dissolved in a very small quantity of dilute alkali. The salts formed on acidification tend to keep mucin in solution.

tion. The proportion of inorganic matter, and its influence against extraction of mucoid, would naturally remain almost the same in every particle, however small.

These obvious defects in the methods heretofore employed led the present writer to investigate this very simple problem in a way which seemed more favorable to the separation of mucoid. The several difficulties just alluded to have been overcome by very ordinary means, and a substance has been prepared from bone having all the general characters of the glucoproteids.[1]

METHOD OF PREPARATION.

In a few preliminary experiments, merely to test the objections here raised against Young's methods, but with no expectation of more definite results than he obtained, the author used 200–250 grams of powdered femur — made from only the compact portion of the shaft, which had previously been thoroughly scraped with a scalpel for the removal of all superficial connective tissue. These quantities were much larger than Young's. The femur powder was extracted for several days with just enough half-saturated lime water to cover it. On several occasions a very faint turbidity was obtained upon adding to the filtered extract 5 per cent acetic acid or 0.2 per cent hydrochloric acid until the reaction was distinctly acid. Even after standing a long time, the turbidity remained diffuse, and, as in Young's experiments, borrowing his phrase again, "no precipitate came down." But the turbidity was encouraging.

The author next proceeded to remove the salts from the bone as a necessary preliminary to extraction in dilute alkali, and by the following method succeeded in obtaining a surprisingly large yield of glucoproteid from both the femur and the rib of the ox.

The fresh bones, just after removal from the animals, were freed as thoroughly as possible from adherent muscle and connective tissue. In order to prevent putrefactive complications, the marrow, in the case of the femur, was completely cleaned out and the bones then placed in running water for twenty-four hours. At the end of that

[1] The terms mucin, mucoid, and chondromucoid have been used here to refer to connective tissue glucoproteid. Recent researches seem to indicate that the particular substances to which these names have been applied are not as different chemically as had been supposed. See CUTTER and GIES: Proceedings of the American Physiological Society; This journal, 1900, iii, p. vi. Also PANZER: Zeitschrift für physiologische Chemie, 1899, xxviii, p. 363; and LEVENE: *Ibid.*, 1901, xxxi, p. 395.

time the closely adherent connective tissue was somewhat swollen and could easily be completely scraped from the bones with an ordinary heavy scalpel. The inside of the shaft of the femur was again thoroughly swabbed. After this had been accomplished the bones were kept in 0.2–0.5 per cent hydrochloric acid. In the course of a few hours the dilute acid took out the inorganic matter from the surface of the bones just as satisfactorily, although not so rapidly, as much stronger acid could have done. It was better adapted for the purpose, also, because there was no special danger that transformation of mucoid would result from its use, — a fact of which there could be little doubt, because the acidity of the fluid in contact with the bones was constantly diminishing by reaction with the earthy compounds.[1]

After this treatment the bones were scraped twice daily with a stout, well-sharpened scalpel. The superficial decalcified layer was thus easily removed in long, narrow, thin, elastic shavings, exceedingly soft and pliable. The dilute acid was completely renewed after each scraping.[2] The ossein obtained in the first two scrapings was thrown away, for fear it was contaminated with minute particles

[1] This fact was observed repeatedly. The following results of one experiment in this connection show how rapid is the decrease of total acidity. In several preliminary titrations 100 c.c. of a special 0.5 per cent HCl solution was found to be exactly neutralized by 38.2 c.c. of a convenient dilute solution of ammonia; congo red was used as the indicator. A perfectly fresh femur of the usual size, after it had been thoroughly cleaned, was placed in 1000 c.c. of this particular solution of 0.5 per cent HCl. At intervals, after the fluid had been thoroughly stirred, total acidity was determined, with the same alkaline solution, in portions that had been boiled, for a few minutes, for elimination of carbon dioxide:

5.45 P. M. (femur first placed in acid) : 100 c.c. neutralized by 38.2 c.c. NH_4OH.
8.00 P. M. . . . . : 100 c.c. neutralized by 18.2 c.c. NH_4OH.
11.15 P. M. . . . . : 100 c.c. neutralized by 8.1 c.c. NH_4OH.
10.30 A. M. . . . . : 100 c.c. neutralized by 1.3 c.c. NH_4OH.

All determinations were made in triplicate, with varying volumes and the figures obtained agreed closely. These relative results show that at least 50 per cent of the total free acid was neutralized during the first three hours of contact with the bones.

[2] The quantity of dilute acid used for decalcification was about a litre for each portion of femur 6–8 inches in length; only the diaphysis was employed. When placed for a few hours in hydrochloric acid as dilute as 0.05 per cent, very thin, delicate shavings, so light that they float in water and dilute alcohol, may be obtained. Treatment with 0.5 per cent hydrochloric acid permits much more rapid decalcification, however, and makes the scraping process much easier. One half per cent hydrochloric acid was used in most of the experiments described in the second section, p. 402.

of superficial connective tissue elements belonging to the periosteum, which, perhaps, had not been completely removed in the preliminary treatment. The scraping process was continued until only a very thin, translucent layer inclosed the marrow cavity. While the shavings accumulated they were kept in 0.2 per cent hydrochloric acid for thorough decalcification, and for such gelatinization of collagenous elements as might be helpful to disintegration of the tissue and more complete liberation of "cement substance" during subsequent extraction. This treatment also prevented putrefactive changes.[1] At the end of two weeks two scrapings a day of two dozen sections of ox femur a little more than half a foot in length gave 1700 grams of moist ossein. The surplus moisture had been eliminated by cumulative pressure in a meat press.

The shavings were next run through a meat-chopper,[2] and then placed in running water until they were washed free from chloride. Finally the bulky ossein hash was transferred to several stoppered bottles and repeatedly shaken with half-saturated lime water in the proportion of from 2 to 5 c.c. of extractive fluid for every gram of the moist hash. Within ten minutes after the lime water treatment began, the extractive fluid became very frothy on shaking, and with excess of dilute acid a flocculent precipitate was obtained in a small portion. The extraction was continued for forty-eight hours, by the end of which time, it was subsequently found, almost all of the soluble substance had been removed. The filtered extract was then treated with 0.2 per cent hydrochloric acid.[3] The first addition produced heavy turbidity, and, after neutralization, a bulky flocculent precipitate separated at once in moderate excess of 0.2 per cent hydrochloric acid and fell rapidly to the bottom under a water-clear fluid.[4]

From this point the usual method for the purification of mucin was

[1] Subsequent experiments indicated that this acid treatment of the shavings, favoring gelatinization, is not particularly advantageous, perhaps is undesirable. Dilute alcohol (10 per cent) has been found to serve very well for preservative purposes during this preliminary period. See methods, p. 404 *et seq.*

[2] This can be done quite easily before the acid is washed out of the shavings, but is very difficult thereafter.

[3] Preferred to acetic acid as precipitant, because of its greater solvent action on non-glucoproteid material and because former experience has shown that connective tissue mucin is more easily thrown down with it.

[4] The precipitate closely resembled, in appearance and behavior, tendon mucin and chondromucoid.

pursued. The precipitate was several times washed, by decantation, in water made slightly acid with hydrochloric acid, then freed from acid by washing in water, filtered off, later dissolved in half-saturated lime water, reprecipitated with 0.2 per cent hydrochloric acid, repeatedly washed in acidified water, in water, and in alcohol, and lastly treated with boiling anhydrous alcohol-ether (50 per cent) as long as anything dissolved out. The alcohol was washed out with anhydrous ether. The purified substance dried quickly in the air to a very light, white, or faintly cream-colored powder devoid of hygroscopic qualities. Seventeen hundred grams of moist femur ossein yielded a trifle more than 7 grams of the substance; 875 grams of rib shavings gave 3.5 grams. In each case the amount of prepared substance was equal to approximately 0.4 per cent of the moist ossein.[1]

The acid filtrate from the substance thus prepared contains gelatin and a body closely related to, if not identical with, the separated mucoid. Possibly chondroitin sulphuric acid and gelatin combinations, such as Schmiedeberg[2] recognized, are in solution. The author is not sure that nucleoproteid is not contained in it. These matters are under investigation.

DISCUSSION OF MODIFYING FACTORS.

It will be seen from the analytic results given on page 402 that the substance which has been isolated by the method just described is typical glucoproteid. In considering its preparation by this method the author would not ignore the possibility that chondroitin sulphuric acid has combined with some of the gelatin, resulting from the action of the acid on the collagen, to form an artificial glucoproteid. It is well known that such combination of these substances may occur after prolonged contact at body temperature or more quickly in the presence of free acid, and it might be assumed that such syntheses took place in these experiments. Mörner found that chondroitin sulphuric acid has strong affinity for gelatin, in acidified solution, and made use of this tendency to detect the

[1] Various minor improvements of the method of preparation suggested themselves as the work progressed. Notes of these are made in the second section, p. 404 *et seq*.

[2] SCHMIEDEBERG: Archiv für experimentelle Pathologie und Pharmakologie, 1891, xxviii, p. 355.

ethereal compound.[1] Schmiedeberg[2] has given the names "peptochondrin" and glutinchondrin" to the insoluble intermediate combinations of gelatin pepton and chondroitin sulphuric acid, and "chondralbumin" or "chondralbuminoid" to the soluble products, formed in his process of isolating chondroitin sulphuric acid from cartilage. His experiments clearly indicate that various substances containing chondroitin sulphuric acid, similar to chondromucoid, are present in cartilage, probably all of them loose compounds of the acid with simple proteid. Mörner[3] has shown that chondroitin sulphuric acid may combine with simple proteid in the urine, which compound, on acidification, separates as an insoluble substance having most of the qualities of uromucoid. Krawkow[4] has also called attention to the fact that various combinations of chondroitin sulphuric acid may be induced with different proteids.

It has frequently been said that bone contains a trace of chondroitin sulphuric acid, but if any is present as such in osseous tissue, or as a simple alkali salt, it would seem that the author's preliminary treatment in these experiments should have entirely extracted it from the ossein, unless, perhaps, the hydrochloric acid, used to remove inorganic matter, fixed it *in situ* by quickly furnishing it with the requisite amount of gelatin before its solution from the decalcifying tissue. Mörner,[5] it will be recalled, used essentially this same acid treatment to gelatinize the collagen of cartilage in order to extract chondromucoid more completely and easily. After preliminary treatment with distilled water he digested the cartilage shavings in 0.1–0.2 per cent hydrochloric acid at 40° C. to transform insoluble collagen into soluble gelatin, thus disintegrating the tissue somewhat and favoring subsequent extraction of the glucoproteid from the residue with 0.05–0.1 per cent potassium hydroxide. Although it would be expected that this preliminary treatment with water should

[1] C. Th. Mörner: *Loc. cit.* The precipitate of gelatin and chondroitin sulphuric acid is readily soluble in excess of mineral acids. Salts interfere with precipitation of the compound by 0.2 per cent hydrochloric acid. Chondroitin sulphuric acid itself interferes to a certain extent with precipitation of chondromucoid by dilute acid at room temperature. See also, Zeitschrift für physiologische Chemie, 1894, xx, p. 357, and K. A. H. Mörner, cited in note below.

[2] Schmiedeberg: *Loc. cit.*

[3] K. A. H. Mörner: Skandinavisches Archiv für Physiologie, 1895, vi, p. 332.

[4] Krawkow: Archiv für experimentelle Pathologie and Pharmakologie, 1897, xl, p. 195.

[5] C. Th. Mörner: *Loc. cit.*

suffice to dissolve out all of the preformed or loosely combined chondroitin sulphuric acid, it is possible that some of it may have remained in the cartilage in Mörner's experiments, just as some might have remained in the decalcified tissue in the present experiments. Mörner has ignored the matter entirely, and no one else has called attention to such possibility. The question raised in this connection is now being studied. The author inclines to the belief that artificial glucoproteid was not formed in the ossein in the manner just discussed.

It should not be forgotten, of course, in any consideration of this matter, that no one has ever shown definitely the existence of preformed, free chondroitin sulphuric acid in normal bones. Mörner's[1] first researches on the distribution of chondroitin sulphuric acid in the bones of the ox did not disclose its presence. Unlike Schmiedeberg,[2] however, he was able to prepare it from some pathological human cartilaginous and osseous structures — in six cases of enchondroma, in one of chondroma osteoides mucosum tibiae and one of exostosis cartilaginea humeri. Mörner's method of detecting chondroitin sulphuric acid in these investigations, consisting, as it did in part, of treatment with 2 per cent potassium hydroxide, makes it uncertain whether this complex ethereal sulphuric acid existed as such in the bones he analyzed or whether it was derived from preexistent glucoproteid in the extraction process.[3] The present writer thinks the latter view more probable.

Later, Mörner's[4] studies of the content of sulphuric acid in the ash of the bones of the ox, as well as in the acid extract obtained by treatment of bones from the same animal with boiling hydrochloric acid (25 per cent), led to the deduction that the constant trace of SO_3 found, 0.01–0.04 per cent, came from a very slight quantity of chondroitin sulphuric acid, and Mörner assumed that these indirect methods gave positive proof of the presence of this substance in bone, contrary to the former negative results, because of the "greater delicacy" they possessed over his original direct estimations. His methods of detection do not warrant the belief, however, that the SO_3

[1] C. Th. Mörner: Zeitschrift für physiologische Chemie, 1895, xx, p. 357.

[2] Schmiedeberg: *Loc. cit.*

[3] Levene has separated a substance similar to chondroitin sulphuric acid from tendon mucin and other mucoids. Cleavage was accomplished by essentially the same treatment — with 2 per cent sodium hydroxide: Zeitschrift für physiologische Chemie, 1901, xxxi, p. 395. See also Schmiedeberg, *loc. cit.*, for similar facts.

[4] C. Th. Mörner: Zeitschrift für physiologische Chemie, 1897, xxiii, p. 311.

came directly from preformed chondroitin sulphuric acid or an alkali salt. It might have come indirectly from glucoproteid, which, if present, would have been decomposed into simple proteid and SO_3 combinations during the treatment in each of the processes used.[1] Bielfeld[2] recently found as much as 0.076 per cent of SO_3 in the ash of fœtal bones and attributed this increase over Mörner's figures to a greater amount of chondroitin sulphuric acid in the embryonic tissue. It is quite as reasonable to assume, however, that the SO_3 detected by Bielfeld was originally a part of chondroitin sulphuric acid in constituent glucoproteid. Krawkow[3] also states that he found chondroitin sulphuric acid in the diaphysis of the femur of the horse, sheep, and ox. He decalcified with hydrochloric acid; he does not state the strength of the acid employed, but it may have been sufficient to decompose mucoid. Subsequently the prepared ossein was digested in artificial gastric juice (with probable formation of "peptochondrin," etc.), and chondroitin sulphuric acid was extracted from the undigested residue, after treatment with potassium hydroxide (amount and strength not stated), in continuation of Schmiedeberg's process. The methods Krawkow employed make it probable that the ethereal compound was derived from antecedent complex material, and his results prove nothing regarding preformed chondroitin sulphuric acid, or the presence in bone of a simple salt of the same.

PROPERTIES OF OSSEOMUCOID.

The substance prepared by the method previously outlined has the general qualities of the glucoproteids, and for the sake of convenient reference the author proposes for it the name osseomucoid, although he believes that it is quite as nearly related to the mucins of tendon and ligament[4] as is chondromucoid of cartilage.[5]

[1] See VANDEGRIFT and GIES: This journal, 1901, v, p. 287, for similar facts connected with SO_3 in the ash of ligament and for related points. Krawkow has separated chondroitin sulphuric acid by destructive method from ligamentum nuchæ as well as from bone.

[2] BIELFELD: Zeitschrift für physiologische Chemie, 1898, xxv, p. 350.

[3] KRAWKOW: *Loc. cit.*

[4] RICHARDS and GIES: Proceedings of the American Physiological Society; This journal, 1901, v, p. xi. Also, CUTTER and GIES: *Loc. cit.*

[5] Long after the completion of the experiments described under this head, and shortly before this paper was sent to the editor, the author received Cohnheim's Chemie der Eiweisskörper (1900) and was surprised to find, on page 285, the following: "The ground-work of bone, apart from a very slight quantity of mucoid

Osseomucoid dissolves readily in 0.05 per cent sodium carbonate and in 5 per cent sodium chloride, from which solutions it may be precipitated with mineral or organic acids. It appears to dissolve only slightly in cold 0.2 per cent hydrochloric acid. The moist substance is acid to litmus, lacmoid, and congo red. When the pure product, which had been precipitated with hydrochloric acid, was thoroughly decomposed in dilute nitric acid no chlorine reaction could be obtained in the fluid with silver nitrate. Like tendon and liga-

(mucin) and chondroitin sulphuric acid *which perhaps are not contained in true bone*, consists of collagen, etc." Cohnheim bases this statement regarding possible presence of mucoid on the authority of some observations of Morochowetz (Verhandl. d. Heidelberger naturh.-med. Vereins, N. F., i, p. 480, 1876), whose opinion in this particular connection seems to have received no attention at the time (the text-books of his day do not refer to it), and appears to have been entirely overlooked until Cohnheim brought it to light again (see historical review, p. 387). The only other reference to Morochowetz's work the author has had access to, in the absence of the original paper, is the abstract in the Jahresbericht über die Fortschritte der Thierchemie, 1877, p. 37, where, it may be seen, the article was entitled: "Zur Histochemie des Bindegewebes." Unfortunately, the abstract fails to mention bone among the tissues examined, which suggests, of course, that Morochowetz's result or statement in connection with it was a minor one. From the title of the paper it may be inferred that if any work was done on bone it was purely histochemical in nature and that no mucoid substance was really separated or accurately identified. Besides — and this is a point of considerable significance in this connection — the body which Morochowetz identified in the various other tissues under examination and which he called mucin, did not, he says, contain sulphur, a statement clearly indicating inaccurate chemical observation, since all of the connective tissue mucins contain a relatively large proportion of sulphur. From Cohnheim's statement it may also be judged that the mucoid to which Morochowetz referred was not definitely ascertained to be a part of true osseous tissue. On discovering the statement in Cohnheim's book, the author wrote at once to his colleague, Dr. H. C. Jackson, lately in Professor Hofmeister's laboratory, for detailed information as to the contents of Morochowetz's paper. Dr. Jackson consulted the original in the Strassburg library and, thanks to his kindness, the author is able to say that Morochowetz claimed to have obtained mucin (a sulphur-free glucoproteid!) from several forms of connective tissue, such as cornea and cartilage. The only form of bone studied was embryonic in structure and consequently contained much pure cartilage. Morochowetz states he obtained the same substance from fœtal bone that he had previously identified in various forms of cartilage. His deductions are to be referred rather to cartilage, therefore, than to true bone.

Since the above was given to the printer the author received, through the courtesy of Dr. Leon Asher, of Bern University, a reprint of Morochowetz's paper in the Heidelberg Verhandlungen. A study of the same confirms all that has been said here regarding it.

ment mucins, and chondromucoid, it dissolves in dilute alkali, and when sufficient substance is suspended in the liquid, neutralization of the latter results with formation of an alkali salt of the proteid, which is soluble in neutral fluid. Osseomucoid gives the biuret, Millon's, and the xanthoproteic reactions very distinctly. Neutral solutions of its salts are not coagulated on boiling. It gives only a slight sulphide reaction with lead acetate after decomposition in hot potassium hydroxide. The fluid containing the products of its decomposition by boiling 2 per cent hydrochloric acid, however, gives a heavy precipitate of barium sulphate with barium chloride in the presence of free hydrochloric acid, and strong reduction of Fehling's and Nylander's solutions may be obtained after neutralization. This carbohydrate substance yields osazone crystals with phenylhydrazin. Osseomucoid is partly digested in "pepsin-hydrochloric acid;" the anti-albumid-like residue probably contains substance similar to peptochondrin. On hydration in boiling mineral acid, anti-albumid, albuminate, proteose and pepton are formed and have been identified.

The original preparations, one from the rib, the other from the femur, of the ox, were partially analyzed, with the results shown in the table on page 402.[1]

The discovery of a mucoid constituent of bone naturally suggests numerous lines of investigation, some of which have already been indicated. In what quantity, for example, does osseomucoid exist in bone at various stages of development? Is it peculiar to some bones or is it found in all? How has it affected previous analyses of bone gelatin, of bone ash, etc.? What is its biological significance; its relation, if any, to pathological formations, its exact place in the glucoproteid classification; its inner make-up, composition reactions, etc. These and other related problems are under investigation and the author hopes to present detailed results of these studies in the near future. The following sections, on composition and heat of combustion, give complete results of some of the work in this general plan.

[1] The analyses were incomplete, only because the bulk of each preparation was used for the qualitative determinations which were necessary for ascertaining the general properties of the substance. The methods employed were the same as those outlined on p. 403 of the following section. Customary quantities were used. Sulphur was not determined in the ash because bone contains merely traces of sulphate and the reagents were free from it. Probably only that derived, on oxidation, from the proteid itself, would be found in the ash. Complete analytic results are given in the succeeding section.

In concluding this section, the author wishes to acknowledge his indebtedness to Mr. Christian Seifert, assistant in this laboratory, for much valuable help. Mr. Seifert carefully prepared, under the author's supervision, all of the bone shavings used in these experiments and cheerfully accomplished that arduous task at the cost of considerable personal inconvenience.

PERCENTAGE COMPOSITION.

Preparation.	Nitrogen.	Total sulphur.	Sulphur combined as SO_3.	Total phosphorus.	Ash phosphorus.	Ash.
A. Rib.	12.78	1.68	0.98	0.086	0.051	2.28
	12.99	1.75	0.91	0.031	0.039	2.19
	12.80					
	12.91					
B. Femur	13.38	1.89	1.04	0.108	0.057	2.62
	13.41	1.87	1.11	0.054	0.061	2.57
	13.45					
Calculated for ash-free substance.[1]						
A.	13.17	1.76	0.97	0.013		
B.	13.77	1.93	1.11	0.022		

II. COMPOSITION OF OSSEOMUCOID.[2]

The results of the preliminary analyses seemed to establish beyond doubt the general glucoproteid nature of osseomucoid. Complete elementary analysis was necessary, however, to determine definitely its chemical relationships. We have made such analyses of a number of additional products from the femur of the ox, which were prepared

[1] Reference to phosphorus content, and other deductions as to chemical relationship, are deferred to the succeeding section, where more complete analyses are given. See p. 412.

[2] HAWK and GIES: Proceedings of the American Physiological Society (Baltimore meeting, December, 1900). This journal, 1901, v, p. xv. Previous reports noted on p. 387.

and purified, with several variations, as will be indicated, by the method already given.[1] The results obtained in this work harmonize, it will be seen, with the original deductions.

METHODS OF ANALYSIS.

Carbon and hydrogen. — Estimations were made, with all due precautions, by the method of oxidation in properly arranged combustion tubes, the gaseous products formed in the process passing through a layer of granulated copper oxide and over a reduced copper spiral. The absorbing apparatus consisted of three U-tubes of suitable size, containing concentrated sulphuric acid in the first, for the absorption of water, soda lime in the second and soda lime, with pumice stone moistened by sulphuric acid, in the third, for the absorption of carbon dioxide.[2] The soda lime was prepared as recommended by Benedict.[3] The tubes of the absorbing apparatus were wiped with cloth, in all cases, before weighing, and finally weighed upon a counterpoised balance until constant figures were obtained.[4]

Nitrogen. — Nitrogen was determined by the Kjeldahl process. Digestion of the substance in concentrated sulphuric acid was completed with small quantities of metallic mercury. Before distillation with excess of caustic soda, the mercury was precipitated with potassium sulphide. In the titrations, congo red was used as the indicator.

Total sulphur and phosphorus. — These elements were determined by the well known fusion methods. Fusion was made in silver crucibles (over alcohol flames in the sulphur determinations), with solid potassium hydroxide and potassium nitrate, each free from phosphorus and sulphur.[5]

Sulphur combined as SO_3. — Sulphur in the form of ethereal sulphuric acid was determined as follows: The substance was digested with about 175 c.c. of 2 per cent hydrochloric acid over an alcohol flame for six hours in a flask connected with a reflux condenser. At the end of the boiling process, when cleavage was complete, the

[1] See p. 393.

[2] Benedict: Elementary organic analysis, 1900, p. 34.

[3] Benedict: Journal of the American Chemical Society, 1899, xxi, p. 393.

[4] An important precaution. Considerable variation in the results may occur when it is not observed.

[5] When traces of these elements were present in the reagents, their quantities were carefully determined and corrections made accordingly.

acidity of the fluid was reduced somewhat with pure ammonium hydroxide, although the mixture was left distinctly acid. It was then filtered for the separation of antialbumid-like substance which had formed in small proportion during the process. The sulphuric acid in the hot filtrate and washings finally was precipitated with barium chloride, and the figures for sulphur obtained from the barium sulphate in the usual manner.[1]

Ash. — Inorganic matter was estimated by direct incineration of the substance in a platinum crucible over a very low flame. Phosphorus of the ash was determined in nitric acid solution of the same by the customary method involving the use of "molybdic solution" and "magnesia mixture."[2]

RECORDS OF ANALYSIS.[3]

Preparation No. 1. — Bones in 0.3 per cent HCl. 2,700 grams moist shavings accumulated in 0.2 per cent HCl. Before extraction in lime water, acid was removed by washing in large volumes of water. When decanted fluid no onger gave acid reaction to litmus, ossein hash was extracted in half-saturated lime water, 4 c.c. of dilute alkali per gram of substance, for forty-eight hours. End of that time, extract neutral; gave only slight precipitate on acidification with 0.2 per cent HCl. Acid had not been completely washed out by decantation method. Hash placed in half-saturated lime water again; same quantity for same time. Second extract gave excellent precipitate on acidification with 0.2 per cent HCl. Slight precipitate of first extract discarded, only second purified. Dissolved in half-saturated lime water, filtrate opalescent. Reprecipitated once with 0.2 per cent HCl. Washed in water, alcohol, ether, etc. Purified product snow-white, very light, amorphous powder. 6.5 grams. Dried to constant weight at 100-110° C. and analyzed with following results:

Carbon and Hydrogen.[4] 0.1520 gram substance gave 0.2667 gram CO_2 = 47.85 per cent C, and 0.0952 gram H_2O = 7.01 per cent H; 0.1728 gram substance gave 0.3046 gram CO_2 = 48.08 per cent C, and 0.1078 gram H_2O = 6.98 per cent H.

[1] Great care was taken to prevent introduction of sulphate during the method of preparation of the osseomucoid analyzed. The reagents used were entirely free from SO .

[2] Sulphur of the ash was not determined. See note, p. 401.

[3] Very brief reference to the more important details of preparation precedes the analytic data of each particular sample of osseomucoid. The method given on p. 393 is followed in a general way for each preparation.

[4] Osseomucoid is so light and bulky that larger quantities of substance could hardly be used conveniently in these determinations. Special care was exercised, herefore, in all the analyses.

Nitrogen. 0.2606 gram substance gave 0.0369 gram N = 14.15 per cent N; 0.2557 gram substance gave 0.0361 gram N = 14.11 per cent N; 0.2520 gram substance gave 0.0354 gram N = 14.06 per cent N.

Total Sulphur. 0.2518 gram substance gave 0.0204 gram $BaSO_4$ = 1.12 per cent (?) S; 0.2530 gram substance gave 0.0249 gram $BaSO_4$ = 1.36 per cent S; 0.2510 gram substance gave 0.0252 gram $BaSO_4$ = 1.38 per cent S.

Sulphur combined as SO_3. 0.2390 gram substance, after boiling in HCl, gave 0.0103 gram $BaSO_4$ = 0.59 per cent S; 0.2418 gram substance, after boiling in HCl, gave 0.0085 gram $BaSO_4$ = 0.49 per cent S.

Ash. 0.3134 gram substance gave 0.0070 gram Ash = 2.24 per cent Ash; 0.2560 gram substance gave 0.0054 gram Ash = 2.11 per cent Ash; 0.2572 gram substance gave 0.0064 gram Ash = 2.49 per cent Ash.

Total Phosphorus. 0.2509 gram substance gave 0.0009 gram $Mg_2P_2O_7$ = 0.099 per cent P; 0.2516 gram substance gave 0.0007 gram $Mg_2P_2O_7$ = 0.078 per cent P.

Ash Phosphorus. 0.8266 gram substance left 0.0187 per cent Ash, which gave 0.0008 gram $Mg_2P_2O_7$ = 0.029 per cent P.

PERCENTAGE COMPOSITION OF THE ASH-FREE SUBSTANCE.[1]

								Average.
C	48.97	49.20	....	....	....	....	...	49.08
H	7.17	7.14	....	....	....	....	....	7.16
N	....	....	14.48	14.44	14.39	....	....	14.44
S	....	....	....	....	....	1.39	1.41	1.40
O	....	....	....	....	....	....	....	27.92

Preparation No. 2. — Preliminary treatment same as in Prep. No. 1, except that bones were decalcified in 0.5 per cent HCl. 1,900 grams moist shavings. Profiting by previous experience, however, acid was washed out in running water. Extraction made in 10 c.c. half-saturated lime water for each gram of ossein; continued twenty hours. 2.5 per cent acetic acid used to precipitate. Substance separated in large flocks and settled out more slowly than when thrown down by dilute HCl. Dissolved in half-saturated lime water. Filtrate slightly turbid or opalescent in spite of repeated filtration. Reprecipitated once with 2.5 per cent acetic acid in moderate excess. Washed in water, alcohol, etc. Partly gummy on drying. 5.7 grams dried at 100-110° C. and analyzed, with appended results:

Carbon and Hydrogen. 0.1273 gram substance gave 0.2216 gram CO_2 = 47.48 per cent C, and 0.0815 gram H_2O = 7.16 per cent H; 0.1306 gram substance gave 0.2276 gram CO_2 = 47.53 per cent C, and 0.0777

[1] Reference to phosphorus content is made on p. 412.

gram H_2O = 6.66 per cent (?) H; 0.1280 gram substance gave 0.2242 gram CO_2 = 47.77 per cent C, and 0.0834 gram H_2O = 7.29 per cent H.

Nitrogen. 0.2522 gram substance gave 0.0348 gram N = 13.79 per cent N; 0.2188 gram substance gave 0.0305 gram N = 13.94 per cent N; 0.2484 gram substance gave 0.0349 gram N = 14.02 per cent N.

Total Sulphur. 0.2037 gram substance gave 0.0210 gram $BaSO_4$ = 1.42 per cent S; 0.2035 gram substance gave 0.0202 gram $BaSO_4$ = 1.37 per cent S.

Sulphur combined as SO_3. 0.2021 gram substance, after boiling in HCl, gave 0.0089 gram $BaSO_4$ = 0.61 per cent S; 0.2035 gram substance, after boiling in HCl, gave 0.0105 gram $BaSO_4$ = 0.71 per cent S.

Ash. 0.2556 gram substance gave 0.0066 gram Ash = 2.58 per cent Ash; 0.2528 gram substance gave 0.0064 gram Ash = 2.53 per cent Ash.

Total Phosphorus. 0.2012 gram substance gave 0.0006 gram $Mg_2P_2O_7$ = 0.083 per cent P; 0.3127 gram substance gave 0.0005 gram $Mg_2P_2O_7$ = 0.045 per cent P.

Ash Phosphorus. 0.5084 gram substance left 0.0130 gram Ash, which gave 0.0007 gram $Mg_2P_2O_7$ = 0.038 per cent P.

PERCENTAGE COMPOSITION OF THE ASH-FREE SUBSTANCE.[1]

									Average.
C	48.72	48.77	49.01	....	....	....	....	....	48.83
H	7.35	....	7.48	....	....	....	....	....	7.42
N	....	....	....	14.15	14.30	14.38	....	....	14.27
S	....	....	....	....	....	....	1.46	1.41	1.43
O	....	....	....	....	....	....	....	....	28.05

Preparation No. 3. — Preliminary treatment same as for Prep. No. 2. 2,900 grams moist shavings. Two extractions made; first for twenty hours, second for seventy-two hours. Osseomucoid precipitated by 0.2 per cent HCl. Much less substance precipitated from second extract than from first. Combined and dissolved in 0.05 per cent KOH. Filtrate slightly opalescent. Thrice reprecipitated by 0.2 per cent HCl.[2] Then washed once in 0.1 per cent HCl, lastly in H_2O, etc. 11.2 grams light cream colored powder. Dried, etc., with following analytic results:

Carbon and Hydrogen. 0.1106 gram substance gave 0.1858 gram CO_2 = 45.82 per cent C, and 0.0681 gram H_2O = 6.89 per cent H; 0.1143 gram substance gave 0.1946 gram CO_2 = 46.43 per cent (?) C, and

[1] It will be observed that the composition of the product precipitated by acetic acid (Prep. No. 2) is essentially the same as that prepared with 0.2 per cent hydrochloric acid (Prep. No. 1).

[2] Extra reprecipitation seems to have resulted in lowering of the percentage of carbon and nitrogen, and raising that of sulphur and oxygen. See p. 407.

0.0698 gram H_2O = 6.83 per cent H; 0.0970 gram substance gave 0.1627 gram CO_2 = 45.75 per cent C, and 0.0620 gram H_2O = 7.15 per cent H; 0.1075 gram substance gave 0.1810 gram CO_2 = 45.92 per cent C, and 0.0680 gram H_2O = 7.08 per cent H.

Nitrogen. 0.2790 gram substance gave 0.0366 gram N = 13.13 per cent N; 0.3281 gram substance gave 0.0433 gram N = 13.20 per cent N; 0.2651 gram substance gave 0.0348 gram N = 13.12 per cent N.

Total Sulphur. 0.2526 gram substance gave 0.0336 gram $BaSO_4$ = 1.83 per cent S; 0.2516 gram substance gave 0.0332 gram $BaSO_4$ = 1.82 per cent S.

Sulphur Combined as SO_3. 0.2434 gram substance, after boiling in HCl, gave 0.0183 gram $BaSO_4$ = 1.03 per cent S; 0.2438 gram substance, after boiling in HCl, gave 0.0181 gram $BaSO_4$ = 1.02 per cent S.

Ash. 0.2602 gram substance gave 0.0039 gram Ash = 1.50 per cent Ash; 0.2589 gram substance gave 0.0040 gram Ash = 1.54 per cent Ash.

Total Phosphorus. 0.2504 gram substance gave 0.0009 gram $Mg_2P_2O_7$ = 0.100 per cent P; 0.2506 gram substance gave 0.0004 gram $Mg_2P_2O_7$ = 0.045 per cent P; 0.2874 gram substance gave 0.0005 gram $Mg_2P_2O_7$ = 0.048 per cent P.

Ash Phosphorus. 0.5191 gram substance left 0.0079 gram Ash, which gave 0.0003 gram $Mg_2P_2O_7$ = 0.016 per cent P.

Percentage Composition of the Ash-free Substance.

										Average.
C	46.53	....	46.46	46.63	....	....	....	....	....	46.54
H	7.00	6.94	7.26	7.19	....	....	....	....	....	7.10
N	....	....	....	....	13.33	13.40	13.32	....	....	13.35
S	....	....	....	....	....	....	....	1.86	1.85	1.85
O	....	....	....	....	....	....	....	....	....	31.16

Preparation No. 4. — Same preliminaries as for Prep. No. 2. 3,950 grams moist shavings. Extraction in 10 c.c. half-saturated lime water for each gram of ossein; continued seventy-two hours. Osseomucoid precipitated with 0.2 per cent HCl. Dissolved in slight excess of 0.05 per cent NaOH and reprecipitated five times; each solution filtered. Filtrate at first turbid or opalescent as each time heretofore. After the pores of the filter paper became clogged, however, the filtrate was collected more slowly, but came through as clear as water, though yellowish in color.[1] About three-

[1] Possibly the observed differences in analytic results between this and the previous preparations were due to the presence of bone corpuscles, etc., in the latter, which had not been completely removed in the process of filtration. See also foot-note, p. 406.

fourths of final solution obtained water clear; turbid portion discarded. After fifth reprecipitation substance was thoroughly stirred up in 8 litres of 0.2 per cent HCl. There was no particular diminution in quantity, although the flocks seemed to shrink somewhat and become heavier and more granular. Acid washed out with H_2O. Final purification same as heretofore. In spite of losses, 7.7 grams osseomucoid obtained; very light, cream colored. Analyzed in the usual way, the appended results were obtained:

Carbon and Hydrogen. 0.1124 gram substance gave 0.1906 gram CO_2 = 46.25 per cent C, and 0.0669 gram H_2O = 6.66 per cent H; 0.1311 gram substance gave 0.2216 gram CO_2 = 46.14 per cent C, and 0.0797 gram H_2O = 6.81 per cent H.

Nitrogen. 0.2670 gram substance gave 0.0320 gram N = 11.97 per cent N; 0.2810 gram substance gave 0.0339 gram N = 12.06 per cent N.

Total Sulphur. 0.2526 gram substance gave 0.0406 gram $BaSO_4$ = 2.21 per cent S; 0.2534 gram substance gave 0.0373 gram $BaSO_4$ = 2.03 per cent S; 0.3032 gram substance gave 0.0406 gram $BaSO_4$ = 1.84 per cent (?) S; 0.3290 gram substance gave 0.0503 gram $BaSO_4$ = 2.10 per cent S.

Sulphur Combined as SO_3. 0.3227 gram substance, after boiling in HCl, gave 0.0259 gram $BaSO_4$ = 1.10 per cent S; 0.3237 gram substance, after boiling in HCl, gave 0.0251 gram $BaSO_4$ = 1.04 per cent S.

Ash. 0.2662 gram substance gave 0.0012 gram Ash = 0.45 per cent Ash; 0.2656 gram substance gave 0.0012 gram Ash = 0.45 per cent Ash.

Total Phosphorus. 0.3022 gram substance gave 0.0004 gram $Mg_2P_2O_7$ = 0.044 per cent P; 0.3028 gram substance gave 0.0002 gram $Mg_2P_2O_7$ = 0.018 per cent P.

Ash Phosphorus. 0.5318 gram substance left 0.0024 gram Ash, which gave 0.0003 gram $Mg_2P_2O_7$ = 0.016 per cent P.

PERCENTAGE COMPOSITION OF THE ASH-FREE SUBSTANCE.

								Average.
C	46.46	46.35	....	....	....	....	....	46.40
H	6.69	6.84	....	....	....	....	....	6.77
N	....	....	12.02	12.11	....	....	....	12.06
S	....	....	....	....	2.22	2.04	2.11	2.12
O	....	....	....	....	....	....	....	32.65

Preparation No. 5. Bones decalcified in 0.5 per cent HCl. In sixteen days 4,410 grams moist shavings obtained. Shavings each day were placed in 0.1 per cent HCl; on the following day, and thereafter until used, in 25 per cent alcohol. Latter was acid from acid in shavings. Acid washed out with water by decantation until pieces of the ossein hash no longer reacted acid to litmus. 6 c.c. half-saturated lime water used to extract, for each gram of ossein. After two hours, extract was nearly neutral; showing that acid in interior of pieces

had not been completely washed out.[1] Sufficient 10 per cent KOH was then added, drop by drop with thorough shaking, to make approximately 0.05 per cent KOH in the fluid. After twelve hours the alkalinity had again perceptibly diminished; 2 c.c. half-saturated lime water for each gram of ossein finally added. Extractive period, from beginning, was fifty-two hours. Extract in the end very frothy. Was diluted with equal volume of water, and osseomucoid precipitated from diluted solution with 0.2 per cent HCl. Reaction was made only very slightly acid; precipitation purposely left incomplete, the turbid portion yielding small amount of flocculent precipitate on further acidification. This was discarded. Main precipitate dissolved in half-saturated lime water and reprecipitated eight times. Just before final precipitation with 0.2 per cent HCl, the filtrate, after passing through the same filter paper repeatedly, was obtained as clear as water. In the end poured into 0.2 per cent HCl drop by drop, with instantaneous precipitation. Substance finally washed in sixteen litres 0.2 per cent HCl and twenty-four litres 0.1 per cent HCl, with thorough stirring; eventually in water, alcohol, etc. During the washing in water, some of the product persisted in floating, as had been the case in all previous preparations. In this particular case the floating portion was finally skimmed off and discarded. 17.8 grams of cream colored fluffy powder were obtained. Dried and analyzed:

Carbon and Hydrogen. 0.1247 gram substance gave 0.2180 gram CO_2 = 47.68 per cent C, and 0.0718 gram H_2O = 6.44 per cent H; 0.1492 gram substance gave 0.2615 gram CO_2 = 47.80 per cent C, and 0.0877 gram H_2O = 6.58 per cent H; 0.1615 gram substance gave 0.2809 gram CO_2 = 47.44 per cent C, and 0.0938 gram H_2O = 6.50 per cent H.

Nitrogen. 0.3026 gram substance gave 0.0355 gram N = 11.75 per cent N; 0.3022 gram substance gave 0.0352 gram N = 11.64 per cent N.

Total Sulphur. 0.5674 gram substance gave 0.1020 gram $BaSO_4$ = 2.47 per cent S; 0.5306 gram substance gave 0.0969 gram $BaSO_4$ = 2.51 per cent S.

Sulphur combined as SO_3. 0.4026 gram substance, after boiling in HCl, gave 0.0452 gram $BaSO_4$ = 1.54 per cent S; 0.4018 gram substance, after boiling in HCl, gave 0.0572 gram $BaSO_4$ = 1.96 per cent (?) S; 0.3512 gram substance, after boiling in HCl, gave 0.0382 gram $BaSO_4$ = 1.50 per cent S.

Ash. 0.3542 gram substance gave 0.0010 Ash = 0.28 per cent Ash; 0.3518 gram substance gave 0.0009 gram Ash = 0.26 per cent Ash; 1.329 gram substance gave 0.0043 gram Ash = 0.32 per cent Ash.

Total Phosphorus. 0.6371 gram substance gave 0.0002 gram $Mg_2P_2O_7$ = 0.008 per cent P; 0.9381 gram substance gave 0.0007 gram $Mg_2P_2O_7$ = 0.021 per cent P.

Ash Phosphorus. 1.329 gram substance left 0.0043 gram Ash, which gave 0.0007 gram $Mg_2P_2O_7$ = 0.015 per cent P.

[1] See foot-note, p. 410.

PERCENTAGE COMPOSITION OF THE ASH-FREE SUBSTANCE.[1]

								Average.
C	47.82	47.94	47.58	....	....	....	....	47.78
H	6.46	6.60	6.52	....	....	....	....	6.53
N	....	....	....	11.78	11.67	....	....	11.72
S	....	....	....	....	....	2.48	2.52	2.50
O	....	....	....	....	....	....	....	31.47

Preparation No. 6. Bones in 0.5 per cent HCl eighteen days. 6,680 grams moist shavings obtained by end of that time. As they were made they were placed in 10 per cent alcohol, repeatedly renewed to remove acid during period of accumulation. Alcohol washed out later in water by decantation. Extraction in half-saturated lime water, 8 c.c. per gram of ossein. End of four hours, extract nearly neutral. 10 per cent KOH added as before to make 0.05 per cent KOH in extract. After eighteen hours, extract again nearly neutral. 10 per cent KOH added to make total of 0.1 per cent KOH. Alkalinity gradually decreased; due not only to combining power of osseomucoid but also, probably, to failure to completely wash out HCl.[2] Ossein in dilute alkali for ten days. Powdered thymol prevented putrefactive change. Extract finally obtained as perfectly clear filtrate. Diluted with four volumes water and this treated with equal volume 0.4 per cent HCl. Immediate precipitation in large flocks, which became smaller and more granular after thorough stirring, and quickly settled out. Precipitate dissolved in fifth-saturated baryta water and reprecipitated with 0.4 per cent HCl nine times. Tenth reprecipitation made by filtering the $3\frac{1}{2}$ litres of the baryta solution of substance into twenty litres of 0.2 per cent acid. Each drop solidified on contact and fell quickly to the bottom in globular form. Globules were broken up on stirring. Thoroughly washed in 0.3, 0.2 and 0.1 per cent HCl, later in water, etc., as usual. Final product very light, snow-white powder: 29.75 grams. Following results of analysis were obtained:

Carbon and Hydrogen. 0.1862 gram substance gave 0.3176 gram CO_2 = 46.52 per cent C, and 0.1114 gram H_2O = 6.65 per cent H; 0.1877 gram substance gave 0.3190 gram CO_2 = 46.36 per cent C, and 0.1128 gram H_2O = 6.68 per cent H; 0.1449 gram substance gave 0.2469 gram CO_2 = 46.47 per cent C, and 0.0906 gram H_2O = 7 per cent H; 0.1649 gram substance gave 0.2802 gram CO_2 = 46.34 per cent C, and 0.1013 gram H_2O = 6.87 per cent H.

[1] See foot-notes, pp. 406 and 407.

[2] It is evident that the decantation process must be repeated very frequently if all acid is to be washed out. Filtered running water serves best for this purpose.

Nitrogen. 0.3000 gram substance gave 0.0360 gram N = 12 per cent N; 0.3000 gram substance gave 0.0357 gram N = 11.90 per cent N; 0.3000 gram substance gave 0.0360 gram N = 12 per cent N.

Total Sulphur. 0.3887 gram substance gave 0.0734 gram $BaSO_4$ = 2.59 per cent S; 0.2761 gram substance gave 0.0502 gram $BaSO_4$ = 2.50 per cent S.

Sulphur combined as SO_3. 0.3045 gram substance, after boiling in HCl, gave 0.0344 gram $BaSO_4$ = 1.55 per cent S; 0.3355 gram substance, after boiling in HCl, gave 0.0379 gram $BaSO_4$ = 1.55 per cent S.

Ash. 0.2658 gram substance gave 0.0006 gram Ash = 0.23 per cent Ash; 0.2650 gram substance gave 0.0006 gram Ash = 0.23 per cent Ash; 1.3781 gram substance gave 0.0036 gram Ash = 0.26 per cent Ash.

Total Phosphorus. 0.6840 gram substance gave 0.0002 gram $Mg_2P_2O_7$ = 0.008 per cent P.

Ash Phosphorus. 1.3781 gram substance left 0.0036 gram Ash, which gave 0.0009 gram $Mg_2P_2O_7$ = 0.018 per cent P.

Percentage Composition of the Ash-free Substance.[1]

										Average.
C	46.63	46.47	46.58	46.45	....	....	....	....	....	46.53
H	6.67	6.69	7.01	6.89	....	....	....	....	....	6.81
N	....	....	....	....	12.03	11.93	12.03	....	....	11.99
S	....	....	....	....	....	....	....	2.60	2.51	2.55
O	....	....	....	....	....	....	....	....	....	32.12

Preparation No. 7. Fifty sections of femur decalcified in particularly dilute HCl — 0.05 per cent.[2] Scraped twice daily. Shavings, as they were collected, were placed directly into 3–5 litres of water, 12–24 hours, and then in 10 per cent alcohol until sufficient quantity accumulated. At end of three weeks 2,500 grams very thin, narrow, elastic shavings obtained. After hashing, the finely divided ossein was extracted in half-saturated lime water, 20 c.c. per gram of hash, for seventy-two hours. Alkalinity had perceptibly diminished by end of that time. Water clear filtrate obtained. With 0.2 per cent HCl in excess finely flocculent precipitate at once. Same purification process as for Prep. No. 6. Reprecipitated only five times. Final product very light, white powder; 5.2 grams. Analytic results as follows :

Carbon and Hydrogen. 0.2470 gram substance gave 0.4304 gram CO_2 = 47.51 per cent C, and 0.1487 gram H_2O = 6.69 per cent H; 0.1952

[1] See foot-notes, pp. 406 and 407.

[2] The analytic results of this preparation agree very well with those for preparations Nos. 5 and 6, and indicate that the acid used in decalcifying has had no particular influence on the products separated.

gram substance gave 0.3389 gram CO_2 = 47.35 per cent C, and 0.1158 gram H_2O = 6.59 per cent H.

Nitrogen. 0.1754 gram substance gave 0.0212 gram N = 12.05 per cent N; 0.2431 gram substance gave 0.0296 gram N = 12.18 per cent N.

Total Sulphur. 0.4482 gram substance gave 0.0783 gram $BaSO_4$ = 2.40 per cent S; 0.6320 gram substance gave 0.1158 gram $BaSO_4$ = 2.52 per cent S.

Sulphur combined as SO_3. 0.6171 gram substance, after boiling in HCl, gave 0.0678 gram $BaSO_4$ = 1.51 per cent S; 0.5009 gram substance, after boiling in HCl, gave 0.0501 gram $BaSO_4$ = 1.37 per cent S.

Ash. 0.7256 gram substance gave 0.0022 gram Ash = 0.30 per cent Ash; 0.2891 gram substance gave 0.0008 gram Ash = 0.28 per cent Ash.

Total Phosphorus. 0.5661 gram substance gave 0.0005 gram $Mg_2P_2O_7$ = 0.025 per cent P.

Ash Phosphorus. 1.0147 gram substance left 0.0030 gram Ash, which gave 0.0010 gram $Mg_2P_2O_7$ = 0.027 per cent P.

Percentage Composition of the Ash-free Substance.

							Average.
C	47.65	47.49	....	....	....	....	47.57
H	6.71	6.61	....	....	....	....	6.66
N	....	....	12.09	12.22	....	....	12.15
S	....	....	.....	....	2.41	2.53	2.47
O	....	....	....	....	....	....	31.15

Summaries and Discussion of Analytic Results.

Content of phosphorus. — Before reviewing the general results of the analyses of the seven preparations we have carefully studied, special attention should be directed to the data on phosphorus content. The averages of our figures for percentage amount are here summarized: —

Substance.	Preliminary preparations.		Preparations completely analyzed.							Averages.
	Rib.	Femur.	1.	2.	3.	4.	5.	6.	7.	All.
Dry.	0.058	0.081	0.088	0.064	0.046	0.031	0.014	0.008	0.025	0.046
Ash.	0.045	0.059	0.029	0.038	0.016	0.016	0.015	0.018	0.027	0.029
Ash free.	0.013	0.022	0.049	0.026	0.030	0.015	....	....	....	0.017

It is very evident, from these results, that osseomucoid is a substance free from phosphorus. Not only are the above quantities entirely too small to have any particular significance, but all of them are within the ordinary variations in accuracy of the method of determination itself, and fluctuations may be due to unavoidable analytic error. Such traces as are indicated by the very painstaking determinations we have made undoubtedly are a part of the ash and not of the organic substance itself. The higher figures for the earlier preparations might be interpreted to mean phosphorized-proteid impurity. The differences are too slight, however, to warrant any such conclusion.[1]

Sulphur combined as SO_3. — We have not yet attempted to separate chondroitin sulphuric acid from osseomucoid, but the large proportion of combined SO_3 detected in, and separated from osseomucoid strongly indicates the presence of such a radicle in its molecule, particularly also because of the acid reaction of the proteid itself. The recent results obtained by Panzer,[2] on ovarial mucoid (paramucin), and Levene,[3] on various connective tissue and glandular glucoproteids, further suggest the probability that such an acid radicle will eventually be separated from osseomucoid. The percentage quantities of sulphur combined as SO_3 in all our preparations are here summarized, for ash-free substance, and the general averages contrasted with the amounts in chondromucoid and the mucins of ligament and tendon : — [4]

Preliminary preparations.		Preparations completely analyzed.							Averages.		Chondromucoid.	Tendon mucin.	Ligament mucin.
Rib.	Femur.	1.	2.	3.	4.	5.	6.	7.	All.	4–7.	Averages.		
0.97	1.11	0.55	0.68	1.05	1.08	1.53	1.55	1.44	1.11	1.40	1.76	1.43	1.07

[1] The tendon mucins analyzed by Dr. Gies, several years ago, contained 0.17 per cent P (average), which was also found to equal the percentage of phosphate in the ash. This observation has since been verified by Mr. Cutter, and identical results obtained for ligament mucin by Dr. Richards, in this laboratory. See also Krawkow's figures for percentage of amyloid : Krawkow, *loc. cit.*

[2] Panzer : *Loc. cit.*

[3] Levene : *Loc. cit.*

[4] C. Th. Mörner, Cutter and Gies, Richards and Gies : *Loc. cit.*

General Review. — The appended table summarizes the results for average percentage composition of osseomucoid (ash-free substance) and gives average composition of preparations 1–7; also of preparations 4–7, inclusive, the latter having been specially grouped together because of the greater attention given to their purification, by repeated reprecipitation, as has already been indicated: —

Elements.	Individual preparations.							Averages.	
	1.	2.	3.	4.	5.	6.	7.	1–7	4–7
C	49.08	48.83	46.54	46.40	47.78	46.53	47.57	47.53	47.07
H	7.16	7.42	7.10	6.77	6.53	6.81	6.66	6.92	6.69
N	14.44	14.27	13.35	12.06	11.72	11.99	12.15	12.85	11.98
S	1.40	1.43	1.85	2.12	2.50	2.55	2.47	2.05	2.41
O	27.92	28.05	31.16	32.65	31.47	32.12	31.15	30.65	31.85

The above results emphasize the glucoproteid character of osseomucoid, for, like practically all of these compound proteids, osseomucoid has a relatively low content of carbon and nitrogen, with a comparatively large proportion of sulphur and oxygen — due to the content of carbohydrate (probably polysaccharide) and sulphuric acid radicles; both rich in oxygen, the latter in sulphur.

Lack of particular uniformity in percentage composition, however, is evident on comparing the analytic results for the individual preparations. This want of analytic harmony cannot be due to nucleoproteid impurity, — our results for content of phosphorus show that conclusively,[1] — nor does it seem probable that admixture of other soluble proteid can be the cause, for bone contains too little such material to warrant that belief. We have already considered the possibility of chondroitin sulphuric acid combining with any gelatin made during the process of decalcifying, to form different products of varying solubilities, but, as has already been suggested, there is no reason to believe that bone contains sufficient chondroitin sulphuric acid to

[1] The content of phosphorus is too low for an assumption that either nucleoalbumin (0.4–0.8 per cent P) or phosphoglucoproteid (0.45 per cent P) was admixed. Comparatively large quantities of the substance contained the merest trace of iron. Undoubtedly this minute amount is to be recognized as inorganic impurity.

effect such a result.[1] We varied our method of preparation somewhat each time a new product was made for analysis, as may be seen in the records of analytic results, but, unless it be assumed that osseomucoid is very unstable, like submaxillary mucin, for example, and therefore easily influenced by the mild chemical treatment to which it was subjected, these changed conditions would not account for altered composition. We have seen, however, that osseomucoid behaves like tendon mucin and chondromucoid. We have every confidence in the accuracy of our methods of analysis and their manipulation.

Hammarsten,[2] it will be remembered, found that frequent precipitation of submaxillary mucin resulted in a lowering of the percentage of carbon and nitrogen of the purified product because of fractional elimination of nucleoalbumin. Our preparations 4–7 were given particular attention in this regard, with general results similar to those obtained by Hammarsten, and it may be that we have had to deal with unsuspected proteid impurity, which could only be, and perhaps was finally, eliminated by repeated reprecipitation. In the absence of direct evidence of such impurity, however, — and every condition seems to be against its occurrence, — we think our results justify the conclusion that the mucin substance of bone varies in composition just as the glucoproteid from other sources does, and that the figures in our analyses represent the make-up of several of these very closely related bodies. Such a conclusion not only accords with our analytic results but harmonizes also with the deductions drawn, under similar conditions for other tissues and products, by various observers.[3]

There appear to be many forms of glucoproteid. In all probability the acid and carbohydrate radicles of the mucoids have the power of uniting with various proteids in varying proportions to form different compounds, and while they can easily be arranged into general groups as we classify them to-day, in inner make-up they are doubtless multifarious. Such a conception of the chemical nature of the mucin substances would account for the wide variations that have been observed in the elementary composition not only of apparently the same substance, but also of very nearly related products from differ-

[1] See p. 396.

[2] HAMMARSTEN: Zeitschrift für physiologische Chemie, 1888, xii, p 163.

[3] CHITTENDEN and GIES: The journal of experimental medicine, 1896, i, p. 186. Also, SCHMIEDEBERG, K. A. H. MÖRNER, CUTTER and GIES, KRAWKOW, RICHARDS and GIES: *Loc. cit.*

GENERAL SUMMARY. AVERAGE PERCENTAGE COMPOSITION.

Elements.	Glucoproteids.										Simple proteid.
	Osseomucoid.		Chondromucoid.[1]	Tendon mucin.[2]	Cornea mucoid.[3]	Umbilical mucoid.[4]	Submaxillary mucin.[5]	Ovarial mucoid.[6]	Amyloid.[7]	Serum mucoid.[8]	Serum albumin.[9]
	1–7.	4–7.									
C	47.53	47.07	47.30	48.76	50.16	51.33	48.84	51.76	49.44	47.60	53.08
H	6.92	6.69	6.42	6.53	6.97	6.63	6.80	7.76	6.79	7.10	7.11
N	12.85	11.98	12.58	11.75	12.79	14.13	12.32	10.70	13.92	12.93	15.93
S	2.05	2.41	2.42	2.33	2.07	1.04	0.84	1.09	2.79	2.38	1.90
O	30.65	31.85	31.28	30.63	28.01	26.87	31.20	28.69	27.06	29.99	21.98

[1] C. Th. Mörner: Skandinavisches Archiv für Physiologie, 1889, i, p. 210.
[2] Chittenden and Gies: *Loc. cit.*
[3] C. Th. Mörner: Zeitschrift für physiologische Chemie, 1893, xviii, p. 213.
[4] Jernström: Jahresbericht über die Fortschritte der Thier-Chemie, 1880, x, p. 34.
[5] Hammarsten: *Loc. cit.*
[6] Mitjukoff: Centralblatt für die medicinischen Wissenschaften, 1895, xxxiii, p. 737.
[7] Krawkow: *Loc. cit.*
[8] Zanetti: Jahresbericht über die Fortschritte der Thier-Chemie, 1897, xxvii, p. 31.
[9] Michel: *Ibid.*, 1895, xxv, p. 11.

ent tissues. Until we know more about the inner nature of simple proteid, and of such complex substances as chondroitin sulphuric acid which readily unite with proteid in the normal and pathological metabolic changes in the tissues, it will be difficult to reach, from analytic results, conclusions more definite regarding various glucoproteids than those we have been able to draw from our analyses of osseomucoid.

Compared results. — In the general summary, on page 416, of analytic figures for tissue mucoids, direct comparison may be made with the osseomucoid averages. The figures for crystallized serum albumin are also given for convenient comparison of the collated analytic data with similar results for simple proteid.

III. Heat of Combustion of Osseomucoid, Tendon Mucin and Chondromucoid.

HISTORICAL.

In any consideration of the metabolism of energy in the body, the combustion equivalents of the food and excreta are factors of fundamental importance. It is now generally agreed, we believe, by all who have given special attention to such studies, that careful determinations of the potential energy, as expressed in calories, of all the constituents of the tissues should be made, if various important phases of metabolism are to be more thoroughly comprehended.

Although the "fuel values" of numerous albuminous mixtures, and some proteid substances, taken from the animal body have been very carefully estimated, no attention appears to have been paid, in this connection, to the glucoproteids, members of which group of bodies constitute so large a proportion of the interfibrillar or intercellular substance of various forms of connective tissue. We considered it desirable, therefore, to determine the combustion equivalent of osseomucoid and also of related glucoproteid, not only for the general thermochemical interest such results would have, but in the belief, also, that the caloric values obtained would throw further light on the chemical relationships of these tissue proteids, and ultimately be of worth in any metabolic study of their syntheses and transformations.

The researches of Stohmann, B. Danilewsky, Rubner, Berthelot and Atwater, and their pupils, have shown that the combustion equivalents of the chemically pure animal proteids thus far studied vary from averages of 5270 calories for gelatin and 5298.8 calories for

pepton, to 5961.3 calories for elastin; with egg albumin, at 5735.2 calories, representing about the mean value.[1] The work of these observers also indicates in a general way that the higher the percentage of carbon in the proteid, the greater its combustion equivalent; the greater the proportion of oxygen, on the other hand, the lower the heat of combustion. Thus elastin, which, we have seen, has the highest equivalent, contains about 55 per cent of carbon and 20 per cent of oxygen; pepton, with a much lower equivalent, contains roughly 50 per cent of carbon and 26 per cent of oxygen; albumin, having an average combustion equivalent, contains approximately 52.5 per cent of carbon and 23 per cent of oxygen.

Considerable variation is to be noted on comparing the figures for calories obtained for the same compound by different observers. This fact may be attributed, however, to different degrees of purity of the products burned, as well as to variations in the accuracy of the methods employed. Thus the caloric value of "ossein" is 5039.9 according to Stohman and Langbein[2] and 5410.4 according to Berthelot and André[3] — a difference of 370.5 calories. But as "ossein" is in strictness a tissue residue, not a pure chemical substance, these variations are not at all surprising.

The only strictly compound proteid investigated by combustion methods thus far is hæmoglobin. Its potential energy appears to be relatively high, varying from 5885.1[2] to 5914[3] calories. The com-

[1] The first of these figures was obtained by Atwater (see foot-note, p. 419). The rest were determined by Stohmann and Langbein, with the improved Berthelot method, and are taken from the table in the Centralblatt für Physiologie for 1892 (vi), p. 157. B. Danilewsky obtained somewhat lower figures for pepton, an average of 4900 calories (Centralblatt für die medicinischen Wissenschaften, 1885, xxiii, p. 678), but as these were derived by the older Thompson-Stohmann process, which was not as accurate as the Berthelot method, the values given by Stohmann and Langbein are probably more trustworthy. Fibroin is the only native proteid thus far studied which has a combustion equivalent lower than that of pepton. According to Stohmann and Langbein it is 4979.6 calories. Berthelot and André found it to be 5095.7 (Centralblatt für Physiologie, 1890, iv, p. 609). An excellent résumé of combustion methods and results is given by ATWATER: Methods and results of investigations on the chemistry and economy of food (Bulletin No. 21, Office of Experiment Stations, U. S. Department of Agriculture), 1895, p. 113; also by BUNGE: Lehrbuch der physiologischen und pathologischen Chemie, 1894, p. 62, and by GAUTIER: Leçons de chimie biologique normale et pathologique, 1897, p. 788.

[2] STOHMANN und LANGBEIN: Centralblatt für Physiologie, 1892, vi, p. 156.

[3] BERTHELOT et ANDRÉ: *Ibid.*, 1890, iv, p. 609.

bustion equivalent of milk casein, classified, by some, as pseudonucleoproteid, varies from 5629.2[2] to 5858.3[1] calories.

Of the results thus far obtained in calorimetric experiments the most important for us in this particular connection are those for "chondrin." Stohmann and Langbein have found the combustion equivalent of "chondrin" to be 5130.6 calories;[1] Berthelot and André[2] place it at 5345.8 calories.[3] This difference of 211.8 calories may be attributed to variations in the composition of the product burned, for "chondrin," with approximately 50 per cent of carbon and 28 per cent of oxygen, is a mixture consisting mostly of cartilage gelatin, chondromucoid and chondroitin sulphuric acid. It is almost impossible to make two preparations of the mixture having the same composition and in which the proportions of the components are alike. It is to be observed, however, that, even if the higher figures be accounted more correct, the value expressed by them is still about as low as any thus far determined for animal proteid — even for the *hydrated* forms such as pepton. The lowered potential energy of "chondrin," as well as its lowered percentage of carbon and the raised proportion of oxygen, may be reasonably attributed in great part to the carbohydrate portions of the contained chondroitin sulphuric acid and chondromucoid.[4]

METHOD OF DETERMINATION.

The determinations of heat of combustion in our own experiments were made in a Berthelot bomb calorimeter as modified and improved by Atwater and Blakeslee. Most of the experimental work in this connection was done by Mr. Hawk, in the chemical laboratories of Wesleyan University, the privileges of which were very kindly extended for the purpose by Professor Atwater, to whom we are

[1] STOHMANN und LANGBEIN: *Loc. cit.*

[2] BERTHELOT et ANDRÉ: *Loc. cit.*

[3] B. DANILEWSKI, working with the older and less accurate method, found it to be 4909 calories: Centralblatt für die medicinischen Wissenschaften, 1885, xxiii, p. 678.

[4] The values for heat of combustion of connective tissue collagens have never been determined. For the hydration product of mixed collagens, commercial gelatin, the value is 5,270 calories. ATWATER: Report of the Storrs (Conn.) Agricultural Experiment Station, 1899, p. 92 (Fish gelatin = 5493 calories: B. DANILEWSKY, *loc. cit.*). Cartilage gelatin has not been studied, in this connection. The combustion equivalent of disaccharides averages about 3900 calories; of polysaccharides about 4200 calories.

greatly indebted, also, for many courtesies and much valuable assistance.

Combustions of pure substances of known calorific power were made at intervals to test the apparatus and manipulations. The customary method of ignition, by means of iron wire, was used, and the

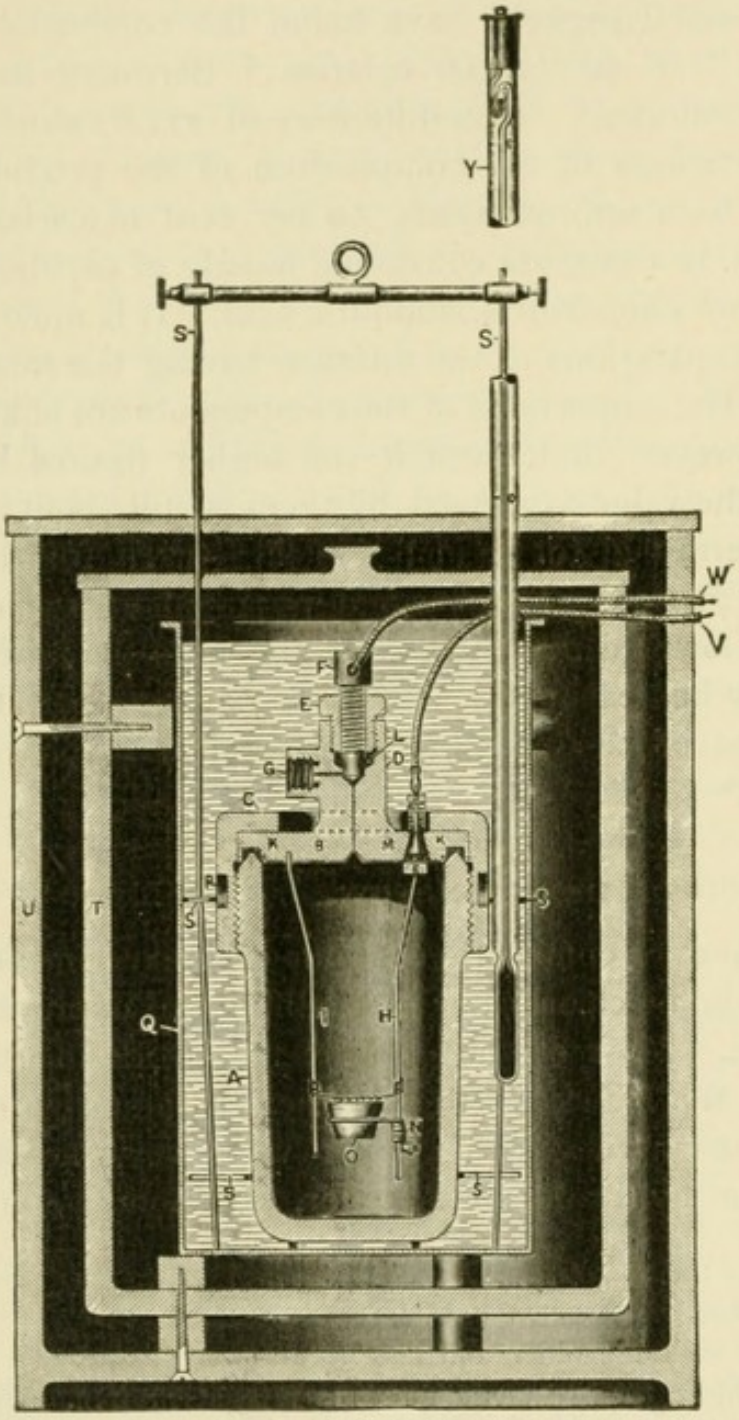


FIGURE 1. — Atwater-Blakeslee bomb calorimeter and accessory apparatus as arranged for combustions. — The platinum lined bomb of steel, holding oxygen and the substance to be burned, is immersed in water contained in a metal cylinder (Q); the latter is surrounded by concentric covered cylinders (T, U) of indurated fibre. Air spaces between the outer cylinders favor retention of heat in the water. The water is kept in motion with the aid of a stirrer (SS) driven by a small electric motor, thus equalizing temperature. Oxygen is forced into the empty bomb through the side passage (G) in the neck (D). Perfect closure of this passage is made by the valve screw (F). The electric current, for fusing the iron wire over the substance to be burned in the capsule (O), is conveyed by the insulated wires (W, V), one of which (W) is connected with the valve screw (F) and thus with one of the platinum wires inside the bomb (I), and the other (V) with the insulated platinum wire (H) which passes through the cover of the bomb. The thermometer is graduated to hundredths of a degree, and is capable of being read to thousandths with a magnifying lens.[1]

[1] For full description see ATWATER and BLAKESLEE: Report of the Storrs (Conn.) Agricultural Experiment Station, 1897, p. 199.

necessary correction made for its heat of combustion. Proper correction was also made for the thermal changes due to oxidation of the nitrogen of the proteid to nitric acid. The quantities of proteid employed in each determination varied from 0.6 to 1.0 gram. Each sample burned completely without special difficulty.

Two of the best of our completely analyzed preparations of osseomucoid were burned in the bomb. Samples of preparations No. 5 and No. 6 (see preceding section) were selected for the purpose. All but one of the tendon mucins employed for the same purpose were prepared and analyzed by Cutter and Gies,[1] and represent the glucoproteids, made by fractional precipitation methods, from both the sheath and the shaft of the tendo Achillis of the ox. The mucin of preparation, "c 8" was made and analyzed several years ago by Chittenden and Gies.[2] The preparations of chondromucoid which we oxidized in the calorimeter were made by Mörner's[3] method, especially for this work. Preparation "a 9" represents the mixed mucoid from three successive extractions of cartilage from the nasal septum of the ox; preparation "b 10" only the glucoproteid in the second extract of a separate portion of cartilage from the same source. Elementary analyses, in duplicate, were made by the methods given on page 403.

EXPERIMENTAL RESULTS.

In the summary on page 422 the figures in duplicate determinations, under "heat of combustion," represent small calories at constant volume per gram of substance dried at 100–110° C. to constant weight; the analytic figures represent elementary composition of perfectly anhydrous substance; complete averages and other data are also included.

DISCUSSION OF DATA.

The striking feature of the results for heat of combustion is the fact that they are uniformly low. The general averages fall far below the figures for potential energy of all the common proteids, including the hydrated forms, and even beneath the smallest equivalent recorded for fibroin (see page 418). This result was naturally to be expected,

[1] CUTTER and GIES: *Loc. cit.* The complete analytic data given here for these preparations anticipate the detailed publication of the results obtained.

[2] CHITTENDEN and GIES: *Loc. cit.*

[3] C. TH. MÖRNER: Skandinavisches Archiv für Physiologie, 1889, i, p. 210.

COMBUSTION EQUIVALENTS AND ELEMENTARY COMPOSITION OF GLUCOPROTEIDS.

Preparation.	Direct determinations.									Averages: Calculated for ash-free substance.					
	Heat of combustion. Small calories.			Average percentage composition of ash-free substance.					Ash.	Heat of combustion. Small calories.				Carbon.	Oxygen.
	Per gram.			C	H	N	S	O	Per cent.	Per gm.	For subst'nce containing 1 gm. carbon.	Group averages.			
	I	II	Average							X	Y	X	Y	Per cent.	
A. Osseomucoid.															
No. 5.—1	4915	4927	4921	47.78	6.53	11.72	2.50	31.47	0.29	4935	10329				
No. 6.—2	5029	5044	5037	46.53	6.81	11.99	2.55	32.12	0.24	5049	10850	4992	10589	47.16	31.79
B. Tendon mucin.															
a. From shaft 3	4925	4940	4933	47.47	6.68	12.58	2.20	31.07	0.69	4967	10463				
4	4963	4930	4947	47.23	6.56	11.78	1.81	32.61	0.80	4986	10558				
5	4921	4934	4928	47.61	6.60	12.66	1.85	31.18	1.04	4979	10459				
b. From sheath 6	4908	4920	4914	48.92	6.83	12.64	2.80	28.81	0.75	4951	10121				
7	5044	5036	5040	48.25	6.54	12.69	2.34	30.18	1.78	5131	10635				
c. From shaft and sheath 8	5010	5007	5009	48.74	6.46	11.80	2.35	30.65	0.67	5042	10345	5009	10430	48.04	30.75
C. Chondromucoid															
a. Of several extracts 9	4835	4850	4843	46.15	6.51	11.95	2.28	33.11	0.30	4857	10525				
b. Of second extract 10	4895	4888	4892	45.58	6.80	12.38	2.55	32.69	0.34	4909	10769	4883	10647	45.87	32.90
General Averages	4944	4948	4946	47.43	6.63	12.22	2.32	31.40	0.69	4981	10505	4981	10505	47.43	31.40

however, because of the decreased proportion of carbon and nitrogen, and the raised percentage of sulphur and oxygen produced in these compound substances by the union of proteid with carbohydrate and sulphuric acid radicles in their construction. The general average equivalent falls about midway between the figures for calorific value of polysaccharide and albumin.

Very little stress can be laid on the differences shown in the above table for the separate groups, because they are entirely too slight, and quite within the limits of unavoidable experimental error. On the other hand, the group agreement is so decided in the main that further experimental evidence is furnished, we think, of the chemical similarity and close relationship of the three substances, or groups of substances, under examination. It is interesting, also, to find that such differences as are expressed in the group averages run parallel with the fluctuations in amount of carbon and oxygen, the equivalents increasing as the percentage of carbon rises, and falling as the oxygen goes up in proportion.

The above average figures for composition and combustion equivalent are brought into direct comparison below with a similar average given by Stohman and Langbein: —[1]

Investigators.	Substances.	Average percentage composition.					Combustion equivalent. Small calories.
Stohmann and Langbein.	Numerous animal and vegetable proteids; not including mucoids.	C	H	N	S	O	5711
		52.64	7.08	16.00	1.03	23.20	
Hawk and Gies.	Connective tissue glucoproteids only.	C	H	N	S	O	4981
		47.43	6.63	12.22	2.32	31.40	

The general relation of our results to those obtained for other common proteids and albuminous mixtures is so clearly shown in the table[2] of averages on page 424 that further comment is unnecessary.

[1] STOHMANN and LANGBEIN: *Loc. cit.*

[2] Results not our own are selected from those for many substances burned and analyzed by Berthelot and André: *Loc. cit.*

Substance.	Combustion equivalent. Small calories.	Combustion equivalent. Large calories.	Percentage of carbon.	Percentage of oxygen.
	Per gram.	For substance containing 1 gm. of carbon.		
Chondromucoid.	4883	10.65	45.87	32.90
Tendon mucin.	5009	10.43	48.04	30.75
Osseomucoid.	4992	10.59	47.16	31.79
Hæmoglobin.	5914	10.62	55.51	17.62
Egg albumin.	5691	10.99	51 77	24.15
"Ossein."	5414	10.81	50.10	24.60
"Chondrin."	5346	10.54	50.89	23.03
Fish gelatin.	5242	10.80	48.53	25.54
Fibroin.	5097	10.60	48.09	27.41

IV. Summary of Conclusions.

1. A substance, designated as osseomucoid, having the chemical and physical qualities of mucin and chondromucoid, may be extracted from the rib and femur of the ox with lime water. Such extraction may be made most satisfactorily from ossein prepared, in the form of shavings, from bones which have previously been partly decalcified with very dilute acid (0.05–0.5 per cent HCl).

This discovery makes it evident that ordinary compact bone, like the other forms of connective tissue, does contain mucin substance, and further, contrary to Young's deduction, that in the process of ossification the connective tissue matrix is *not* completely removed.

2. The percentage composition of seven preparations of osseomucoid varied between the following extremes, with the subjoined general averages for the seven, also for the four agreeing quite closely and to which particular attention was given in the process of purification: —

	C	H	N	S	O
Extremes:	49.08–46.40	7.42–6.53	14.44–11.72	1.40–2.55	27.92–32.65
Average 1–7:	47.53	6.92	12.85	2.05	30.65
Average 4–7:	47.07	6.69	11.98	2.41	31.85

It is probable that there are two or more glucoproteids in bone, judging from the variations noted in the results for percentage composition.

Osseomucoid does not contain phosphorus. Between 1 and 1.6 per cent of its sulphur may be split off as SO_3 on boiling in dilute hydrochloric acid.

3. The energy liberated on oxidation of the mucin substances, as represented by osseomucoid, tendon mucin, and chondromucoid, is less than that for any other form of proteid except fibroin. The average of twenty duplicate determinations for ash-free substance is 4981 small calories per gram, just midway between the average equivalents for albumin and polysaccharide.

The average potential energy of osseomucoid (4992), tendon mucin (5009), and chondromucoid (4883) is found to be so nearly the same for each substance that additional experimental evidence is furnished of the very close chemical relationship of these connective tissue glucoproteids. Slight and variable differences in the content of carbon and oxygen in these substances appear to account for the minor fluctuations in the figures for combustion equivalent.

The average elementary ash-free percentage composition of the ten samples of typical glucoproteid studied by the combustion method is :

C	H	N	S	O
47.43	6.63	12.22	2.32	31.40

The figures for elementary ash-free composition of the preparations of tendon mucin and chondromucoid studied in this connection agree quite well with those for similar products analyzed several years ago by Mörner and by Chittenden and Gies. The observed analytic variations are comparatively slight, but suggest that tendon and cartilage each contains several closely related mucin substances.

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CHEMICAL STUDIES OF ELASTIN, MUCOID, AND OTHER PROTEIDS IN ELASTIC TISSUE, WITH SOME NOTES ON LIGAMENT EXTRACTIVES.[1]

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COMPREHENSION of function is dependent on knowledge of structure and composition. The influence of any tissue on the other parts of the body is more easily understood as our appreciation of the varieties and relations of its constituent elements increases. Elastic tissues have received little analytic attention. They have been overlooked by reason, apparently, of their seeming metabolic passivity and because they serve mainly mechanical functions.

The earlier observers regarded the cervical ligament as an extravascular tissue, for the most part, with practically no special chemical

[1] Some of the results of this research have already been given in the Proceedings of the American Physiological Society: This journal, 1900, iii, p. v.; 1901, v, p. xi.

activity and believed that it consisted almost wholly of the albuminoid elastin. Recently, however, it has been found in this laboratory[1] that the ligamentum nuchæ of the ox contains not only the large percentage of water and elastin, and the slight amounts of inorganic matter, collagen, and fat assumed to be present by the earlier investigators, but also appreciable quantities of mucoid,[2] coagulable proteid and crystalline extractives. These later results indicate that the production of elastin is the feature of ligament metabolism, and they indicate, further, that the chemical changes normally occurring in yellow elastic tissue are greater than had been supposed.

We have recently subjected the various constituents of elastic tissue to a somewhat detailed study. The particular form of tissue from which the constituents were prepared in all our experiments was the ligamentum nuchæ of the ox.

I. Ligament Elastin.

Preparation. *Historical.* — Tilanus[3] was probably the first to analyze elastic tissue. In his earlier preparations of "pure tissue" small pieces of the cervical ligament of the cow were first extracted in cold water to remove traces of blood and inorganic matter, and then dehydrated (and fat eliminated) with alcohol and ether. This product was hardly anything better than "prepared" ligament. In a second preparation he extracted in boiling dilute acetic acid in addition. Extraction with the acid doubtless removed all of the coagulable proteid and most of the collagen, but probably left behind most, or at least much, of the mucoid. The residue prepared in this way (after thorough removal of acid by washing in water and then dehydrating), unlike the product obtained by the first method, was said to be free of sulphur. Tilanus assumed it to be a pure chemical substance — elastin — and gave it the formula $C_{52}H_{80}N_{14}O_{14}$. In both of the methods used by Tilanus the tissue extractives were doubtless completely eliminated.

W. Müller[4] improved Tilanus's methods by adding treatment in boiling dilute alkali and cold dilute mineral acid to the preparation

[1] Vandegrift and Gies: This journal, 1901, v, p. 287.

[2] We use the word "mucoid" in the sense first suggested by Cohnheim. See Cutter and Gies: This journal, 1901, vi, p. 155 (foot-note).

[3] Tilanus: See Mulder, Versuch einer allgemeinen physiologischen Chemie, Zweite Hälfte, 1844–51, p. 595.

[4] W. Müller: Zeitschrift für rationelle Medicin, dritte Reihe, 1861, x, p. 173.

process. He alternately boiled finely divided ligamentum nuchæ from the horse and ox in dilute acetic acid and in dilute potassium hydroxide, and then extracted in cold dilute hydrochloric acid.[1] Such treatment tended to remove the residual collagen and all of the mucoid, but also favored decomposition of the elastin. Müller states that his purified product was fibrous in microscopic appearance and seemed to be unaffected by the alkali treatment.

Horbaczewski[2] made still further modification of the method used by Müller by introducing repeated extraction of the cervical ligament of the ox in boiling water. The treatment in boiling water thoroughly transformed insoluble collagen into soluble gelatin although it made subsequent extraction of coagulated proteid more difficult. Horbaczewski continued all of his extractions for longer periods than any of his predecessors. Subsequently, Chittenden and Hart,[3] commenting on Horbaczewski's work and the method of elastin preparation used by him wrote as follows: "So vigorous is the method of treatment, that it appears almost questionable whether a body belonging to a group noted for ease of decomposition might not suffer some change in such a long process of preparation."

Chittenden and Hart[4] compared elastin made from the ligamentum nuchæ of the ox by Horbaczewski's method with that obtained in their own process, which was the same except that the substance was not extracted in alkali. The chief difference noted was that the elastin which had been treated with potassium hydroxide contained no sulphur, whereas that which had not been extracted with alkali contained 0.3 per cent. For the first time the danger in the use of hot alkali was appreciated and pointed out.[5] At the same time the probable presence of mucoid was overlooked. There is no reason for believing that the mucoid could have been completely removed from the tissue pieces without the aid of alkali.

Bergh[6] recently obtained elastin from the cervical ligament by Horbaczewski's method, but added, also, digestion in pepsin-hydro-

[1] This was the method then commonly used for the preparation of resistant tissue elements like cellulose and chitin.

[2] Horbaczewski: Zeitschrift für physiologische Chemie, 1882, vi, p. 330.

[3] Chittenden and Hart: Studies from the Laboratory of Physiological Chemistry, Yale University, 1887–88, iii, p. 19.

[4] Chittenden and Hart: *Loc. cit.*

[5] Objections had also been raised from another standpoint by Zollikofer: Annalen der Chemie und Pharmacie, 1852, lxxxii, p. 169.

[6] Bergh: Zeitschrift für physiologische Chemie, 1898, xxv, p. 337.

chloric acid. Elastin is readily digestible in gastric juice,[1] however, so that this modification of treatment could hardly have dissolved very much that the acid and alkali did not remove, except elastin itself. Aside from determining the presence of sulphur in his own preparation of elastin and in Grübler's (a commercial product from the same source and prepared by Horbaczewski's method), Bergh made no attempt to ascertain the elementary composition of ligament elastin.

The ligament elastins made in various studies of this albuminoid by other observers (whose analytic results are referred to below), were all from the same source — ligamentum nuchæ of the ox. Morochowetz[2] made his products by Müller's method. Stohmann and Langbein[3] obtained theirs by the Horbaczewski process. Zoja,[4] Mann,[5] and Eustis[6] each used the method of Chittenden and Hart.

The following summary gives the average analytic results for percentage composition of the ash-free products prepared from ligament by the above methods.[7]

	Method of preparation.	C	H	N	S	O
Tilanus:						
	(*a*) Not extracted with acid	54.65	7.26	17.41	0.34	20.34
	(*b*) Extracted with acid	55.65	7.41	17.74	....	19.20
Müller[8]	Extracted with hot alkali	55.46	7.41	16.19	....	20.94
Horbaczewski[9]	Extracted with hot alkali	54.32	6.99	16.75	....	21.94

[1] See page 111.

[2] Morochowetz: St. Petersburger medicinische Wochenschrift, neue Folge, 1886, iii, p. 135.

[3] Stohmann und Langbein: Journal für praktische Chemie, neue Folge, 1891, lxiv, p. 353.

[4] Zoja: Zeitschrift für physiologische Chemie, 1897, xxiii, p. 236.

[5] Mann: Archiv für Hygiene, 1899, xxxvi, p. 166.

[6] Chittenden (for Eustis): Proceedings of the American Physiological Society, 1899, This journal 1900, iii, p. xxxi.

[7] For the composition of elastins from other sources than ligament see Vandegrift and Gies, *loc. cit.*, also Cohnheim: Chemie der Eiweisskörper, 1900, p. 293. We have no occasion in this review to refer to elastins which were not analyzed. Various observers have engaged in chemical studies of elastin without satisfying themselves of the purity of their products.

[8] Müller found 0.08 per cent of sulphur in his elastin, but assumed it to be due to accidental impurity.

[9] The analytic results credited to Etzinger by Charles in his "Elements of Physiological and Pathological Chemistry" (1884, p. 129), were those obtained by Horbaczewski. Etzinger made no analyses of ligament elastin. See Zeitschrift für Biologie, 1874, x, p. 84.

	Method of preparation	C	H	N	S	O
MOROCHOWETZ[1]	Extracted with hot alkali	?	?	?	0.63	?
CHITTENDEN and HART:						
	(*a*) Prepared by Horbaczewski's method	54.24	7.27	16.70	....	21.79
	(*b*) Their own, without extraction in alkali	54.08	7.20	16.85	0.30	21.57
STOHMANN and LANGBEIN:						
	Extracted with hot alkali	55.03	7.20	16.91	0.18	20.68
ZOJA	Not extracted with hot alkali . . .	?	?	16.96	0.28	?
MANN	Not extracted with hot alkali . . .	?	?	16.52	?	?
EUSTIS[2]	Not extracted with hot alkali . . .	54.42	7.40	16.65	0.14	21.39

It will be observed, from the preceding statements and summary, that as a general rule extraction with hot alkali resulted in the preparation of a sulphur-free product. On the other hand, methods which did not include alkali extraction gave elastins containing sulphur. The exceptions resulted, probably, when the alkali treatment was not as prolonged or thorough as customarily.

That treatment in hot alkali is apt to cause decomposition is now almost self-evident. Referring to this subject, Chittenden and Hart stated that "treatment with acid of the alkaline solution obtained in preparing elastin by Horbaczewski's method, plainly showed the presence of hydrogen sulphide." Did this sulphur come from the elastin and is elastin a sulphur-containing body, or did it arise from another substance originally in the ligament?[3]

The only constituents of elastic tissue which seem to require

[1] It has been shown by CHITTENDEN and HART that in elastoses there is a diminution of the content of carbon, and an increase in the content of oxygen, proportional to the extent of proteolysis. In spite of this fact, MOROCHOWETZ's analyses of elastose gave the following results:

C	H	N	S	O
55.90	7.29	16.68	0.62	19.50

He did not completely analyze the original elastin — only sulphur was determined as given above. It seems necessary to conclude that the elastin used by MOROCHOWETZ was an impure product and that the figures above for sulphur are inaccurate.

[2] EUSTIS made only a partial analysis. We are greatly indebted to him for a sample of his product, from which we obtained the remaining results. The individual ash-free determinations, by the methods we used farther on, were as follows:

C	H	N	S
54.52	7.47	16.64	0.12
54.32	7.32	16.66	0.15

[3] See page 105.

treatment with alkali, in addition to acid, in order to effect their complete solution are mucoid, and traces of nucleoproteid. These bodies as they are situated, resist the action of acids, the former particularly, and their removal from compact tissue particles is easy only when alkali is used. They are readily soluble in cold dilute lime-water, which has no effect on the elastin.[1]

Improved method. — Our improved method of preparing ligament elastin includes extraction in cold lime-water instead of destructive treatment with boiling potassium hydroxide, and may be given briefly as follows: Ligamentum nuchæ was cut into strips, these very finely minced in a meat chopper[2] and the resultant hash thoroughly washed in cold running water for from twenty-four to forty-eight hours. Traces of blood, lymph, and much coagulable proteid, with extractives, were removed in this process. The finely divided tissue was then thoroughly extracted for from forty-eight to seventy-two hours in large excess of cold half-saturated lime-water, renewed occasionally, for complete removal of residual simple proteid,[3] and also mucoid and nucleoproteid. After the alkali had been thoroughly removed by washing in water, the minced substance was boiled in water, with repeated renewals, until only traces of dissolved proteid (elastoses) could be detected in the washings. The tissue was then boiled in ten per cent acetic acid for a few hours, treated with five per cent hydrochloric acid at room temperature for a similar period, again extracted in hot acetic acid and in cold hydrochloric acid, finally washed free of acid in water, and then kept in boiling alcohol and

[1] It will be remembered that half-saturated lime-water and very weak alkaline fluids of approximately the same strength have been repeatedly used for extractive purposes in the past because they manifest no destructive action on compound proteids and albuminoids at room temperature.

[2] The ordinary hashing machine can be very advantageously used for this purpose. It not only finely divides the tissue but also tends to loosen the fibres in all of the particles, and thus greatly favors extraction of interfibrillar substance. Previous observers make no mention of the use of special mincing apparatus. In some of the preceding work the ligament was merely cut into small pieces with a knife.

[3] Our results with the simple proteids of ligament (page 118) suggest that in the preparation of elastin due regard must be paid to the fact that the fresh ligament contains at least 0.6 per cent of soluble and coagulable proteid. It certainly cannot be an easy matter to remove all this from the fibrous meshwork, particularly *after* the tissue has been boiled in water, and possibly some of the variations in the figures reported for the composition of elastin and the nature of its decomposition products may be due to such impurity not completely eliminated.

ether until dehydration was complete, and all fat and extractive substance had been removed.[1]

The elastin particles prepared in this way were soft and porous and could easily be ground in a mortar to a cream-colored, very light powder.[2] Under the microscope the particles were seen to consist uniformly of typical elastic fibres. No extraneous matter was held in the meshes of these.

In order to study the effect of the above modified method of preparation, as well as to obtain further information on the composition of elastin, we made several samples of elastin both by the Chittenden and Hart method and our own and subjected the products to comparative analysis. The analytic methods employed were the same as those recently described in detail in a paper from this laboratory.[3]

Elementary composition. Preparation No. 1. — Preparations 1–4 were made by the Chittenden and Hart process as follows: Finely minced tissue (100 grams) was boiled in water until practically nothing more dissolved.[4] This process required about ten changes of 1 litre of water and a total of seventy-five hours for completion. The substance was next warmed in 1 litre of ten per cent acetic acid for one and one-half hours on a water bath. It was kept in the same fluid eighteen hours longer at room temperature and then boiled for four hours directly over a flame. The acid was then thoroughly washed out and the substance kept in five per cent hydrochloric acid for eighteen hours at room temperature. After the mineral acid had been thoroughly removed the treatment in the acids, with appropriate washing, was repeated. Finally, dehydration and removal of fat and extractive matter were effected in boiling alcohol-ether in the usual manner. The analytic results follow:

Carbon and Hydrogen. 0.2909 gm. substance gave 0.5752 gm. CO_2 and 0.1906 gm. H_2O = 53.93 per cent C and 7.28 per cent H; 0.2538 gm. substance gave 0.5078 gm. CO_2 and 0.1659 gm. H_2O = 54.56 (?) per

[1] Further details will be found with each preparation under records of analysis, pages 99 and 101. See also page 111.

[2] Compare with the experience of HORBACZEWSKI and of CHITTENDEN and HART, who evidently had not succeeded in completely dehydrating.

[3] HAWK and GIES: This journal, 1901, v, p. 387.

[4] One variation here from the CHITTENDEN and HART process is to be noted. We put the cleaned ligament through a *hashing machine*. The tissue used by CHITTENDEN and HART was "chopped quite fine." The more finely divided the tissue the easier and more complete the extraction, of course. See pages 98 and 104.

cent C and 7.26 per cent H; 0.2603 gm. substance gave 0.5159 gm. CO_2 and 0.1703 gm. H_2O = 54.05 per cent C and 7.27 per cent H; 0.2591 gm. substance gave 0.5118 gm. CO_2 and 0.1681 gm. H_2O = 53.87 per cent C and 7.21 per cent H.

Nitrogen. 0.2909 gm. substance gave 0.0469 gm. N = 16.12 per cent N; 0.3527 gm. substance gave 0.0565 gm. N = 16.01 per cent N.

Sulphur. 1.2540 gm. substance gave 0.0153 gm. $BaSO_4$ = 0.17 per cent S; 0.9790 gm. substance gave 0.0141 gm. $BaSO_4$ = 0.20 per cent S; 0.6661 gm. substance gave 0.0119 gm. $BaSO_4$ = 0.25 per cent S.

Ash. 0.4504 gm. substance gave 0.0038 gm. Ash = 0.84 per cent Ash; 0.3424 gm. substance gave 0.0025 gm. Ash = 0.73 per cent Ash.

PERCENTAGE COMPOSITION OF THE ASH-FREE SUBSTANCE.

										Average.
C	54.35	....	54.48	54.30	....	....	....	....	....	54.38
H	7.34	7.32	7.33	7.27	....	....	....	....	....	7.32
N	....	....	....	....	16.25	16.13	....	....	....	16.19
S	....	....	....	....	....	....	0.17	0.20	0.25	0.21
O	....	....	....	....	....	....	....	....	....	21.90

Preparation No. 2.

Carbon and Hydrogen. 0.2572 gm. substance gave 0.5067 gm. CO_2 and 0.1702 gm. H_2O = 53.73 per cent C and 7.35 per cent H; 0.3730 gm. substance gave 0.7383 gm. CO_2 and 0.2408 gm. H_2O = 53.98 per cent C and 7.17 per cent H; 0.4186 gm. substance gave 0.2711 gm. H_2O = 7.22 per cent H; 0.4614 gm. substance gave 0.9096 gm. CO_2 and 0.2968 gm. H_2O = 53.77 per cent C and 7.15 per cent H.

Nitrogen. 0.4863 gm. substance gave 0.0811 gm. N = 16.67 per cent N; 0.2892 gm. substance gave 0.0481 gm. N = 16.62 per cent N; 0.2521 gm. substance gave 0.0426 gm. N = 16.88 per cent N.

Sulphur. 0.5535 gm. substance gave 0.0089 gm. $BaSO_4$ = 0.22 per cent S; 0.7942 gm. substance gave 0.0112 gm. $BaSO_4$ = 0.19 per cent S.

Ash. 0.5009 gm. substance gave 0.0030 gm. Ash = 0.60 per cent Ash; 0.5364 gm. substance gave 0.0031 gm. Ash = 0.58 per cent Ash.

PERCENTAGE COMPOSITION OF THE ASH-FREE SUBSTANCE.

										Average.
C	54.05	54.30	....	54.09	....	....	....	....	....	54.15
H	7.39	7.21	7.26	7.19	....	....	....	....	....	7.26
N	....	....	....	....	16.77	16.71	16.98	....	....	16.82
S	....	....	....	....	....	....	....	0.22	0.20	0.21
O	....	....	....	....	....	....	....	....	....	21.56

Preparation No. 3.

Carbon and Hydrogen. 0.2562 gm. substance gave 0.5101 gm. CO_2 and 0.1694 gm. H_2O = 54.30 per cent C and 7.35 per cent H.

Nitrogen. 0.3305 gm. substance gave 0.0550 gm. N = 16.64 per cent N; 0.3577 gm. substance gave 0.0596 gm. N = 16.67 per cent N.

Sulphur. 1.1549 gm. substance gave 0.0128 gm. $BaSO_4$ = 0.15 per cent S; 0.7953 gm. substance gave 0.0100 gm. $BaSO_4$ = 0.17 per cent S.

Ash. 0.6690 gm. substance gave 0.0045 gm. Ash = 0.67 per cent Ash; 0.5782 gm. substance gave 0.0038 gm. Ash = 0.66 per cent Ash.

Percentage Composition of the Ash-free Substance.

						Average.
C	54.67	....	....	....	....	54.67
H	7.40	....	....	....	....	7.40
N	....	16.75	16.78	....	....	16.76
S	....	....	....	0.15	0.17	0.16
O	....	....	....	....	....	21.01

Preparation No. 4.

Carbon and Hydrogen. 0.2571 gm. substance gave 0.5084 gm. CO_2 and 0.1671 gm. H_2O = 53.93 per cent C and 7.22 per cent H.

Nitrogen. 0.3386 gm. substance gave 0.0562 gm. N = 16.59 per cent N; 0.2545 gm. substance gave 0.0426 gm. N = 16.72 per cent N.

Sulphur. 0.9068 gm. substance gave 0.0163 gm. $BaSO_4$ = 0.25 per cent S; 1.0077 gm. substance gave 0.0163 gm. $BaSO_4$ = 0.22 per cent S.

Ash. 0.4931 gm. substance gave 0.0052 gm. Ash = 1.05 per cent Ash; 0.4412 gm. substance gave 0.0050 gm. Ash = 1.13 per cent Ash.

Percentage Composition of the Ash-free Substance.

						Average.
C	54.52	....	....	....	....	54.52
H	7.30	....	....	....	....	7.30
N	....	16.77	16.90	....	....	16.83
S	....	....	....	0.25	0.22	0.24
O	....	....	....	....	....	21.11

Preparation No. 5. — Preparations 5–8 were made by our own method. 100 grams of ligament strips were washed in cold running water 24–48 hours. The strips were next run through a hashing machine and the hash thoroughly extracted several times (for 3 days) in half-saturated lime-water. The last extract did not become turbid on acidification. The alkali was completely washed out of the hash

with water. The rest of the process — boiling in water, etc., was the same in time, order, and character as that for preparations 1–4.

Carbon and Hydrogen. 0.2448 gm. substance gave 0.4819 gm. CO_2 and 0.1648 gm. H_2O = 53.69 per cent C and 7.48 per cent H; 0.2627 gm. substance gave 0.5142 gm. CO_2 and 0.1776 gm. H_2O = 53.38 per cent C and 7.51 per cent H; 0.4568 gm. substance gave 0.8922 gm. CO_2 and 0.2916 gm. H_2O = 53.27 per cent C and 7.09 per cent H.

Nitrogen. 0.3735 gm. substance gave 0.0620 gm. N = 16.59 per cent N; 0.2420 gm. substance gave 0.0400 gm. N = 16.51 per cent N; 0.2498 gm. substance gave 0.0417 gm. N = 16.69 per cent N.

Sulphur. 1.0358 gm. substance gave 0.0119 gm. $BaSO_4$ = 0.16 per cent S; 0.5907 gm. substance gave 0.0075 gm. $BaSO_4$ = 0.17 per cent S.

Ash. 0.3943 gm. substance gave 0.0029 gm. Ash = 0.74 per cent Ash; 0.3907 gm. substance gave 0.0036 gm. Ash = 0.92 per cent Ash.

Percentage Composition of the Ash-free Substance.

									Average.
C	54.14	53.83	53.72	....	....	....	....	....	53.90
H	7.54	7.57	7.15	....	....	....	....	....	7.42
N	....	....	....	16.73	16.65	16.83	....	....	16.74
S	....	....	....	....	....	....	0.16	0.17	0.16
O	....	....	....	....	....	....	....	....	21.78

Preparation No. 6.

Carbon and Hydrogen. 0.3285 gm. substance gave 0.6550 gm. CO_2 and 0.2161 gm. H_2O = 54.38 per cent C and 7.31 per cent H; 0.2539 gm. substance gave 0.5036 gm. CO_2 and 0.1654 gm. H_2O = 54.09 per cent C and 7.24 per cent H; 0.3343 gm. substance gave 0.6662 gm. CO_2 and 0.2202 H_2O = 54.35 per cent C and 7.32 per cent H.

Nitrogen. 0.4117 gm. substance gave 0.0701 gm. N = 17.02 per cent N; 0.2965 gm. substance gave 0.0510 gm. N = 17.18 per cent N; 0.2797 gm. substance gave 0.0478 gm. N = 17.08 per cent N.

Sulphur. 1.3763 gm. substance gave 0.0128 gm. $BaSO_4$ = 0.13 per cent S; 1.1255 gm. substance gave 0.0121 gm. $BaSO_4$ = 0.15 per cent S.

Ash. 0.9620 gm. substance gave 0.0008 gm. Ash = 0.08 per cent Ash; 1.0230 gm. substance gave 0.0009 gm. Ash = 0.09 per cent Ash.

Percentage Composition of the Ash-free Substance.

									Average.
C	54.43	54.14	54.40	....	....	....	....	....	54.32
H	7.32	7.25	7.33	....	....	....	....	....	7.30
N	....	....	....	17.03	17.20	17.09	....	....	17.11
S	....	....	....	....	....	....	0.13	0.15	0.14
O	....	....	....	....	....	....	....	....	21.13

Preparation No. 7.

Carbon and Hydrogen. 0.2584 gm. substance gave 0.5120 gm. CO_2 and 0.1685 gm. H_2O = 54.04 per cent C and 7.25 per cent H.

Nitrogen. 0.4656 gm. substance gave 0.0764 gm. N = 16.42 per cent N; 0.4482 gm. substance gave 0.0744 gm. N = 16.60 per cent N.

Sulphur. 0.8678 gm. substance gave 0.0096 gm. $BaSO_4$ = 0.15 per cent S; 0.8896 gm. substance gave 0.0080 gm. $BaSO_4$ = 0.12 per cent S.

Ash. 0.5082 gm. substance gave 0.0038 gm. Ash = 0.75 per cent Ash; 0.3540 gm. substance gave 0.0030 gm. Ash = 0.85 per cent Ash.

Percentage Composition of the Ash-free Substance.

						Average.
C	54.47	....	....	....	....	54.47
H	7.30	....	....	....	....	7.30
N	....	16.55	16.73	....	....	16.64
S	....	....	....	0.15	0.12	0.14
O	....	....	....	....	....	21.45

Preparation No. 8.

Carbon and Hydrogen. 0.2552 gm. substance gave 0.5000 gm. CO_2 and 0.1666 gm. H_2O = 53.43 per cent C and 7.25 per cent H.

Nitrogen. 0.3169 gm. substance gave 0.0536 gm. N = 16.90 per cent N; 0.4482 gm. substance gave 0.0431 gm. N = 16.84 per cent N.

Sulphur. 0.8235 gm. substance gave 0.0087 gm. $BaSO_4$ = 0.15 per cent S; 0.5679 gm. substance gave 0.0059 gm. $BaSO_4$ = 0.14 per cent S.

Ash. 0.4533 gm. substance gave 0.0032 gm. Ash = 0.71 per cent Ash; 0.3851 gm. substance gave 0.0031 gm. Ash = 0.81 per cent Ash.

Percentage Composition of the Ash-free Substance.

						Average.
C	53.84	....	....	....	....	53.84
H	7.31	....	....	....	....	7.31
N	....	17.03	16.96	....	....	17.00
S	....	....	....	0.15	0.14	0.14
O	....	....	....	....	....	21.71

The results for elementary composition of our eight preparations are brought together in the appended general summary. No great differences in the average composition of the preparations of each group are to be found. In fact the general analytic harmony is very striking and rather unexpected. The significant feature is to be seen in the figures for sulphur. The quantity is slight throughout, with

the content of sulphur in preparations 5–8 regularly lower than that of preparations 1–4.[1]

GENERAL SUMMARY OF ELEMENTARY COMPOSITION.

Elements.	Preparations 1–4. Made by the method of CHITTENDEN and HART.					Preparations 5–8. Made by the method of RICHARDS and GIES.					Gen'l av.
	1	2	3	4	Av.	5	6	7	8	Av.	
C	54.38	54.15	54.67	54.52	54.43	53.90	54.32	54.47	53.84	54.14	54.29
H	7.32	7.26	7.40	7.30	7.32	7.42	7.30	7.30	7.31	7.33	7.33
N	16.19	16.82	16.76	16.83	16.65	16.74	17.11	16.64	17.00	16.87	16.76
S	0.21	0.21	0.16	0.24	0.21	0.16	0.14	0.14	0.14	0.14	0.18
O	21.90	21.56	21.01	21.11	21.39	21.78	21.13	21.45	21.71	21.52	21.44

The following summary affords ready comparison in this connection with related results for average elementary composition: —

	C	H	N	S	O
Ligament elastin:					
HORBACZEWSKI	54.32	6.99	16.75	....	21.94
CHITTENDEN and HART . .	54.08	7.20	16.85	0.30	21.57
RICHARDS and GIES	54.14	7.33	16.87	0.14	21.52
Aorta elastin:					
SCHWARZ[2]	54.34	7.08	16.79	0.38	21.41
BERGH	53.99	7.54	15.20	0.60	22.67

Reactions. — We have little to add in this connection to what has already been noted. We have found, however, that elastin is not as resistant to acids and alkalies as it is generally considered to be. When the original tissue is very finely and thoroughly divided with a meat chopper, as was the case for the first time in our experiments, the particles undergo some solution in the acids used in the extraction process. The *purified powdered* substance is slightly soluble even in cold 0.2 per cent hydrochloric acid on long standing and dissolves very quickly and completely in 1 per cent potassium hydroxide on warming. These results suggest that the state of division of the tissue in preparation of elastin greatly influences solubility and thereby also purification. We believe that the agreement in composition between the two groups of our products was

[1] See references under "Sulphur content," page 105.

[2] SCHWARZ: Zeitschrift für physiologische Chemie, 1894, xviii, p. 487.

dependent largely on the particularly fine division of the tissue employed. The acids used for extractive purposes were given an excellent opportunity to decompose and completely dissolve interfibrillar extraneous matter.

Sulphur content. — It will be recalled that in the older methods of elastin preparation extraction of the elastic tissue by boiling in dilute alkali for several hours was a part of the process and that, although the resultant substance varied somewhat in composition, it was free from sulphur in a majority of cases. Chittenden and Hart were the first, as we have already pointed out, to call attention to the probability that sulphur is really an integral part of elastin, and that on boiling with alkali the constituent sulphur is removed. By avoiding the use of alkali Chittenden and Hart prepared elastin with a content of sulphur amounting to 0.3 per cent. They said in this connection, "Whether pure elastin does contain sulphur or whether the 0.3 per cent present in preparation B (made by their own method) is a constituent of some adhering proteid, removable by alkali, we are not at present prepared to say, but deem it probable that elastin does contain a small amount of sulphur."

Zoja and Eustis have recently confirmed the Chittenden and Hart result. Schwarz lately found about the same amount of sulphur in elastin from the aorta, but states that all was removable on boiling with 1 per cent potassium hydroxide and that the residual product was identical with the original body. Bergh has also obtained as much as 0.55 per cent of sulphur in aorta elastin prepared by the old alkali extraction method.

The results for sulphur content of all our preparations are given on page 106.

It will be seen that the average sulphur content of the five preparations made according to the older method was 0.20 per cent, whereas the elastin made by our own process, from which we had positively excluded the presence of mucoid and coagulable proteid, shows a percentage of sulphur amounting to 0.15 per cent, an average difference of 0.05 per cent in favor of the improved method. This difference, slight though it is, is fairly constant throughout. The analyses were made with the very greatest care. Our results seem to show conclusively that sulphur, in minute quantity at least, is a component part of pure ligament elastin.

Schwarz, it will be remembered, found that after treatment of aorta elastin with boiling one per cent potassium hydroxide for four hours

all of the sulphur (0.38 per cent) was split off in a form which could be precipitated as lead sulphide, leaving a sulphur-free, insoluble elastin having all of the properties of the original substance. Ligament elastin seems to be a different substance. On decomposing samples of our eight preparations in one per cent potassium hydroxide as Schwarz did, no sulphur in the form of sulphide could be detected

Elastin made by the CHITTENDEN and HART method.			Elastin made by the RICHARDS and GIES method.		
Number of preparation.	Percentage of sulphur.[1]		Number of preparation.	Percentage of sulphur.[1]	
	Direct determinations.	Average.		Direct determinations.	Average.
1	0.17 0.25 0.20	0.21	5	0.16 0.17	0.16
2	0.22 0.20	0.21	6	0.13 0.15	0.14
3	0.15 0.17	0.16	7	0.15 0.12	0.14
4	0.25 0.22	0.24	8	0.15 0.14	0.15
9[2]	0.16 0.18	0.17			
General average . .		0.20	General average . .		0.15

[1] The ash of each preparation was slight in amount. The ash contained an appreciable proportion of sulphur — an average of 0.11 per cent of the proteid of each group of preparations. This was doubtless derived in great part from the organic sulphur during incineration.

[2] This preparation was not completely analyzed, and therefore was not included in the series under elementary composition, page 104. It contained only 0.54 per cent ash.

in any of them, even when the whole volume of alkaline fluid was used for the test. A sample of the elastin prepared by Eustis, by the older method, however, which did not include preliminary treatment with lime-water for removal of mucoids, etc., gave decided sulphide reaction under similar circumstances. Our preparations completely dissolved in the warm alkali.

These facts indicate that the small amount of sulphur contained in

pure elastin is held in a form of combination not convertible into sulphide by treatment with boiling alkali.

Distribution of nitrogen. — The nitrogen of the proteids appears to exist in various amino forms, none of it being in nitro or nitroso combination. Some of it is easily split off in the form of ammonia by acid and by alkali. Usually, however, the largest quantity is obtainable on decomposition in the form of monamido acids and a considerable proportion is frequently separable in diamido combination.

No attempts to ascertain the distribution of nitrogen in the elastin molecule were made until very recently.[1] Soon after Kossel[2] had stated his belief that all proteids would yield hexone bases on decomposition Bergh[3] attempted to isolate lysin and arginin from among the cleavage products obtained from elastins of the cervical ligament and the aorta. His attempts resulted negatively.[4]

Hedin[5] by essentially the same methods came to the same negative result. He was unable, also, to identify histidin. These results would imply that elastin does not contain a protamin radicle.

Kossel and Kutscher,[6] however, by an improved method, subsequently isolated arginin from among the decomposition products of ligament elastin and thus directly contradicted the conclusions of Bergh and Hedin. The quantity of arginin isolated by them was unusually small — much less than that for most of the other proteids. Not long ago these same observers[7] were able to separate and identify lysin also among the bases obtainable from elastin.

The lack of agreement between Bergh and Hedin on the one side and Schwarz and Kossel and Kutscher on the other led to the study made by Eustis,[8] under Chittenden's direction, of the proportion of basic nitrogen split off from elastin on decomposition with hydrochloric acid and stannous chloride. Following the method adopted

[1] Horbaczewski studied some of the decomposition products from a different standpoint: Jahresbericht der Thier-Chemie, 1885, xv, p. 37. Schwarz made a study of aorta elastin similar in this respect to that of Horbaczewski: Schwarz, *loc. cit.*

[2] Kossel: Zeitschrift für physiologische Chemie, 1896–97, xxii, p. 176.

[3] Bergh: *Loc. cit.*

[4] Schwarz had previously found "lysatinin" (lysin + arginin) among the cleavage products of aorta elastin. *Loc. cit.*, p. 497.

[5] Hedin: Zeitschrift für physiologische Chemie, 1898, xxv, p. 344.

[6] Kossel and Kutscher: Zeitschrift für physiologische Chemie, *Ibid.*, p. 551.

[7] Kossel and Kutscher: *Ibid.*, 1900–01, xxxi, p. 165.

[8] Chittenden (for Eustis): *Loc. cit.*

by Schulze[1] and numerous other investigators, Eustis, in five experiments on the same preparation of elastin, obtained the following divergent results for percentage of nitrogen in the form of organic bases:

1	2	3	4	5
0.86	17.69	15.57	6.50	15.14

These discordant data, while they indicated that elastin does yield hexone bases, led to the conclusion that the method then in use for the separation of the hexone bases by phosphotungstic acid and determination of the nitrogen therein, was unreliable for quantitative purposes.

The divergence of the results obtained by Eustis made it seem desirable for us in this work to repeat his experiments, with elastin made by the method of preparation he used and also with products purified by our own method.

The preparations used for this particular purpose were Nos. 1, 3, and 7. (See page 104). The method of cleavage was the same as that used by Eustis. Decomposition took place (in the presence of one gram of stannous chloride) in 20 per cent hydrochloric acid in quantity equal to 4 c.c. per gram of substance used. The mixture was boiled each time in a reflux condenser for ninety-six hours. Separation of tin, precipitation with phosphotungstic acid and the other steps to quantitative determination were the same in detail as those taken in this connection by Schulze. The substance which remained in the acid mixture as an insoluble residue, very slight in quantity (possibly melanin), contained a mere trace of nitrogen.

In addition to a study by Schulze's procedure, we also made a similar decomposition of preparation No. 6 by the method of Kossel and Kutscher.[2] 100 grams of substance was boiled in a solution of 300 grams of pure concentrated sulphuric acid in 600 grams of water for fourteen hours in a reflux condenser. Further details of the separations were the same as those in the experiments of Kossel and Kutscher.

In the following table we give the essential data obtained by both methods, the figures expressing averages of closely agreeing results:

[1] SCHULZE: Zeitschrift für physiologische Chemie, 1898, xxiv, p. 276.

[2] KOSSEL und KUTSCHER: Zeitschrift für physiologische Chemie, 1900–01, xxxi, p. 165. The authors show that this method gives more abundant yield of hexone bases than any other.

Number of preparation.	Weight of ash-free elastin used.	Nitrogen.						
		Grams.				Percentage of total.		
	Grams.	Total.	Ammonia.	Monamido acids.	Bases.	Ammonia.	Monamido acids.	Bases.
A–3	13.4361	2.2371	0.0507	2.1351	0.0666	2.26	95.44	2.98
1	11.4472	1.8533	0.0434	1.8238	0.0420	2.34	98.42	2.26
7	11.5549	1.9227	0.0333	1.8311	0.0593	1.73	95.23	3.08
B–6	105.2000	18.1012	0.2572	17.0081	0.9153	1.42	93.96	5.06
				Average		1.94	95.76	3.34

It will be noted that although there is some variation in the percentage figures, there is yet a striking agreement among them. The latter fact is true even though two methods were employed and very different quantities of elastin were taken for each series of determinations. It is to be noticed also that the nitrogen in the bases was greatest for preparation No. 6 as determined by the Kossel and Kutscher method, a result in harmony with the claim of these observers that their process furnishes the most abundant supply of hexone bases. The uniformity of our results is in striking contrast to the disagreement of those obtained by Eustis.

Although the strictest quantitative accuracy cannot be claimed for the methods employed,[1] it does seem warrantable to conclude from our results in this connection that elastin contains an appreciable amount of nitrogen which on proper decomposition may be identified as nitrogen in the form of hexone bases.

In addition to the above results somewhat more specific data as to hexone bases were obtained with preparation No. 6 in a continuation of the Kossel and Kutscher method previously used. These afford the direct comparison made with similar results obtained by

[1] See papers in the Zeitschrift für physiologische Chemie, 1898–1901 (vols. xxv–xxxi) by Hedin, Gulewitsch, Henderson, Friedmann, Kutscher, and Schulze and Winterstein.

Kossel and Kutscher on histon, salmin, zein and gelatin, among other products,[1] in the appended summary:

Substance.	Percentage of total nitrogen.				Percentage of weight of original substance.			
	Histidin.	Arginin.	Lysin.	Ammonia.	Histidin.	Arginin.	Lysin.	Ammonia.
Ligament elastin	0.170	1.380	—[1]	1.375	0.027	0.197	—[1]	0.287
Commercial gelatin	?	16.600	?	1.400	?	9.300	5-6[2]	0.300
Zein	1.410	3.760	0	13.530	0.810	1.820	0	2.560
Histon (thymus)	1.790	25.170	8.040	7.460	1.210	14.360	7.700	1.660
Salmin	0	87.800	0	0	0	84.300	0	0

[1] Unusual difficulty was experienced in our attempts to separate lysin quantitatively. By difference our figures for nitrogen of lysin were 6.65 per cent of the total. We do not include them in the above table, because we have no confidence in their accuracy. The microscopic appearance of the histidin dichloride and arginin nitrate prepared by us was typical. The quantities obtained were too slight for satisfactory analysis.

[2] Approximate value.

Elastin appears to be characterized by containing a comparatively small quantity of hexone radicles. Our results indicate that histidin as well as the lysin and arginin found by Kossel and Kutscher may be split off from this albuminoid on appropriate treatment.

Is elastin a "fat-proteid compound?" — Nerking[2] has very recently found that various proteids as they are commonly prepared, contain fatty or fatty acid radicles. He did not examine elastin in this connection. We have done so, with entirely negative result.

Samples of preparations Nos. 5 and 6 were used for the purpose. The amounts of substance taken were 5.6747 gms. of No. 5 and 8.7429 gms. of No. 6. After two weeks' continuous extraction in anhydrous ether in a Soxhlet extractor, only 0.0015 gm. of extractive substance (dried in vacuo) could be obtained from No. 5; only 0.0013 gm. from No. 6. After digesting each preparation in pepsin-

[1] Their work on elastin in this connection was only qualitative. Lysin was isolated and identified. *Loc. cit.*, p. 205.

[2] NERKING: Archiv für die gesammte Physiologie, 1901, lxxxv, p. 330.

hydrochloric acid, in continuation of Dormeyer's method, as was done by Nerking in his work, and then thoroughly extracting the digestive mixture in the customary manner with ether, only 0.0017 gm. of ether-soluble matter was obtained from No. 5, only 0.0013 gm. from No. 6. Thus, in the double extraction process only 0.0032 gm. of ether-soluble matter (0.056 per cent) was obtained from No. 5; only 0.0026 gm. (0.03 per cent) from No. 6. These amounts are too minute, however, to mean anything positive — are, in fact, within the limits of unavoidable errors of extractive work. The pepsin used in the digestive process contained 0.5 to 1 mgm. of ether-soluble matter, which should be subtracted from the above totals in each case. At most the merest trace of soluble matter could have existed in either of the preparations. Thus it is certain that elastin as prepared by the method we employed does not contain fat or fatty acid, either in ordinary molecular combination or as an admixture.[1] These experiments have nothing to do, of course, with the question of fatty radicles contained *within* the proteid molecule.

Digestibility. — In the preceding determinations of possible fat admixture it was necessary to digest the elastin. Our preparations were readily digested in gastric juice, a result quite in harmony with the earliest observations of Etzinger.[2] Thus samples of preparations Nos. 5 and 6, weighing respectively 5.6747 gms. and 8.7429 gms., completely dissolved, in twenty-four hours, in mixtures of 300 c.c. of 0.2 per cent hydrochloric acid and 0.2 gm. of commercial pepsin scales (very active preparation) kept at 40°C. Cumulative power of combining with the acid was manifested by the products as is the case with other proteoses and peptones.[3] At the end of twenty-four hours only the merest turbidity remained in the fluid, showing that only a very slight amount of antialbumid had formed.

In the work of Chittenden and Hart on elastin and elastoses, elastin peptone could not be detected among the products of pepsin and trypsin proteolysis, even though zymolysis continued under

[1] Posner and Gies: Proceedings of the American Physiological Society, 1901, This journal, vi, p. xxix. This result indicates that the difficulty experienced by Horbaczewski and Chittenden and Hart in completely removing "fat-like matter" from their elastins was due to the compact character of the pieces of their preparations. Dehydration was complete in our own (page 99), with the result that fat extraction in purifying was comparatively easy. See Chittenden and Hart, *Loc. cit.*, p. 21.

[2] Etzinger: Zeitschrift für Biologie, 1874, x, p. 84.

[3] Chittenden: Digestive proteolysis, 1894, p. 52.

favorable conditions for several days. Peptone was absent also from the products formed on hydration of their elastin with very dilute acid. Although they were unable to detect peptone among the proteolytic products of elastin, Chittenden and Hart seem to have assumed its probable formation under favorable conditions, however, for toward the end of their paper they say: "Under the conditions of our experiments, no appreciable amount of true peptone was formed in any of the digestions; at least, nothing approaching a peptone in reactions was to be found in any of the digestive fluids, after saturation with ammonium sulphate. We propose, later, to attempt a study of the elastin peptone, using for this purpose the elastoses just described as well as elastin itself, and more vigorous digestive fluids, both peptic and tryptic."[1] Chittenden and Hart found that Horbaczewski's "elastin peptone" was in reality deutero-elastose and that his "hemi-elastin" is the same as proto-elastose.

After our digestive mixtures had been duly extracted with ether, in accordance with the original aim of the experiments immediately preceding, we allowed proteolysis to continue for about six weeks. Ether was added to the acid mixture occasionally to prevent bacterial changes. At the end of that period the elastose precipitate obtained on saturating the boiling neutral fluid with ammonium sulphate was surprisingly large. The filtrate was also made alternately acid and alkaline and thoroughly boiled each time while saturated with ammonium sulphate.[2] Only very slight additional proteose precipitates were obtained in this way. Ammonium sulphate was removed from the filtrate with alcohol and barium carbonate in the customary manner. The final filtrate gave a strong biuret reaction with a *slight* amount of cupric sulphate and an *excess* of potassium hydroxide. The amount of peptone precipitable by absolute alcohol was comparatively slight, although more than could have arisen, directly or by auto-digestion, from the pepsin preparation used at the outset.

In a special experiment in this connection 8.15 gms. of preparation No. 6 were digested in a solution of 900 c.c. of 0.4 per cent hydrochloric acid and 2 gms. of the very active commercial pepsin used above. Toluol was added to the mixture as a preservative, although the acid would have prevented bacterial action. Complete solution

[1] CHITTENDEN and HART: *Loc. cit.*, p. 36. See also CHITTENDEN: Digestive proteolysis, 1894, p. 72.

[2] KÜHNE: Zeitschrift für Biologie, 1892, xxix, p. 1.

of the elastin occurred within twenty-four hours. At the end of seventeen days a large proportion of elastose was separated by saturation of the neutral, acid and alkaline fluid with ammonium sulphate. Separated quantitatively in absolute alcohol containing ether the ash-free substance recovered as elastose (albuminate and antialbumid were absent at this stage of the digestion) was 7.43 gms., showing that at least 0.7 gm. of the original elastin had been transformed into peptone.[1] The final ammonium sulphate filtrate gave a strong biuret reaction when large excess of potassium hydroxide was present in the fluid. Some of the peptone contributing to this biuret reaction must, however, have arisen from the pepsin preparation.

These experiments show that elastoses are particularly resistant to progressive proteolysis through the action of pepsin, although they demonstrate that a small proportion of true peptone is formed from them during prolonged periods of favorable contact with the enzyme.[2]

The precipitate obtained from the above digestive mixtures on saturation with ammonium sulphate contained both primary and secondary elastoses. It retained the color of the original elastin. Judging from the reactions of solutions of the mixed proteoses, the amount of proto-elastose was relatively large. Such solutions, when concentrated, became heavily turbid on warming, as Horbaczewski[3] and subsequently Chittenden and Hart observed. Turbidity was decided even when tubes containing the clear concentrated fluid were immersed in water at 38°C or held under the tongue. Such turbid solutions cleared up again on cooling. The clear *concentrated* solution gave heavy precipitates with small quantities of concentrated nitric acid, picric acid, potassio-mercuric iodide, and other proteid precipitants, but such precipitates were only partially, if at all, soluble on warming. When these reagents were added to *dilute* solutions, however, the precipitates which were formed at once *dissolved* on

[1] All weights were made of substance dried to constant weight at 100°–105° C.

[2] In similar experiments, concluded after this paper had gone to the editor, 8 grams of elastin yielded only 1.38 gram of crude elastose when the digestion had proceeded for forty-six days. After digesting for seventy days 10 grams of elastin yielded less than 1 gram of elastose. A small proportion of proto-elastose was contained in the latter mixture. Large proportions of peptone were formed. These results harmonize with, and emphasize the conclusions above.

[3] Horbaczewski, Chittenden, and Hart: *Loc. cit.* See also, Morochowetz, Schwarz: *Loc. cit.*

warming and *reappeared* on cooling, just as in the case of other proteoses. Addition of excess of concentrated sodium hydroxide to the concentrated proteose solution was followed by heavy precipitation of some of the proteid, the precipitate persisting even when the solution was boiled.

The above reactions appear to have been due to proto-elastose, which seems to be a peculiar member of the proteose family.

Heat of combustion. — The potential energy of the proteids, expressed in calories, varies from about 5,000 to 6,000 small calories per gram of substance. Proteids such as peptone and osseomucoid, with comparatively small content of carbon, have the lowest combustion equivalents, whereas bodies like hæmoglobin, with relatively large proportion of carbon, have the very highest. The heat of combustion of any albuminous substance depends largely on the amounts and combinations of carbon and oxygen contained in it. The figures for composition of elastin suggest that its heat of combustion is relatively great.

The only previous observations on elastin made in this connection were those published by Stohmann and Langbein.[1] These observers worked with elastin made by Horbaczewski's method. The combustion equivalent was determined by the improved Berthelot method, and averaged 5,961.3 small calories per gram of ash-free substance — the highest equivalent for animal proteid.

Last June, while enjoying the freedom of Professor Atwater's laboratory, we made a thermochemical study of some of our products.[2] We wish here to express our thanks to Professor Atwater for his help and encouragement in this work and to acknowledge, also, our indebtedness to his assistants, Messrs. E. M. Swett and Emil Osterberg, for experimental aid.

The following table summarizes our results for the preparation of ligament elastin made by Mr. Eustis by the Chittenden and Hart process, for one made by us by the same method (No. 2), and for two preparations made by our own method (Nos. 5 and 6); it also includes the results obtained by Stohmann and Langbein:

[1] STOHMANN and LANGBEIN: *Loc. cit.*

[2] The apparatus used and method employed were the same as those previously described in This journal: 1901, v, p. 419. Quantities of 0.7–0.8 gram were burned at a time. The figures in the table are for substance dried to constant weight at 105°–110° C.

Preparation.	Direct determinations. Heat of combustion. Small calories per gram.			Averages: Calculated for ash-free substance.[1] Percentage composition.					Heat of combustion. Small calories.	
	I	II	Av.	C	H	N	S	O	Per gm.	For substance containing 1 gm. of carbon.
Eustis . . .	5933	5947	5940	54.42	7.40	16.65	0.14	21.39	5960	10952
Richards and Gies .										
Prep. No. 2.	5849	5821	5835	54.15	7.26	16.82	0.21	21.56	5870	10840
Prep. No. 5.	5840	5871	5855	53.90	7.42	16.74	0.16	21.78	5904	10954
Prep. No. 6.	5923	5909	5916	54.32	7.30	17.11	0.14	21.13	5967	10985
Average			5886	54.20	7.34	16.83	0.16	21.47	5925	10933
Stohmann and Langbein . .				55.03	7.20	16.91	0.18	20.68	5961	10832

[1] The percentage of ash in Eustis' preparation was 0.34; in our own it varied between 0.08 and 0.83. The Stohmann and Langbein preparation contained 0.07 per cent ash.

The general relation of the above results to those for other proteids, is seen at a glance in the following summary:

Substance.	Average percentage composition.					Heat of combustion. Small calories.	
	C	H	N	S	O	Per gram.	For substance containing 1 gram of carbon.
Ligament elastin[1] . . .	54.36	7.32	16.85	0.17	21.31	5932	10912
Various animal and vegetable proteids, not including glucoproteids[2] .	52.64	7.08	16.00	1.03	23.20	5711	10849
Connective tissue mucoids[3]	47.43	6.63	12.22	2.32	31.40	4981	10505

[1] The figures for ligament elastin are the averages of the results obtained by Stohmann and Langbein and in our own experiments.

[2] Averages obtained by Stohmann and Langbein.

[3] Averages obtained by Hawk and Gies: This journal, 1901, v, p. 423.

II. Mucoid.

Although a few indefinite statements regarding mucoid in ligament[1] were made shortly after Rollett's detection of this substance among the proteids of tendon, no attempts to separate and identify such a substance in elastic tissue were recorded before this work was begun. It seems that its presence had been inferred, not shown. Vandegrift and Gies have lately found that the quantity of mucoid in the ligamentum nuchæ of the ox averages 0.525 per cent of the fresh and 1.237 per cent of the dry tissue.[2] The quantity of mucoid in ligament is considerably less than in tendon.[3]

Our mucoid preparations were made by the method used by Chittenden and Gies.[4] Quantities of ligament hash varying from three to nine kilos were employed at a time. Much of the mucoid was lost mechanically in the purification process. Special care was taken to reprecipitate from solution in potassium hydroxide (0.05 per cent) or half-saturated lime-water several times; also, to wash thoroughly and to dehydrate and purify in boiling alcohol-ether.

We have not made an extended analytic study of ligament mucoid, but the following facts show its near relationship to the other connective tissue mucoids.[5]

In physical appearance the purified product is practically the same as tendomucoid or osseomucoid, although the latter substances can be dehydrated more easily. It gives the proteid color reactions very distinctly. It yields reducing substance and ethereal sulphate on decomposition with two per cent hydrochloric acid. The reducing substance forms dextrosazone-like crystals with phenylhydrazine, a fact indicating the presence of glucosamine among the hydration products. Among the other products resulting from its hydration in dilute acid are an antialbumid-like body, acid albuminate, proteoses and peptone. It is digestible in pepsin-hydrochloric acid and leaves a residue containing considerable reducing substance. Its sulphur may be obtained both as sulphate and sulphide.

[1] Kühne: Lehrbuch der physiologischen Chemie, 1868, p. 363.

[2] Vandegrift and Gies: *Loc. cit.*

[3] Buerger and Gies: This journal, 1901, vi, p. 219.

[4] Chittenden and Gies: Journal of experimental medicine, 1896, i, p. 186.

[5] Mead and Gies: Proceedings of the American Physiological Society, 1901, This journal, 1902, vi, p. xxviii.

Ligament mucoid is soluble in 0.05 per cent solution of sodium carbonate, half-saturated lime-water and 5 per cent sodium chloride. It is insoluble in 0.1 per cent hydrochloric acid, but is somewhat soluble in 0.2 per cent solution of the same. It is less resistant to acid than the mucoid from tendon or bone and somewhat more difficult to precipitate completely from its solution. The pure substance does not contain phosphorus. It is acid to litmus, neutralizes dilute alkali and has the same general precipitation reactions as the other connective tissue mucoids. None of our preparations contained chlorine.

The percentage amounts of nitrogen and sulphur in mucoids furnish favorable data for general comparisons of composition. The summary below gives our results in this connection, together with the proportion of ethereal sulphur. In the analyses the usual amounts of substance, dried to constant weight at 100°–110° C were taken. The quantity of ash in the preparations varied between 1.04 per cent and 1.90 per cent. The ash consisted mostly of calcium and of phosphoric acid. The quantity of total phosphorus in preparation B (the only one analyzed in this connection) was 0.18 per cent. The phosphorus of the ash of preparation B amounted to 0.16 per cent of the proteid.

Preparations.	A	B	C	D	E	General Averages.
Nitrogen	12.80 13.01	13.40 13.64	13.74 13.66	13.90 13.82	13.27 13.22	
	12.90	13.52	13.70	13.86	13.25	13.44
Total sulphur	2.05 2.09	1.77 1.68	1.49 —	1.37 1.27	1.45 1.40	
	2.07	1.73	1.49	1.32	1.42	1.61
Sulphur as SO_3 . . .	1.32 1.17	1.02 —	0.90 —			
	1.25	1.02	0.90	....	....	1.06

The percentage content of nitrogen appears to be uniformly higher in ligament mucoid than in related connective tissue glucoproteids. The content of sulphur is somewhat lower. It is to be noted, how-

ever, that experiments recently completed in this laboratory[1] indicate that there is more than one mucoid in tendon and bone, some of the glucoproteid separable from these tissues having as much as fourteen to fifteen per cent of nitrogen. We are inclined to believe, from the above results, that the same deduction regarding variability of general composition may be made with respect to mucoid substance in ligament also. It is possible, of course, that our preparations have been contaminated somewhat with coagulable proteid or other impurity we failed to remove. At the same time we used every precaution to prevent admixture.

III. Coagulable Proteids.

The simple proteids of the connective tissues have received very little attention. Those who have worked with the albuminoid constituents have usually confined their studies to those particular substances, and the various papers on the mucoids have made only incidental reference to the albumins and globulins.

We were surprised at the outset of these studies by the comparatively large amount of coagulable proteid present in ligament. In two preliminary quantitative determinations with the ligamentum nuchæ of the ox we found that the coagulable proteid was equal on an average to 0.64 per cent of the fresh tissue.[2] The quantities of coagulable proteid in tendon and cartilage, we found, were much less, and, moreover, were very difficult to separate and determine satisfactorily.[3]

[1] Cutter and Gies, Hawk and Gies: *Loc. cit.*

[2] Additional results are given by Vandegrift and Gies: *Loc. cit.*

[3] Using the methods employed with ligament (to be described on page 119), we found that aqueous extracts of the tendo Achillis of the ox contained only two coagulable proteids — one separated at 54°–57° C., corresponding to "(2)" in ligament: the other at 73° C, apparently the same as "(4)" in ligament. (See page 120). Loebisch, touching on this matter incidentally in his preparation of tendomucoid, referred to what he called serum globulin and a proteid coagulating at 78° C. He took no special pains, however, to remove the blood completely before making the extraction in water. See, Zeitschrift für physiologische Chemie, 1886, x, p. 43, foot-note.

Extracts of hyaline cartilage, in the few experiments we tried, gave negative results. On boiling, the extracts became opalescent. Flocks did not form, even with a fairly strong acidity. Chondromucoid and chondroitin sulphuric acid were present, of course. These bodies doubtless interfered with coagulation of such albumin or globulin as may have been contained. Von Mering obtained merely

In order to determine, if possible, the number and character of the simple proteids present in ligament we made use of various common methods, among them the process of fractional coagulation. For this purpose several extracts were made — aqueous and saline. Five per cent solution of magnesium sulphate was used generally for the latter type.

In the preparation of these extracts only such ligaments were used as seemed to be free from blood in all parts. The tissue was freed of extraneous matter and at first cut into narrow strips, which were kept in running water for from twelve to twenty-four hours. This treatment removed blood and lymph. The strips were then run through a meat chopper and the finely minced substance treated with enough extractive fluid to just cover it. At the end of from twelve to twenty-four hours, after repeated stirring, the fluid was strained through cloth and filtered. Each extract obtained in this way was always free from hæmoglobin, as examination with the spectroscope demonstrated, — a result implying also the absence of most, if not all, lymph proteids as well. Such extracts were either practically neutral in reaction or weakly alkaline to litmus. On heating, the solutions became very turbid and after addition of a trace of acid, flocculent separation in a water-clear fluid took place. All extracts contained such saline matter in solution as was found by us previously in ligament ash.

In determining the temperatures of coagulation the apparatus recommended by Gamgee[1] and commonly used in such work was employed, and 20–40 c.c. of the extract, made very faintly acid with acetic acid, was taken for each series of observations. The temperature was raised very gradually, and as soon as turbidity ensued the flame was removed and the solution kept at that temperature, or raised very slowly until the precipitate became flocculent. At this point the temperature was kept constant for from one-half to three-quarters of an hour, and then the solution filtered. The filtrates in each case were as clear as water. Upon raising the temperature beyond the previous maximal point the fluid remained clear until it had reached a temperature several degrees higher, when suddenly the next turbidity ensued.

Working in this way we obtained separations at the following temperatures :

the same opalescence on boiling. See, Ein Beitrag zur Chemie des Knorpels, 1873, p. 7. (Inaugural-Dissertation, Strassburg.)

[1] GAMGEE: Text-book of the physiological chemistry of the animal body, 1880, i, p. 15.

No.	Extremes of temperature.[1]	Average temperature.
1.	31°–49° C.	40° C.
2.	51°–61° C.	56° C.
3.	60°–70° C.	65° C.
4.	74°–76° C.	75° C.
5.	77°–85° C.	82° C.

All of these were obtained from each of the above types of extracts; (1), (4), and (5) were comparatively slight in amount.

The question naturally arose whether the precipitates separating at the above temperatures represented individual proteids in the tissue. Direct elementary chemical analysis would not have sufficed to answer this question definitely, for only very minor differences in composition exist among the albumins and globulins. Nor would a study of the decomposition products of these coagula have afforded any more definite conclusions. (See pages 126 and 127.)

We have sought the solution of the problem in fractional separation experiments by the methods repeatedly used by Hofmeister, Kauder, and others, particularly for the differentiation of albumins and globulins. Our results in this connection, on extracts made by the method previously detailed, are briefly summarized below:

A. *Aqueous extracts treated with $(NH_4)_2SO_4$ in substance.*

(*a*) When the aqueous solutions were *half-saturated* with $(NH_4)_2SO_4$, a fairly heavy precipitate was obtained, which consisted theoretically in whole or for the most part of globulin, albumin not being precipitated by this proportion of $(NH_4)_2SO_4$ (see page 124). The $MgSO_4$ solution of this precipitate contained bodies (1), (2), and (4) in the table above.

(*b*) In the aqueous solution of this same precipitate (*a*), bodies (1), (3), (4), and (5) were thrown down on heating. Precipitates (1) and (3) were comparatively heavy, the others were slight. Diluted with an equal volume of water, this aqueous solution of precipitate (*a*) gave bodies (1), (3), (4), and (5).

(*c*) The filtrate from precipitate (*a*) was *saturated* with $(NH_4)_2SO_4$. The substance thrown out of solution in this way was dissolved in water and the solution heated. It gave precipitates (2), (3), (4), and (5).

B. *$MgSO_4$ extracts treated with $MgSO_4$ in substance.*

When the $MgSO_4$ extracts were saturated with $MgSO_4$, a heavy pre-

[1] The extremes represent the limits of *all* our observations. As a rule the separations occurred at or about the mean temperature, with comparatively long intervals.

cipitate was obtained, which, dissolved in 5% $MgSO_4$ solution, contained products (1) and (2). The filtrate from the $MgSO_4$ precipitate, on heating, gave bodies (2), (3), (4), and (5).

Comparison of the figures for coagulated products under **A** and **B** will show that of the total number of bodies in the aqueous and saline extracts of ligament only one can be completely separated by saturation with $MgSO_4$ or by half-saturation with $(NH_4)_2SO_4$, viz. — the one which separates at or about 40° C. (1). All the other substances are to be found in the filtrates from the precipitates formed on addition of $MgSO_4$ to saturation or of $(NH_4)_2SO_4$ to half-saturation.

C. *Continuous fractional precipitation of aqueous and $MgSO_4$ extracts with $MgSO_4$ and $(NH_4)_2SO_4$ in substance, and with saturated solution of $(NH_4)_2SO_4$.*

We have attempted to make a closer differentiation of the coagulable proteids contained in ligament extracts than was possible by the methods under A and B. The extracts for these experiments were made by the method outlined on page 119. The extract to be tested was accurately neutralized. To a measured portion of it was added, a few grams at a time, the salt used for precipitative purposes. As soon as a flocculent precipitate had formed it was filtered off and washed with a solution of the precipitating substance of a strength equivalent to that of the mother liquid. To the filtrate, plus enough of the washings to make it up to the original volume, were again added weighed quantities of the salt. When a second precipitate had appeared it was treated in a manner exactly similar to that to which the first was subjected. This process was continued till the solution was saturated or until all proteid had been removed. The precipitates were then dissolved in a small quantity of water with the aid of the saline matter adhering to them, and subjected to fractional coagulation in the usual manner. The results for the globulins are appended:

(*a*) 5% $MgSO_4$ extract. Volume 100 c.c. Solid substance used to precipitate was $MgSO_4$.

Results: Precip. I. 5 gms. = turbidity; 25 gms. = heavy flocculent precipitate.

Precip. II. 35 gms. = turbidity; 53 gms. to saturation = flocks.

Coagulations: Solution of Precip. I. 44°–47° C. (1)
Solution of Precip. II. 64° C. (3)
Nothing more from either I or II on boiling.

(*b*) Aqueous extract was treated with an equal volume of saturated solution of $(NH_4)_2SO_4$. The resultant precipitate (globulin?) was dis-

solved in water and treated with a very slight amount of dilute acetic acid. A precipitate corresponding to separation No. 1 in the coagulation series formed as a result of this treatment. The same was filtered off and the filtrate carefully neutralized. This neutral filtrate was used below in (*c*) and (*d*).

(*c*) Neutral filtrate obtained in (*b*). Volume 100 c.c. Precipitating salt $MgSO_4$.

Results: Precip. I. 20 gms. = turbidity; 42 gms. = flocculent precipitate.

Precip. II. 43 gms. = turbidity; 50 gms. = flocculent precipitate.

Precip. III. 56 gms. = turbidity; 63 gms. = flocculent precipitate.

Precip. IV. 73 gms. = turbidity; saturation + acid = final precipitate.

Coagulations: Solution of Precip. I. 51°–58° C. (2); 65°–67° C. (3).

Solution of Precip. II. 68°–69° C. (3).

Solution of Precip. III. 66°–67° C. (3).

Solution of Precip. IV. 54°–56° C. (2); 67°–70° C. (3).

If this method gives evidence of the presence of distinct proteids in a solution, as various observers believe, we seem to have dealt in this instance with at least two substances.

(*d*) Neutral filtrate obtained in (*b*), previously used in (*c*). Volume 100 c.c. For precipitation purposes, instead of $MgSO_4$ in substance, saturated solution of $(NH_4)_2SO_4$ was used.[1]

Results: Precip. I. 100 c.c. of the original filtrate + 65 c.c. saturated solution of $(NH_4)_2SO_4$ = turbidity; on standing, flocks separate.

Precip. II. 100 c.c. original filtrate + 82 c.c. saturated solution $(NH_4)_2SO_4$ = precipitate.

Precip. III. 100 c.c. original filtrate + 91 c.c. saturated solution $(NH_4)_2SO_4$ = precipitate.

Precip. IV. 100 c.c. original filtrate + 100 c.c. saturated solution $(NH_4)_2SO_4$ = precipitate.

[1] In this series addition of $(NH_4)_2SO_4$ solution was made cautiously until turbidity began. On standing, the precipitate became flocculent. This was filtered off and the total volume made up to the original amount with an appropriate quantity of $(NH_4)_2SO_4$ solution of equal strength. This fluid was then treated carefully with more saturated solution until further precipitation occurred. The intervals between initial turbidities were quite marked, though less so than in the experiments under (*a*) and (*c*).

At this point, according to the theoretical differences between albumins and globulins, all the globulin-like substance ought to have been removed from the solution (half-saturated with $(NH_4)_2SO_4$). The addition of larger proportions of $(NH_4)_2SO_4$ to the solution gave further precipitates as follows:

Results (continued):

Precip. V. 100 c.c. original filtrate + 125 c c. saturated solution $(NH_4)_2SO_4$ = precipitate.

Precip. VI. 100 c.c. original filtrate + 142 c.c. saturated solution $(NH_4)_2SO_4$ = precipitate.

Precip. VII. 100 c.c. original filtrate + 150 c.c. saturated solution $(NH_4)_2SO_4$ = precipitate.

No further precipitation was obtainable, either with more $(NH_4)_2SO_4$, by the addition of acid or on boiling.

Coagulations:

Solution of Precip.	I.	61°–63° C. (3)
Solution of Precip.	II.	66°–67° C. (3)
Solution of Precip.	III.	66°–67° C. (3)
Solution of Precip.	IV.	56°–58° C. (2)
Solution of Precip.	V.	53°–59° C. (2)
Solution of Precip.	VI.	56°–57° C. (2); 64°–68° C. (3)
Solution of Precip.	VII.	58°–60° C. (2); 67°–70° C. (3)

A study of the results under C shows that among the substances extractable from ligament by $MgSO_4$ solution or water is one which is precipitable from $MgSO_4$ extract by addition of 25 gms. of $MgSO_4$ to 100 c.c. of extract, or from a dilute saline solution by trace of acid at about 40° C. (1), or by larger amount of acid at room temperature.

A second substance, presumably a globulin, was precipitated by 53 gms. of $MgSO_4$ from $MgSO_4$ extracts and coagulated at about 65° C. (3). This substance, apparently, may also be separated from the aqueous solution of the precipitate obtained on half-saturation of aqueous extract with $(NH_4)_2SO_4$ or by the addition of $MgSO_4$ in quantities varying from 20 gms. per 100 c.c. of extract to the saturation quantity for the same volume. It was also obtained from such solution by additions of from 65 to 150 c.c. of saturated solution of $(NH_4)_2SO_4$ per 100 c.c. of proteid solution.[1]

[1] This substance appears to be comparable to fibrinoglobulin, also to serum albumin. See COHNHEIM, *Loc. cit.*, pp. 143 and 161.

There is apparently another substance, separating at about 56° C. (2) and precipitable from solution in water by 42 gms. of $MgSO_4$ per 100 c.c. of proteid solution; also by from 73 gms. of $MgSO_4$ to the saturation equivalent for the same volume of proteid fluid. It is precipitated also by 100–150 c.c. of saturated solution of $(NH_4)_2SO_4$ per 100 c.c. of proteid extract. From its coagulation temperature it would seem to be comparable to fibrinogen.[1]

The two other proteids in the extracts of A and B coagulated at about 75° C. (4) and 82° C. (5). Like (1) they occurred in only very small amounts. They correspond to the albumins ("serins") found in ox-serum, by Halliburton, coagulating at 77° C. and 84° C. respectively.[2]

Of these five products the one separating at the lowest temperature is not a coagulum. (See page 125). The proteid which separates at about 65° C. is also peculiar. It begins to separate from its solution when 82 c.c. of saturated solution of $(NH_4)_2SO_4$ are added per 100 c.c. of its own, and is not completely precipitated till the amount of admixed saturated $(NH_4)_2SO_4$ solution reaches 150 c.c. per 100 c.c. of proteid fluid. According to the generally accepted observations of Hofmeister, Kauder, and others on the proteids of serum, globulins are precipitated by the addition of 92 c.c. of saturated $(NH_4)_2SO_4$ solution to 100 c.c. of proteid fluid, whereas the precipitation of albumins does not begin until more than 128 c.c. have been added. This substance, in respect to its behavior toward ammonium sulphate partakes, therefore, of the characteristics of both globulin and albumin.[3] The fractional precipitation and coagulation methods are not of sufficient definiteness in result for us to contend that the precipitates we have obtained are not mixtures of albumins and globulins.[4]

[1] Compare with the serum albumins studied by MICHEL: Jahresbericht der Thier-Chemie, 1895, xxv, p. 11. See also HAMMARSTEN: Lehrbuch der physiologischen Chemie, 1899, p. 132.

[2] HALLIBURTON: Jahresbericht der Thier-Chemie, 1884, xiv, p. 126; 1886, xvi, p. 344. The first of these also corresponds to serum globulin in coagulation temperature, but serum globulin is precipitated on half-saturation with ammonium sulphate, the above bodies were not.

[3] This solution was completely saturated. Our analytic results showed the presence of 53.67 per cent of $(NH_4)_2SO_4$. KAUDER'S results for the same were 52.42 per cent. See, Archiv für experimentelle Pathologie und Pharmakologie, 1886, xx, p. 411.

[4] The boundary line between albumins and globulins, never very definitely marked, has been growing less and less distinct. See STARKE: Zeitschrift für Biologie, 1900, xl, p. 494.

These various proteids do not appear to come wholly from residues of serum — the quantity in which they may be obtained seems to be too great to permit of such an assumption. We believe, however, that it is impossible to remove every trace of serum from such a tissue without modifying the chemical character of the contents.

We are not unmindful, in considering the character of these products, of the known influences exerted on the coagulation temperature of proteids by the reaction of the fluid, its degree of acidity, the proportion and character of saline matter in solution, rapidity of heating, presence of foreign soluble organic bodies, concentration, etc. All of these conditioning factors were carefully governed, however, to prevent erroneous deductions.

IV. Nucleoproteid.

We believe that the substance separating at 40° C. (1) in nearly all of the preceding coagulation experiments is, in great part at least, nucleoproteid. That the substance was directly precipitated at that temperature, not coagulated, was apparent from the fact that when the various extracts employed were treated with a slight amount of acetic acid and then allowed to stand over night, a light flocculent precipitate settled out. After its removal only precipitate (2) and the higher bodies previously obtained separated from the filtrate on heating. That this acid precipitate was not a true coagulum was further evidenced by the fact that it dissolved readily in 0.5 per cent sodium carbonate, from which solution it was easily precipitated by slight excess of dilute acid.

When 100 c.c. of the aqueous extract of ligamentum nuchæ was treated with 0.5 c.c. of 36 per cent acetic acid, a bulky flocculent precipitate was obtained which dissolved easily in dilute alkali. This precipitate was not coagulable either in acid or alkaline fluid and after fusion with alkali gave a good phosphate reaction with molybdic solution. Further, after a very large quantity of the aqueous extract of the tissue had been evaporated to a small bulk on the water bath and the heavy precipitate of coagulated proteid filtered off, the viscid filtrate gave an abundant precipitate on the addition of but a few drops of 36 per cent acetic acid. This precipitate dissolved readily in 5 per cent sodium chloride and was reprecipitated on saturation with the same substance. Its solutions would not coagulate in any

medium. The substance so obtained contained phosphorus in organic combination.

Various proteids are precipitable from their solutions on acidification. Those of special interest for us in this connection are glucoproteids, nucleoproteids and globulins. When carefully tested as to its solubility in dilute acid the substance obtained in these experiments was found to be precipitated by moderate excess of 0.2 per cent acetic or hydrochloric acid. Serum globulin and fibrinogen may be precipitated from their solutions by minute quantities of acids. They are readily soluble, however, in moderate excess of the acids just mentioned — in the proportion which was favorable to the precipitation of the substance above. The same would be true of small quantities of albuminate. Furthermore, as has already been pointed out, our acid precipitate, unlike the other bodies just mentioned, contains phosphorus and was non-coagulable.

Connective tissue mucoid has much the same characteristics as this substance. Mucoid, however, is a phosphorus-free glucoproteid, and on boiling with acids yields reducing substances. When our acid-precipitated product was boiled for several hours with 2 per cent hydrochloric acid, the fluid neutralized, and tested with Fehling's solution, only a trace of a reduction occurred. Our substance could not, therefore, be mucoid, although the slight reduction suggests that a trace of mucoid might have been admixed with it.[1]

A special preparation of this acid precipitate was made as follows: Aqueous extract of 8 kilos of ligamentum nuchæ was obtained as in the method given on page 119, and to it was added 0.5 c.c. of 36 per cent acetic acid per 100 c.c. of extract. The flocculent precipitate which formed on standing was dissolved in 0.3 per cent solution of sodium carbonate. This fluid was neutralized and then acetic acid added until precipitation occurred. 1 to 1.3 c.c. of 36 per cent acetic acid was required per 100 c.c. of fluid to effect the same — a total acidity which would have dissolved globulins readily. This precipitate was again dissolved and was reprecipitated in the same manner, after which it was washed free of acid and dehydrated, and purified as usual in alcohol and ether. 4.5 gms. (0.056 per cent of the fresh tissue) were obtained.

[1] Aqueous extracts of the tissue are in reality extracts in dilute saline solution, the salts of the tissue contributing their solvent power. Mucoid is somewhat soluble in such extracts. Possibly, however, the reducing substance was derived from the nucleoproteid.

Analysis of this product gave the following results for percentage content of phosphorus in the ash-free substance:[1] (1) 0.49, (2) 0.45; average, 0.47.

These figures for phosphorus content are somewhat lower than they are for most nucleoproteids. Mucoid impurity, as we have already suggested, may have partially accounted for this lowering of phosphorus content.

That the substance was not a "cell nucleo-albumin"[2] was shown by the results of the following experiment: About 2 gms. of the substance was decomposed with acid in the usual way and a test made for nuclein bases among the cleavage products, with positive result. "Ammoniacal silver solution" gave the typical flocculent brown precipitate. No precipitate formed, on cooling, in the solution of this precipitate in nitric acid (1.1 specific gravity). On neutralizing however, and rendering slightly alkaline with ammonia, xanthin silver in quantity practically equal to the original precipitate was obtained. Tested with Fischer's modification of Weidel's reaction this precipitate gave positive results for xanthin.[3]

That the substance is nucleoproteid, or at least contains a large proportion of this compound albuminous substance, we feel confident. Although we are not accustomed to associate nucleoproteids with any but glandular tissues, the fact remains that nucleoproteids are to be found in every cell, and therefore must exist in every tissue. Pekelharing[4] has lately found that 0.37 per cent of fresh muscle — a comparable tissue in this connection — consists of a nucleoproteid containing 0.7 per cent of phosphorus.

V. Collagen (Gelatin).

All forms of connective tissues contain collaginous fibres. Eulenberg[5] first demonstrated the presence of collagen in ligamentum nuchæ by obtaining gelatin from it. Recently the quantity was

[1] The merest trace of phosphorus was present in the ash, 4–6 per cent of the total quantity. This was deducted from the figures for total phosphorus. The ash amounted to 0.75 and 0.89 per cent — average, 0.82. 0.5–0.6 gram of substance was used in each of the determinations by the usual methods.

[2] See Cohnheim: *Loc. cit.*, pp. 181–183.

[3] Fischer: Berichte der deutschen chemischen Gesellschaft, 1897, xxx, p. 2236.

[4] Pekelharing: Zeitschrift für physiologische Chemie, 1896–97, xxii, p. 245. See also, Kossel, *Ibid.*, 1882–83, vii, p. 7.

[5] Eulenberg: See Schultze, Annalen der Chemie und Pharmacie, 1894, lxxi, p. 277.

accurately determined and was found to be 7.23 per cent of the fresh and 17.04 per cent of the dry tissue — equal, roughly, to one-fourth the amount of contained elastin.[1]

The presence of so much elastin in ligament makes it impracticable to separate the collagen as such, by the Ewald and Kühne process of digestion with trypsin in alkaline medium.[2] In order to obtain some idea of its character, however, we transformed it into gelatin and then separated and studied the latter.

Preparation of ligament gelatin. — After the cleaned ligament had been put through a meat chopper the hash was thoroughly washed in running water and later thoroughly extracted in half-saturated lime-water. After the alkali had been completely removed with water, the residual tissue was boiled for a short time in distilled water. The filtrate was concentrated somewhat on the water bath and then the gelatin precipitated from it by pouring it into a large excess of alcohol. The typical fibrous precipitate of gelatin was obtained in this way. This was redissolved in water and reprecipitated in alcohol several times, then dehydrated and completely purified in alcohol-ether.

Elementary composition. — The following data were obtained in elementary analysis of one preparation by the methods previously used in this connection for elastin.

Carbon and Hydrogen. 0.2324 gm. substance gave 0.1372 gm. H_2O = 6.56 per cent H; 0.3773 gm. substance gave 0.6860 gm. CO_2 and 0.2250 gm. H_2O = 49.59 per cent C and 6.63 per cent H; 0.3681 gm. substance gave 0.6705 gm. CO_2 and 0.2194 gm. H_2O = 49.68 per cent C and 6.62 per cent H.

Nitrogen. 0.2867 gm. substance gave 0.0501 gm. N. = 17.47 per cent N; 0.3578 gm. substance gave 0.0634 gm. N = 17.72 per cent N.

Sulphur. 0.7370 gm. substance gave 0.03050 gm. $BaSO_4$ = 0.568 per cent S; 0.9417 gm. substance gave 0.03734 gm. $BaSO_4$ = 0.544 per cent S.

Ash. 0.3503 gm. substance gave 0.0058 gm. Ash = 1.65 per cent Ash; 0.2746 gm. substance gave 0.0047 gm. Ash = 1.71 per cent Ash.

[1] VANDEGRIFT and GIES: *Loc. cit.*

[2] EWALD und KÜHNE: Jahresbericht der Thier-Chemie, 1877, vii, p. 281.

PERCENTAGE COMPOSITION OF THE ASH-FREE SUBSTANCE.[1]

								Average.
C	....	50.44	50.53	....	....	....	....	50.49
H	6.67	6.74	6.73	....	....	....	....	6.71
N	....	....	....	17.77	18.02	....	....	17.90
S	....	....	....	....	....	0.58	0.56	0.57
O	....	....	....	....	....	....	....	24.33

The following summary of percentage elementary composition shows the relation of ligament gelatin to bone and tendon gelatin and to purified commercial gelatin, the latter consisting of a mixture of gelatins from bone and other connective tissues:

	C	H	N	S	O
Ligament gelatin . . .	50.49	6.71	17.90	0.57	24.33
Tendon gelatin[2] . . .	50.11	6.56	17.81	0.26	25.24
Commercial gelatin[3] . .	49.38	6.81	17.97	0.71	25.13
Bone gelatin[4]	50.40	6.64	18.34	....	24.64

Recent studies of the composition of connective tissues indicate that there are perhaps three groups of collagens. These appear to be characterized by appreciable differences in elementary composition. Thus the collagens in tendon[5] and bone[6] yield gelatins containing approximately 18 per cent of nitrogen. Corneal collagen[7] contains about 17 per cent of nitrogen. Cartilage collagen yields a gelatin containing little more than 16 per cent of nitrogen.[8] Our results in this connection indicate that the collagen of ligamentum nuchæ is essentially the same as that in tendon and bone.

Heat of combustion. — In two determinations of the heat of combustion of ligament gelatin we obtained an average of 5276 small calories (5261, 5291) as the combustion equivalent. These figures accord very well with those previously obtained by other observers for different gelatins, as will be seen from the following summary,

[1] The sulphur of the ash amounted to 0.17 per cent of the dry proteid. This was not subtracted from the above figures — much of it doubtless arose during incineration.

[2] VAN NAME: Journal of experimental medicine, 1897, ii, p. 124.

[3] CHITTENDEN and SOLLEY: See CHITTENDEN, Digestive proteolysis, 1894, p. 32.

[4] MULDER: See HOPPE-SEYLER, Physiologische Chemie, 1881, p. 100.

[5] VAN NAME: *Loc. cit.*

[6] HOPPE-SEYLER: Physiologische Chemie, 1881, p. 100.

[7] C. TH. MÖRNER: Zeitschrift für physiologische Chemie, 1894, xviii, p. 224.

[8] C. TH. MÖRNER: Jahresbericht der Thier-Chemie, 1888, xviii, p. 221.

which gives also the combustion equivalents of two proteids having equivalents among the very lowest for albuminous substances:

Substance. Dried at 100°–110° C.	Heat of combustion. Small calories.		Percentage composition.	
	Per gram.	For substance containing 1 gm. of carbon.	Carbon.	Oxygen.
Ligament gelatin . .	5276	10450	50.49	24.33
Fish gelatin [1] . . .	5242	10800	48.53	25.54
Commercial gelatin [2] .	5270	....	....	....
Fibrin pepton [3] . . .	5299	10577	50.10	25.79
Tendomucoid [4] . . .	5003	10415	48.04	30.62

[1] Berthelot et Andre: Centralblatt für Physiologie, 1890, iv, p. 611.
[2] Atwater: Report of the Storrs (Conn.) Agricultural Experiment Station, 1899, p. 92.
[3] Stohmann und Langbein: Journal für praktische Chemie, neue Folge, 1891, xliii, p. 375.
[4] Cutter and Gies: *Loc. cit.*

Crystalline Extractives.

In our first report of this work [1] we called attention to the fact that ox ligament contains an appreciable quantity of crystalline extractives. The only crystalline substance whose identity we had definitely determined at that time was creatin, although the general method of detecting nuclein bases had shown the presence of one or more of these bodies also. A continuation of this work has given us more definite results.

Preparation of extract. — The "extract" containing the crystalline substances was obtained in the following manner: 15–20 pounds of ligamenta nuchæ, which were perfectly fresh and which had only mere traces of blood in them, were finely minced in a meat-chopper. The hash was extracted in a moderate amountof water at 40° C. for 12–24 hours — ether or powdered thymol preventing putrefaction. The extract was strained through cloth, then heated to boiling, after which sufficient acid was added to completely separate coagulable

[1] Richards and Gies: *Loc. cit.*

proteid and contained mucoid.[1] That practically no hæmoglobin was present was shown by the fact that the precipitate at this point was entirely white.

The slightly acid filtrate was then neutralized and evaporated on a water bath to a thin syrup. This concentrated extract had all of the physical properties of ordinary "meat extract." It contained traces of proteid (derived gelatin and albuminate probably) but no reducing substance could be detected in it.[2] Chloride and phosphate of sodium and calcium were present in comparative abundance. Sulphate was also detected.

Creatin. — The concentrated extract was diluted with several volumes of water and treated with lead acetate for the removal of inorganic radicles. The excess of lead was precipitated with hydrogen sulphide and the filtrate evaporated to a thin syrup on the water bath. On standing thirty-six hours, typical crystals of creatin formed in good quantity. After filtering and evaporating to greater concentration occasionally a new but smaller crop of crystals was obtained each time.

The fluid concentrated in this way was treated with moderate excess of 90 per cent alcohol and the solid matter tested, together with the separated crystals, for creatin. The crystals and the alcohol precipitate were readily soluble in water. On hydration with acid in the usual way, the fluid gave the typical crystals of creatinin zinc chloride with an alcoholic solution of zinc chloride, and also responded to Weyl's reaction.

Hypoxanthin. — The alcoholic filtrate from the precipitated creatin was next evaporated nearly to dryness to get rid of alcohol, a little water added, the fluid made alkaline, filtered, and then treated with an appropriate quantity of "ammoniacal silver solution." The resultant heavy brown precipitate of nuclein bases, on decomposition with hot nitric acid of 1.1 specific gravity, gave a yellowish filtrate, which, on cooling, deposited a large proportion of crystalline substance, mostly needles of hypoxanthin silver nitrate. The mixture was allowed to

[1] A slight amount of mucoid is always contained in the aqueous extract of ligament. The salts present in the extract exert solvent action on it.

[2] Leucin and tyrosin were detected at this point in microscopic examination of one sample of our extracts. We have assumed that these were formed from proteid by hydration in the process of heating to boiling and subsequent evaporation. Some creatinin was also detected several times. This probably arose from the creatin by hydration.

stand for twenty-four hours for complete precipitation of the crystalline matter.

The filtrate from the crystals still contained nuclein base (doubtless xanthin, which may have been formed from the hypoxanthin), as was shown by the brown precipitate which appeared in small quantity when the fluid was again rendered slightly alkaline.

The crystalline precipitate containing hypoxanthin silver nitrate was decomposed in a warm mixture of water and ammonium sulphide on the water bath, the mixture filtered hot, concentrated on a water bath, there saturated with ammonia and again filtered hot. A comparatively large amount of hypoxanthin could be detected in this filtrate.

Guanin. — The substance insoluble in the ammoniacal fluid yielded beautiful crystals of guanin. These were obtained by Horbaczewski's[1] method of solution in alkali, and treatment with alcohol and acetic acid. The crystals were large and they very closely resembled those of creatinin zinc chloride.

The bulk of the crystalline extractives consisted of creatin, hypoxanthin and guanin. We were unable to prove the presence of adenin and carnin, although we occasionally obtained results by the customary qualitative methods indicating the presence of these substances. No tests were made for other extractives.[2]

It is interesting to note in this connection that guanin has been found to occur in the ligaments of pigs with guanin gout.[3]

The amount of nuclein bases found in these extracts was too great to allow of the assumption that they were derived from the small quantity of blood and lymph remaining in the tissue when the separation was begun. Normal blood contains only traces of nuclein bases[4] and the tissue itself contained at the outset only traces of blood. In tissues, such as muscle, which contain relatively few nuclei, nuclein bases are found in the uncombined state, and in this condition undoubtedly represent late stages in the catabolism of nuclear proteids. Our data show a similar catabolism in ligament, thus leading us to a conclusion which would hardly be suggested by the "passive mechanical functions" of the tissue — a conclusion

[1] Horbaczewski: Zeitschrift für physiologische Chemie, 1897, xxiii, p. 229.

[2] We obtained essentially the same results as those above in continuance of the work on tendon extract already referred to by Buerger and Gies: *Loc. cit.*

[3] Hammarsten: Lehrbuch der physiologischen Chemie, 1899, p. 119.

[4] Kossel: Zeitschrift für physiologische Chemie, 1882–83, vii, p. 22.

which harmonizes, however, with the fact that this tissue contains a variety of substances which represent intermediate stages of chemical differentiation.

Summary of Conclusions.

1. By improved method of preparation several samples of ligament elastin were made, having the following average percentage composition:

C	H	N	S	O
54.14	7.33	16.87	0.14	21.52

All of these preparations contained sulphur. None of it could be split off as sulphide on boiling with caustic alkali.

Only very small proportions of elastin nitrogen could be split off in the form of ammonia and hexone bases on decomposition with acid. Arginin, lysin, and histidin have been identified among the basic bodies separated in this way.

Elastin is not a "fat-proteid compound." No extractive material could be separated from our analyzed preparations by Nerking's process.

Our purified powdered elastin readily digested in pepsin-hydrochloric acid. Elastoses and true peptone were formed, proto-elastose predominating in quantity. The amount of true peptone formed was comparatively small even after long periods of favorable contact of the elastin and elastoses with the enzyme in acid solution, showing that elastoses are particularly resistant to progressive zymolysis.

The average combustion equivalent of four preparations of elastin, determinations in duplicate, was 5925 small calories.

2. Ligament contains mucoid having the general qualities of other connective tissue glucoproteids. Analysis of five preparations gave the following average percentage results:

N	S	S as SO_3
13.44	1.61	1.06

3. Extracts of ligament contain proteid coagulating at 56° C., 65° C., 75° C., and 82° C. Although these figures indicate identity with some of the albuminous substances of the blood, the coagulable proteids of our extracts do not appear to have arisen wholly from contained serum.

4. A slight amount of nucleoproteid is contained in ligament and was detected in aqueous and saline extracts.

5. The gelatin obtained from ligament had the following percentage composition:

C	H	N	S	O
50.49	6.71	17.90	0.57	24.33

These results indicate that the collagen of ligament is identical with that of bone and tendon.

The heat of combustion of ligament gelatin was found to be equal to 5276 small calories.

6. Among the crystalline extractives obtainable from ligamentum nuchæ were creatin, hypoxanthin, and guanin.

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THE COMPOSITION OF TENDON MUCOID.[1]

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IN their paper on the glucoproteid of white fibrous connective tissue Chittenden and Gies[2] stated that the average amount of sulphur in three analyzed preparations of tendon mucoid[3] was 2.33 per cent. Loebisch,[4] who previously had been the only one to analyze this substance completely, found in it an average of but 0.81 per cent of sulphur, and ascribed to it the formula $C_{160}H_{253}N_{32}S_1O_{80}$ with a molecular weight of 3936. Referring to the unexpectedly high results of their sulphur determinations, as compared with those obtained by Loebisch, Chittenden and Gies wrote: "We present these figures

[1] Some of the results of this work were reported before the American Physiological Society. See the Proceedings, CUTTER and GIES: This journal, 1900, iii, p. vi.

[2] CHITTENDEN and GIES: Journal of experimental medicine, 1896, i, p. 186.

[3] Following COHNHEIM'S suggestion (Chemie der Eiweisskörper, 1900, p. 259) we use the term "mucoid," instead of the previously accepted "mucin," to designate this substance. We agree with Cohnheim that, for the sake of definiteness, the term "mucin" may be best applied to the glucoproteids elaborated by true secretory cells, and the term "mucoid" to similar substances in the tissues. In the present unsettled state of our chemical knowledge regarding these bodies, such a distinction is at best of only temporary convenience. The original differences have little importance in the light of the results of recent researches.

[4] LOEBISCH: Zeitschrift für physiologische Chemie, 1886, x, p. 40.

with some doubt in our own minds, but, having obtained them as the result of most careful work, we see no possible explanation other than that this amount of sulphur is actually present in the mucin molecule."[1]

The divergent results of these two investigations naturally throw some doubt on the question of the elementary composition of tendon mucoid. We have attempted not only to ascertain definitely the amount of sulphur in tendon mucoid, but also to explain the previous discrepancy in experimental data relating to sulphur content. In addition to the results in this particular connection, certain others of significance obtained by us may be appropriately given with them.

I. Content of Sulphur and Nitrogen.

Historical. — Rollett[2] was the first to show that tendon contains mucin-like material. He described some of the qualities of the substance, but made no elementary analyses of it. Eichwald[3] merely verified Rollett's qualitative results, in this connection.

Loebisch used Rollett's method to prepare sufficient quantities of tendon mucoid for analysis. Only three preparations were analyzed by Loebisch. But one sulphur determination was made on each, with the following results: (a) 0.82 per cent; (b) 0.80 per cent; (c) 0.82 per cent. Chittenden and Gies, who were the next to analyze this particular glucoproteid material, used improved methods of preparation and purification and, in sulphur analysis, obtained seven concordant results on three purified products, with the following averages: (a) 2.34 per cent; (b) 2.35 per cent; (c) 2.31 per cent. The difference is very striking.

With respect to the amount of nitrogen in tendon mucoid, a similar though not so decided analytic difference was established in these two investigations. Loebisch made only four determinations of nitrogen in his three purified preparations. The average of two closely agreeing results for his first preparation was 11.80 per cent; for the second the single result was 11.84 per cent and for the third it was 11.59 per cent. Chittenden and Gies made ten determinations in three preparations with the following averages of results in close

[1] Chittenden and Gies: *Loc. cit.*, p. 197.

[2] Rollett: Untersuchungen zur Naturlehre des Menschen und der Thiere (Moleschott), 1859, vi, p. 1. Also, *Ibid.*, 1860, vii, p. 190.

[3] Eichwald: Annalen der Chemie und Pharmacie, 1865, cxxxiv, p. 177.

agreement: (a) 11.94 per cent; (b) 11.80 per cent; (c) 11.51 per cent. They found, further, that the nitrogen content of a series of very carefully prepared fractional products varied between 11.51 per cent and 12.26 per cent, data which seem to suggest, though they do not establish, the existence of several related mucoids as components of ordinary tendinous tissue.

Preparation of Fractional Products. — At the outset of these experiments we assumed that tendon contains more than one glucoproteid. This seemed probable for several reasons. Among the latter is the fact that the larger tendons show considerable variation in texture throughout their length. Thus the tendo Achillis of the ox, from which the previously analyzed tendon mucoids were extracted, is comparatively soft and very tough in the main shaft, but toward its connections with the bones becomes more compact, and outwardly somewhat resembles cartilage. The superficial qualities of the thick sheaths enveloping the two large branches of the Achilles tendon in this animal also resemble those of cartilage to a certain extent.

These physical modifications within the tendinous tissue naturally suggest chemical differentiation of the constituents. Previous analytic variations respecting tendon mucoid may have been dependent on extraction of different mixtures of distinct though closely related bodies. Loebisch does not state which portions of the tendons were employed in his work. Chittenden and Gies used sections of the main shaft, together with portions of the two branches and the sheaths of the latter. In our own experiments these parts were extracted separately.

General Method. — In the preparation of mucoid for use in these experiments the Achilles tendon of the ox was employed. Following the usual method, the tissue, immediately after removal from the animals, was thoroughly freed of extraneous matter and cut into very thin cross sections. These pieces were washed in water and then extracted in half-saturated calcium hydroxide. The mixtures were shaken at regular intervals. The mucoid was precipitated from the filtered extract with dilute hydrochloric acid.[1] The precipitated substance was repeatedly washed; first in dilute hydrochloric acid, to

[1] We have always found that mucoids may be precipitated from lime-water or sodium carbonate solution much more satisfactorily with dilute HCl than with any other acid. The substance seems to separate much more quickly and completely in the presence of slight excess of this acid. Chlorides have comparatively slight solvent action on mucoids in the presence of free HCl, unless admixed in excess.

remove all traces of adherent proteid impurity, then in water until it was free of acid. It was next redissolved in dilute alkali and reprecipitated once with dilute hydrochloric acid. The washing process was repeated. Finally the acid-free substance was dehydrated and purified by long-continued treatment with large quantities of boiling alcohol-ether; then dried *in vacuo* and weighed.

First Experiment. Series A and B. — In this experiment two parallel series of fractional extractions were made and the mucoid in each separated and analyzed. 4600 gms. of the main shaft of the tendon about five inches in length, with from two to three inches of its bifurcations, were employed in Series A. In Series B only the sheaths of the branches, weighing 1900 gms., were used. Both lots of finely divided tissue were given identical treatment at each stage of the experiment. All extractions were made with 2 c.c. of half-saturated lime-water per gm. of moist tissue. After the extracts had been strained through cloth, the tendon pieces were thoroughly washed in water to prevent adherent dissolved mucoid from becoming part of the succeeding extract. The first extracts in each series were readily precipitated and brought to the flocculent condition with very slight excess of 0.2 per cent hydrochloric acid. Subsequent extracts, however, became only turbid with large excess of 0.2 per cent HCl — even with an equal volume. It was necessary, therefore, to add stronger acid (1.5% HCl) to separate the mucoid in flocks.[1] In purifying, the substance was redissolved in half-saturated lime-water. Powdered thymol, used in the second experiment also, entirely prevented bacterial action.

The summary, Table I, on page 159, gives additional significant facts relating to these fractional preparations.

A striking feature of these preparations was the fact that precipitation became more and more difficult with each extraction. More acid was required in each successive extract (except the fourth of Series B) to bring the mucoid to the flocculent condition. It will be seen from the data in Tables I and II that this was entirely independent of the proportion of contained mucoid. The alkali could not have effected decomposition, and thereby possible variations, because it was too

[1] In each instance the acid was added slowly in small quantities. The mixtures were thoroughly stirred and allowed to stand for flocks to form. After waiting a sufficient time, more acid was added if separation had not taken place. At first 0.2 per cent HCl was used. If after an equal volume of the acid had been stirred in, flocks failed to separate, 1.5 per cent HCl was added little by little. Separation took place instantly upon reaching the proper amount of acid. On reprecipitating, the same procedure was followed. The proportion of acid required was not recorded in the latter case, but great variations were observed. This method was employed in the second experiment also.

weak. Further, the volumes of fluid in each series were kept constant and the temperature was always about the same, so that the salts formed on acidification of the alkali of the extracts had essen-

TABLE I.

Extract.		Time of extraction.	Amount of HCl present to completely precipitate.[1]	Weight of purified product.[2]
Number.	Volume c.c.	Hours.	Per cent.	Grams.
Series A.				
First	9200	24	0.04	6.52
Second	9200	24	0.18	9.79
Third	9200	24	0.26	3.55
Fourth	9200	48	0.32	3.13
Series B.				
First	3800	24	0.03	4.23
Second	3800	24	0.17	1.65
Third	3800	24	0.46	0.93
Fourth	3800	48	0.37	

[1] The figures for per cent of HCl necessarily present to precipitate in flocks express approximate values. The precise amount of acid neutralized by the $Ca(OH)_2$ was not directly determined. It was the same of course throughout each series. Greater exactness would have emphasized the facts made significant by the above data.

[2] These weights are for substance dried *in vacuo*. The amount of each preparation could not be exactly quantitative, of course, because of slight losses during their purification. The mucoids are very difficult substances to handle and their preparation is decidedly laborious. Every effort was made to reduce inevitable loss to a minimum, however, and, as the loss was relatively the same in each preparation, the weights are entirely reliable for the intended comparisons.

tially the same influence throughout. The extracts were strained quickly at practically the same time and were promptly treated with acid, so that no changes could have occurred by reason of delay in final treatment.

The figures for weights of substance in each extract suggest variable resistance, on the part of the mucoid, to the solvent action of the dilute alkali. None of the extracts were ever saturated and all were distinctly alkaline. The peculiar behavior of these preparations harmonizes with the view that the tissue contains two or more glucoproteids, and that the products separated by the usual method of mucoid extraction are mixtures of different bodies.

(*c*) *Second Experiment. Series C and D.* — A second set of preparations was made in essentially the same way as in the first experiment. 6600 gms. of the main shaft of the tendon and its branches, of the same size as heretofore, were extracted in Series C; 4200 gms. of sheath in Series D. The periods of extraction were shorter at the beginning and longer at the close of this experiment than previously. In purifying, the substance was redissolved in 0.5 per cent sodium carbonate.

The summary of results given in Table II, page 161, connected with preparation, is directly comparable with Table I.

In this experiment, also, successive increase in proportion of acid was necessary for precipitation, the results harmonizing in detail with those of the first experiment. Variations in the quantities of separated mucoid again pointed to variable resistance to the action of the extractive. Fractions of a single substance would hardly act so differently at successive intervals under essentially the same conditions.

Analytic results. — Although the differences in the action of our several products indicated the existence of two or more mucoids in tendinous tissue, more direct evidence than qualitative variation was necessary to justify such a conclusion. We very carefully analyzed these products, with results that confirm the original deduction.

The amounts of nitrogen and sulphur in mucoids furnish excellent data for general comparisons of composition. Table III, on page 162, summarizes our results for percentage content of nitrogen and sulphur in the ash-free substance dried at 105–110° C. to constant weight.[1] The analyses were made by the customary methods — Kjeldahl for the nitrogen; fusion with NaOH over alcohol flame, and precipitation with $BaCl_2$, for sulphur.

[1] The proportion of ash in these preparations was usually much less than 1 per cent. In only four was it more than that, and in none of these did it exceed 1.78 per cent. It consisted mostly of phosphate and chloride; only a trace of sulphate was present.

These results seem to prove that more than one substance has been extracted — that mixtures have been obtained. The results for every member of each series differ decidedly in one respect or

TABLE II.

Extract.		Time of extraction.	Amount of HCl present to completely precipitate.[1]	Weight of purified product.[1]
Number.	Volume cc.	Hours.	Per cent.	Grams.
Series C.				
First	13200	17	0.03	14.56
Second	13200	20	0.15	24.88
Third	13200	26	0.17	17.26
Fourth	13200	30	0.38	2.04
Fifth[2]	13200	65	0.45	4.09
Series D.				
First	8400	17	0.02	11.85
Second	8400	20	0.15	13.41
Third	8400	26	0.45	3.19
Fourth	8400	30	0.39	0.29
Fifth	8400	65	0.35	0.59

[1] See notes under Table I.

[2] A sixth extraction lasting 124 hours was made in Series C. A trifle more than a gram of unpurified substance was obtained. The presence of nearly 1 per cent of HCl was necessary in order to bring it to the flocculent condition. This substance was true mucoid — on decomposition it yielded a reducing substance. It is evident from these results that it is very difficult to completely extract glucoproteid from tendinous tissue.

another from the rest in the group, and this, too, in spite of the fact that the analyses of all were conducted by uniform methods and under conditions as nearly the same as it is possible to attain. The extremes in percentage content are too far apart to be due to unavoidable analytic errors.

TABLE III.

CONTENT OF NITROGEN AND SULPHUR.

Extract.	Experiment I.				Experiment II.			
	Series A.		Series B.		Series C.		Series D.	
Number.	Nitrogen.	Sulphur.	Nitrogen.	Sulphur.	Nitrogen.	Sulphur.	Nitrogen.	Sulphur.
First	13.17 13.33 13.25	2.25 2.36 2.25 2.36 2.31	13.14 12.96 13.05	2.11 2.34 2.22	12.49 12.55 12.70 12.58	2.20 2.20 2.20	12.64 12.64 12.64	2.70 2.91 2.81
Second	12.85 12.94 12.71 12.83	1.81 1.66 1.74	12.41 12.46 12.43	2.67 2.72 2.70	11.77 11.79 11.78	1.75 1.86 1.81	12.68 12.70 12.69	2.28 2.39 2.34
Third	13.29[1] 13.25 13.27	1.49	13.47[1] 13.59 13.70	2.23	12.74 12.70 12.54 12.66	1.84 1.87 1.85	13.89[1] 13.92 13.91	2.47 2.28 2.38
Fourth[2]	13.84[1] 13.88 13.86	1.23 1.41 1.32			13.98[1] 14.06 14.02	1.55	14.06[1]	
Fifth					15.02 14.95 14.98	1.49 1.54 1.52	14.56[1]	
General averages.	13.25	1.87	13.02	2.41	13.11	1.81	13.39	2.51

[1] Not ash-free. There was not sufficient substance left for additional determinations. The average amount of ash in all the other preparations was 1.03 per cent. See note, page 160.

[2] The substance of the third and fourth extracts of Series B was combined for analysis; the amounts were too small to be dealt with separately.

It will be noticed that the nitrogen of the mucoids of the first extracts is greater in amount than in the second — just as was found in the single similar experiment by Chittenden and Gies. With one exception the nitrogen of the mucoid in the second extract is much less in each series than in any of the others of the group but becomes greater with each succeeding extraction. The sulphur, on the other hand, shows gradual decrease in Series A and C, but remains much the same in the other two. The average content of sulphur in the mucoids of Series B and D (prepared from the sheaths) is appreciably higher than in the others. The nitrogen average is practically the same in all.[1]

II. Complete Elementary Composition.

We made complete analysis of several of our preparations in order to obtain additional evidence in the connections just discussed, and to add if possible to our knowledge of general composition.

Closely related members of Series C and D of our preparations were selected for this purpose. The methods of analysis were those commonly in use. We followed those outlined in detail in a recent paper on a similar subject from this laboratory,[2] so that their description may be omitted here. Special care was taken to keep as nearly uniform as possible all conditions known to affect analysis, so that the results would be directly comparable.

No. 1. Mucoid of first extract of Series C.

Carbon and Hydrogen. 0.3550 gm. substance gave 0.6120 gm. CO_2 and 0.2100 gm. H_2O = 47.02 per cent C and 6.57 per cent H; 0.4120 gm. substance gave 0.7140 gm. CO_2 and 0.2480 gm. H_2O = 47.26 per cent C and 6.69 per cent H.

Nitrogen. 0.2275 gm. substance gave 0.0282 gm. N = 12.40 per cent N; 0.1484 gm. substance gave 0.0187 gm. N = 12.61 per cent N; 0.1894 gm. substance gave 0.0236 gm. N = 12.46 per cent N.

Total Sulphur. 0.5665 gm. substance gave 0.0905 gm. $BaSO_4$ = 2.19 per cent S; 0.6547 gm. substance gave 0.1045 gm. $BaSO_4$ = 2.19 per cent S.

Sulphur combined as SO_3. 0.4210 gm. substance, after boiling in HCl, gave 0.0413 gm. $BaSO_4$ = 1.33 per cent S; 0.2880 gm. substance, after boiling in HCl, gave 0.0286 gm. $BaSO_4$ = 1.35 per cent S.

Ash. 0.1727 gm. substance gave 0.0012 gm. Ash = 0.69 per cent Ash.

[1] Compare with results for carbon and oxygen, also, in Table IV, page 168.

[2] Hawk and Gies: This journal, 1901, v, p. 403.

PERCENTAGE COMPOSITION OF THE ASH-FREE SUBSTANCE.[1]

								Average.
C	47.34	47.59	....	....	....	....	....	47.47
H	6.63	6.74	....	....	....	....	....	6.68
N	....	....	12.49	12.70	12.55	....	....	12.58
S	....	....	....	....	....	2.20	2.20	2.20
O	....	....	....	....	....	....	....	31.07

No. 2. Mucoid of second extract of Series C.

Carbon and Hydrogen. 0.1252 gm. substance gave 0.7320 gm. H_2O = 6.50 per cent H; 0.1903 gm. substance gave 0.3292 gm. CO_2 and 0.1122 gm. H_2O = 47.18 per cent C and 6.55 per cent H; 0.1303 gm. substance gave 0.2245 gm. CO_2 and 0.0760 gm. H_2O = 46.99 per cent C and 6.48 per cent H.

Nitrogen. 0.2523 gm. substance gave 0.0295 gm. N = 11.70 per cent N; 0.3037 gm. substance gave 0.0355 gm. N = 11.68 per cent N.

Total Sulphur. 0.6541 gm. substance gave 0.0830 gm. $BaSO_4$ = 1.74 per cent S; 0.7209 gm. substance gave 0.0974 gm. $BaSO_4$ = 1.85 per cent S.

Sulphur combined as SO_3. 0.4798 gm. substance, after boiling in HCl, gave 0.0567 gm. $BaSO_4$ = 1.62 per cent S; 0.3760 gm. substance, after boiling in HCl, gave 0.0437 gm. $BaSO_4$ = 1.59 per cent S.

Ash. 0.1989 gm. substance gave 0.0017 gm. Ash = 0.85 per cent Ash; 0.1200 gm. substance gave 0.0009 gm. Ash = 0.75 per cent Ash.

PERCENTAGE COMPOSITION OF THE ASH-FREE SUBSTANCE.

								Average.
C	....	47.56	47.36	....	....	....	....	47.46
H	6.56	6.60	6.53	....	....	....	....	6.56
N	....	....	....	11.79	11.77	....	....	11.78
S	....	....	....	....	....	1.75	1.86	1.81
O	....	....	....	....	....	....	....	32.39

No. 3. Mucoid of third extract of Series C.

Carbon and Hydrogen. 0.1194 gm. substance gave 0.2063 gm. CO_2 and 0.0709 gm. H_2O = 47.12 per cent C and 6.60 per cent H; 0.0973 gm. substance gave 0.1694 gm. CO_2 and 0.0566 gm. H_2O = 47.48 per cent C and 6.46 per cent H.

[1] Only traces of phosphorus were present, equal in amount to the phosphorus in the ash. This was ascertained for each preparation. The quantity was greatest in this particular product — 0.26 per cent and 0.24 per cent in two determinations.

Nitrogen. 0.2181 gm. substance gave 0.0275 gm. N = 12.61 per cent N; 0.3675 gm. substance gave 0.0462 gm. N = 12.57 per cent N; 0.2831 gm. substance gave 0.0351 gm. N = 12.41 per cent N.

Total Sulphur. 0.7412 gm. substance gave 0.0982 gm. $BaSO_4$ = 1.82 per cent S; 0.6574 gm. substance gave 0.0887 gm. $BaSO_4$ = 1.85 per cent S.

Sulphur combined as SO_3. 0.6686 gm. substance, after boiling in HCl, gave 0.0653 gm. $BaSO_4$ = 1.34 per cent S.

Ash. 0.1720 gm. substance gave 0.0018 gm. Ash = 1.04 per cent Ash.

Percentage Composition of the Ash-free Substance.

								Average.
C	47.62	47.98	....	....	....	....	....	47.80
H	6.66	6.53	....	....	....	....	....	6.60
N	....	....	12.74	12.70	12.54	....	....	12.66
S	....	....	....	....	....	1.84	1.87	1.85
O	....	....	....	....	....	....	....	31.09

No. 4. Mucoid of first extract of Series D.

Carbon and Hydrogen. 0.0770 gm. substance gave 0.1372 gm. CO_2 and 0.0480 gm. H_2O = 48.60 per cent C and 6.93 per cent H; 0.0968 gm. substance gave 0.1721 gm. CO_2 and 0.0578 gm. H_2O = 48.48 per cent C and 6.63 per cent H.

Nitrogen. 0.3946 gm. substance gave 0.0495 gm. N = 12.55 per cent N; 0.3154 gm. substance gave 0.0396 gm. N = 12.55 per cent N.

Sulphur. 0.5967 gm. substance gave 0.1159 gm. $BaSO_4$ = 2.68 per cent S; 0.7591 gm. substance gave 0.1603 gm. $BaSO_4$ = 2.89 per cent S.

Sulphur combined as SO_3. 0.8904 gm. substance, after boiling in HCl, gave 0.0886 gm. $BaSO_4$ = 1.36 per cent S.

Ash. 0.1983 gm. substance gave 0.0015 gm. Ash = 0.75 per cent Ash.

Percentage Composition of the Ash-free Substance.

							Average.
C	48.97	48.87	....	....	....	....	48.92
H	6.98	6.68	....	....	....	....	6.83
N	....	....	12.64	12.64	....	....	12.64
S	....	....	....	....	2.70	2.91	2.80
O	....	....	....	....	....	....	28.81

No. 5. Mucoid of second extract of Series D.

Carbon and Hydrogen. 0.1779 gm. substance gave 0.3101 gm. CO_2 and 0.1028 gm. H_2O = 47.54 per cent C and 6.42 per cent H; 0.0608 gm. substance gave 0.1066 gm. CO_2 and 0.0365 gm. H_2O = 47.82 per cent C and 6.69 per cent H.

Nitrogen. 0.3046 gm. substance gave 0.0380 gm. N = 12.48 per cent N; 0.2545 gm. substance gave 0.0316 gm. N = 12.45 per cent N.

Sulphur. 0.7143 gm. substance gave 0.1226 gm. $BaSO_4$ = 2.35 per cent S; 0.9841 gm. substance gave 0.1608 gm. $BaSO_4$ = 2.24 per cent S.

Sulphur combined as SO_3. 0.7130 gm. substance, after boiling in HCl, gave 0.0805 gm. $BaSO_4$ = 1.55 per cent S.

Ash. 0.3477 gm. substance gave 0.0059 gm. Ash = 1.69 per cent Ash; 0.1665 gm. substance gave 0.0031 gm. Ash = 1.86 per cent Ash.

PERCENTAGE COMPOSITION OF THE ASH-FREE SUBSTANCE.

							Average.
C	48.40	48.67	....	....	....	....	48.54
H	6.54	6.81	....	....	....	....	6.68
N	....	....	12.70	12.68	....	....	12.69
S	....	....	....	....	2.39	2.28	2.34
O	....	....	....	....	....	....	29.75

Discussion of results. — The general summary of our results for complete elementary composition, Table IV, may be compared with similar data obtained in the previous investigations. It will be observed that although there is some variation within each series — very slight in Loebisch's, quite marked in our own — the three group averages are very nearly the same. This is particularly significant in this connection. It suggests that mixtures of generally uniform composition resulted in each of the previous studies. Leobisch varied his method very little and obtained practically uniform products; Chittenden and Gies varied theirs more decidedly, and the result was distinct variation in composition of substance extracted. By the fractional method in our own experiments, still greater differentiation was effected.

We do not mean to suggest that our own products are chemical individuals. They are mixtures, just as all the previously described tendon mucoids have doubtless been. Further research, with more elaborate methods, and particularly with reference to inner groupings of the elements, will be necessary for definite differentiation, if such is possible while we remain in our present profound ignorance of the structure and peculiarities of proteid molecules.[1]

The amounts of nitrogen in our preparations appear to be slightly greater than those previously determined, although the nitrogen con-

[1] HAWK and GIES: *Loc. cit.*, p. 414 *et seq.*

tent of preparation No. 2 (Second extract, Series C), which was the largest in quantity of all our products,[1] conforms closely with the generally accepted figures for content of this element.

The only particularly discordant results in the general averages are those for content of sulphur and oxygen (by difference) obtained by Loebisch. We had hoped that this low figure would be explained by our results, but none of our products contained so little sulphur. Our figures in this connection accord very well with those given by Chittenden and Gies. As has already been stated, Loebisch made only a few analyses — only one determination of sulphur in each of his three preparations. He duplicated results in only half of the analyses he reported.

In referring to the differences in composition observed among their products, Chittenden and Gies stated: "Our results seemingly justify the assumption that white fibrous connective tissue contains more than one mucin, or else that the mucin obtainable from this tissue is prone to carry with it a certain amount of some other form of proteid matter which the ordinary methods of purification are not wholly adequate to remove. . . . There is at the present time no standard of purity with regard to this body, and it is quite as probable that fibrous connective tissue contains two or more mucins as that there is only one mucin in the tissue, and that any deviation from the figures obtained by Loebisch or by us in preparation No. 3 is due to the presence of a larger or smaller amount of proteid impurity."[2]

We can no longer believe that proteid impurity is responsible for the observed variations. In the first place the quantity of soluble proteid in tendon, other than mucoid, is very slight. Experiments in progress in this laboratory indicate that it is less than 0.3 per cent. If, however, it were possible for all of this small quantity to combine permanently with the precipitated mucoids, it could not account for the regular rise and fall of nitrogen content observed in each series of our experiments.[3] Although it is conceivable that the mucoid of the first extract could be so affected, such an assumption would not explain the rise of nitrogen in the third and subsequent extracts, particularly in view of the marked fall of the same in the second. Then, too, each product was so thoroughly washed in excess

[1] See table on page 161.

[2] CHITTENDEN and GIES: *Loc. cit.*, p. 194.

[3] See the table on page 162.

TABLE IV.

AVERAGE PERCENTAGE COMPOSITION OF TENDON MUCOIDS.

Investigators.	LOEBISCH.				CHITTENDEN AND GIES.				CUTTER AND GIES.					
Number of preparation.	1	2	3	Average.	1	2	3	Average.	1	2	3	4	5	Average.
C	48.24	48.34	48.32	48.30	49.29	48.74	48.26	48.76	47.47	47.46	47.80	48.92	48.54	48.04
H	6.44	6.43	6.53	6.47	6.63	6.46	6.49	6.53	6.68	6.56	6.60	6.83	6.68	6.67
N	11.79	11.84	11.59	11.74	11.94	11.80	11.51	11.75	12.58	11.78	12.66	12.64	12.69	12.47
S	0.82	0.80	0.82	0.81	2.34	2.35	2.31	2.33	2.20	1.81	1.85	2.80	2.34	2.20
O	32.71	32.59	32.74	32.68	29.80	30.65	31.43	30.63	31.07	32.39	31.09	28.81	29.75	30.62

All of the above preparations were made by similar methods. Loebisch extracted for 48 hours in half-saturated lime-water in the proportion of 2 c.c. of the latter per gram of moist tissue. His first preparation was precipitated with 1–5 per cent acetic acid, the second with 0.1–0.2 per cent hydrochloric, the third with 1–5 per cent acetic acid. Only the third was purified by reprecipitation from alkaline solution. It was redissolved in 0.5 per cent sodium carbonate and again thrown down with acetic acid.

Chittenden and Gies used the above proportions of tissue and half-saturated lime-water in their first preparation and continued the extraction 48 hours. The tissue was re-extracted for the same time and the precipitates from the extracts united and purified together. They precipitated with 0.2 per cent hydrochloric acid, re-dissolved in half-saturated lime-water and reprecipitated again with dil. HCl. Their second product was separated from tissue which had previously been extracted in 10 per cent sodium chloride solution for 36 hours. From this point exactly the same process was employed as that used for preparation No. 1, except that the substance was redissolved in 0.5 per cent Na_2CO_3. In their third preparation the tissue was first extracted in 10 per cent salt solution for 24 hours, then in the usual proportion of half-saturated lime-water for 60 hours. The mucoid in this extract was treated separately in a special experiment. (By fractional precipitation two products were obtained from it, the first with 12.26 per cent of nitrogen, the second with 11.91 per cent. Compare with the nitrogen of their Preparation No. 3 and our own results.) The tissue was again extracted in the usual amount of dilute lime-water and the mucoid in this second extract purified as Preparation No. 3 (with 11.51 per cent N).

Of our own preparations, Nos. 1, 2, and 3 were the first three fractional products of Series C (made from the main shaft of the tendon and its bifurcations). Nos. 4 and 5 were the first two fractional products of Series D (made from the sheaths of the tendons used in Series C). See page 160.

of 0.2 per cent hydrochloric acid, that unless very intimate and unusual chemical union resulted, lymph proteids must have been quickly and completely dissolved from the precipitates. We know of no other substance in tendon which would resist the washing treatment and, by mechanical admixture or chemical combination, account for the orderly variations observed in the analytic series.[1]

It is much more probable, we think, that an answer to these considerations will be found in the fact that the mucoids are labile bodies of great variety in the tissues and with more than one function to perform. Their acid radicles doubtless make them prone to enter into numerous ion combinations. The very complexity of these substances makes it natural to assume that exactly the same proportions of the constituent radicles would in metabolic changes be the exception rather than the rule.

All of the products separated in these experiments were true glucoproteids, responding to each of the well known reactions and yielding reducing substance in abundance.

We have repeated the experiments of Chittenden and Gies on the osazone substance obtainable with the reducing body and, working with a larger quantity of mixed mucoid products by the same and also improved methods, obtained a crystalline product melting at 182° C.[2] In microscopic appearance the crystals are identical with those of glucosazone. We have not yet been able to free the substance entirely from the brownish globules that occur with it and which persist

[1] Since this paper went to the printer we have seen NERKING'S recent note on fat proteid compounds, in the Archiv für die gesammte Physiologie, 1901, lxxxv, p. 330. His results indicate that various proteid products, which have been purified by the usual methods, contain fat or fatty acid in close combination ; further, that this fatty radicle may be broken off, and extracted, by DORMEYER'S method. No such combination with ovomucoid was shown, but about three per cent of extractive matter was found to be combined with submaxillary mucin. NERKING does not state, however, that the mucin was thoroughly extracted in hot alcohol ether during the preliminary process of purification, in the customary manner. No results are presented for tendon mucoid; but LOEBISCH, and CHITTENDEN and GIES have already called attention to the fact that tendon mucoid when freshly precipitated is admixed with extractive matter that is removable only after long continued extraction. All our preparations were given careful and extended treatment in boiling alcohol-ether, and we do not believe that the variations in composition noted are due to such fat combination. We hope that studies which have lately been in progress in this laboratory, will soon furnish direct evidence concerning this and related questions.

[2] The product obtained by CHITTENDEN and GIES melted at 160° C.

in spite of all our attempts to purify the crystals. It seems certain that glycuronic acid and glucosamin, or very closely related bodies, are formed together in the decomposition of tendon mucoid with hot dilute mineral acid.

III. Relation to other Connective Tissue Glucoproteids.

Composition. — It appears to be definitely established by the numerous results of these and the preceding experiments that the amount of sulphur in tendon mucoid is relatively high — almost the same as in chondromucoid and osseomucoid — and that Loebisch's data in this particular connection can no longer be accepted as correct. We have never been able to prepare a tendon mucoid having less than 1.3 per cent of sulphur.[1]

The sulphur is present in at least two combinations, as in the case of chondromucoid and osseomucoid. After boiling with alkali, lead sulphide may be obtained on addition of lead acetate. The amount combined in the form of SO_3 is relatively large, varying as the analytic data for each preparation show, between 1.33 and 1.62 per cent of the whole molecule. The average amount of SO_3 sulphur in chondromucoid is 1.76 per cent. In osseomucoid it equals 1.40 per cent. Levene[2] has lately separated from tendon mucoid a substance very similar to chondroitin sulphuric acid. The quantity of this substance separable from the mucoid has not been estimated.

Two years ago, in our preliminary report, we made the following statement:[3] "Before these experiments were started, the similarity in the percentage composition of Mörner's chondromucoid and the tendon mucin analyzed by Chittenden and Gies suggested to us that the two substances are perhaps closely related. This was further emphasized by the fact that the osazone crystals they obtained had the same general appearance as the crystals of glucosazone, and, therefore, might have arisen from glucosamin, one of the decomposition products of chondromucoid." Levene's results and our own increase the probability that the two substances are very much the same.

The following summary of average elementary composition shows the general relationship of very nearly identical products:

[1] See table, page 162.

[2] Levene: Zeitschrift für physiologische Chemie, 1901, xxxi, p. 395.

[3] Cutter and Gies: *Loc. cit.*

		C	H	N	S	O
Chondromucoid	Mörner	47.30	6.42	12.58	2.42	31.28
Tendomucoid (*a*)	Chittenden and Gies	48.76	6.53	11.75	2.33	30.63
(*b*)	Cutter and Gies (1) .	47.47	6.68	12.58	2.20	31.07
Osseomucoid	Hawk and Gies . .	47.07	6.69	11.98	2.41	31.85
	Average . .	47.65	6.58	12.22	2.34	31.21

Heat of Combustion. — Heat of combustion furnishes important means of estimating chemical relationships, though its indications are not, perhaps, so delicate as those of elementary analysis. The determinations in these experiments were made by the method described by Hawk and Gies. In Table V we give the heat of combustion of our five completely analyzed preparations, together with comparative

TABLE V.

Combustion Equivalents.

Preparation.	Direct determinations.			Averages for ash-free substance.			
	Heat of combustion. Small calories.			Percentage content.		Heat of combustion. Small calories.	
	Per gram of substance.			Carbon.	Oxygen.	Per gm. of substance.	For substance containing 1 gm. of carbon.
	I	II	Average.				
I. Tendomucoid. No. 1	4925	4940	4933	47.47	31.07	4967	10463
No. 2	4963	4930	4947	47.46	32.39	4986	10506
No. 3	4921	4934	4928	47.80	31.09	4979	10416
No. 4	4908	4920	4914	48.92	28.81	4951	10121
No. 5	5044	5036	5040	48.54	29.75	5131	10571
Average.	4952	4952	4952	48.04	30.62	5003	10415
II. Osseomucoid. Average of two preparations.	4972	4985	4979	47.16	31.79	4992	10589
III. Chondromucoid. Average of two preparations.	4865	4869	4867	45.87	32.90	4883	10647

data from the summary in a recent paper from this laboratory.[1] The figures show only imperfectly the differences among the tendon mucoids. They are valuable chiefly for the indication they furnish that the various glucoproteid products referred to are essentially the same compounds.

We still believe "continued investigation will show that the differences among the mucins, mucoids, and chondroproteids are not as great as their varying physical properties and behavior have suggested, but that each is a combination of proteid with a glucosulphonic acid, the qualities of each compound, just as in the case of the nucleoproteids, being dependent largely on the proportions and character of the proteid and compound acid radicles."[2]

IV. Summary of Conclusions.

The more important conclusions to be drawn from the results of this research are:

1. Tendon contains more than one glucoproteid. The average percentage composition of five preparations of mixed mucoid was as follows:

C	H	N	S	O
48.04	6.67	12.47	2.20	30.62

These figures agree very closely with those published by Chittenden and Gies.

2. The composition of mucoid from the shaft and from the sheath:

	C	H	N	S	O
Shaft (3)	47.56	6.61	12.34	1.95	31.52
Sheath (2)	48.73	6.75	12.66	2.57	29.28

3. Tendon mucoids contain an average amount of sulphur equal to that found by Chittenden and Gies — approximately 2.30 per cent. Not a single product had the very low content of sulphur ascribed to this substance by Loebisch.

4. The average composition of mucoid separated from white fibrous connective tissue by the customary methods is very nearly the same as that of chondromucoid and osseomucoid.

5. Thermochemical studies of the mucoids in tendon, cartilage, and bone emphasize the probability that these bodies are very intimately related.

[1] Hawk and Gies: *Loc. cit.*, p. 422.

[2] Cutter and Gies: *Loc. cit.*

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6

ON THE COMPOSITION AND CHEMICAL PROPERTIES OF OSSEOALBUMOID, WITH A COMPARATIVE STUDY OF THE ALBUMOID OF CARTILAGE.[1]

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At the time of the first announcement of the writer's discovery of glucoproteid among the constituents of osseous tissue,[2] it was pointed out that the method of preparing osseomucoid furnishes residual material particularly well adapted to the study of other organic substances in bone. This method, it may be recalled, consisted, briefly, in preliminary softening of superficial layers of bone by removing inorganic matter with dilute acid (*e.g.*, 0.2 per cent HCl). The softer tissue was next transformed into thin shavings by scraping it with a scalpel, and finally, after hashing the material and washing it free of acid in water, was extracted with dilute alkali, such as half-saturated lime-water. The residual product thus obtained naturally contained collagen, also any other albuminoid constituent possibly present in the tissue; other soluble proteid substances, such as lymph proteids or nucleo-compounds, having been eliminated. The weakness of the acid and alkali used in the process of preparing the ossein

[1] A preliminary account is given in the Proceedings of the American Physiological Society: This journal, 1902, vi, p. xxvii.

[2] Gies: Proceedings, *Ibid.*, 1900, iii, p. vii.

makes it very probable, further, that any albuminoid constituents other than collagen are entirely unaffected chemically by such extraction process.

These observations induced us to study the elastin-like substance of bone. This constituent has been referred to by several investigators. They have given us anything but a clear idea of its qualities, however. In this connection it was found desirable, also, to make a comparative study of the albumoid of cartilage, which has been referred to by various observers quite as indefinitely.

Osseoalbumoid.

Historical. — Numerous investigators have made chemical studies of osseous tissue. In their researches, the organic, proteid residue left behind after solution of the salts in acid, the so-called ossein, has usually been regarded as consisting entirely of collagen. Lymph proteids and nucleo-compounds have been recognized, however, and elastic fibres are admittedly present in normal bone and in ossein, though in comparatively small number.[1]

Broesike[2] some years ago reviewed the data of microchemical study of osseous tissue, and published, also, the results of several experiments by himself, which led him to believe that keratin is among the normal bone constituents. The substance he called keratin was evidently located, in part at least, in the lining of the lacunæ and canaliculi. His conclusion that this substance was keratin was dependent on its seeming indigestibility, and, further, on its lack of solubility in various reagents in which keratin, also, is unaffected chemically.

Smith[3] soon after, under Kühne's supervision, made it very evident that Broesike had fallen into a number of experimental errors, and that, as a consequence, the latter observer's chief chemical deductions were fallacious. Instead of finding ossein indigestible in pepsin-hydrochloric acid, for example, Smith clearly demonstrated, as several others seem also to have done before him, that very little solid matter remains after treatment of the organic elements of bone with an *active* enzyme solution. He observed, further, that such residual substance as is resistant either completely disappears, when subjected

[1] Halliburton: Schäfer's Text-book of physiology, 1898, i, p. 111.

[2] Broesike: Archiv für mikroskopische Anatomie, 1882, xxi, p. 695.

[3] Smith: Zeitschrift für Biologie, 1883, xix, p. 469.

to the influence of a new pepsin-acid solution, or is converted into a slight proportion of nuclein-like material entirely different from the keratins.[1] Although Smith did not establish the identity of the substance which Broesike called keratin, his work suggested that the material was elastin.[2]

With nothing very definite on the point of chemical identity we therefore proceeded with our attempts to isolate sufficient material for analysis.

General method of preparation. — All our preparations were made from the femur of the ox. We have already indicated that the preliminary part of the preparation process consisted first in transforming bone into ossein shavings, then putting the shavings through a hashing machine and extracting the mucoid, nucleoproteids, etc., from the finely divided tissue.[3]

After this treatment, the alkali remaining in the shavings was removed by repeated washing in water. When this process had been completed the hash was heated in water in a large, agate-ware kettle until gelatinization of the collagenous elements was complete. In the later preparations the kettle was kept covered so as to elevate the temperature of the mixture to the highest point possible under the circumstances. When it was desired to renew the hydrating fluid, the mixture was at first strained through fine cloth or a sieve. When it became more finely divided, toward the later stages of the disintegration, filtration on a hard filter sufficed for ready separation of the solid matter. Conclusion of the gelatinization process was determined not only by the almost complete disappearance of fibrous structure from the residual flocks, but also by the nearly negative reaction of the filtered fluid with picric acid. Such slight reaction with this reagent as persisted after a few days' boiling was due undoubtedly to proteoses formed from the residual matter.

[1] The results of Smith's experiments are obviously in harmony with the fact that large quantities of bone are ordinarily digested in the alimentary tract of carnivora. We ourselves have witnessed the complete digestion of small pieces of fresh bone in a large proportion of normal gastric juice taken from a fistula in a dog, only a small proportion of nuclein-like material remaining undissolved.

[2] This has since generally been taken for granted. See Text-books of physiological chemistry by HALLIBURTON (1891, p. 493), GAUTIER (1897, p. 107), NEUMEISTER (1897, p. 454), and HAMMARSTEN (1899, p. 326).

[3] This method was given in detail in the second contribution from this laboratory on the subject of the preparation of osseomucoid. This journal, 1901, v, p. 393.

The resultant product contained the elastin-like substance, which was purified in boiling alcohol-ether in the customary manner.

Modifications of, and additions to this method are noted below under each preparation.

Preparation No. 1. — Our first product was made by the general method just outlined. In this case the ossein shavings were boiled in water for 12 hours and the residue heated continuously in a flask over the boiling water of a bath for 328 hours — as long as the substance appeared to diminish in bulk. The final product was dehydrated, and extraneous matter removed, by treatment in alcohol and ether in the usual process of proteid purification.

The material thus obtained was light and fluffy, and grayish brown in color. The moist substance was lightly flocculent, dark brown, granular for the most part, but consisting in small degree of fibrous fragments — probably elastic material.[1] To our great surprise the supposedly pure product contained 76.32 per cent of ash, mostly calcium phosphate.[2] The ash-free substance contained the following:[3]

C	H
49.81%	6.68%

In pepsin-hydrochloric acid, samples of this product digested very readily, proteoses forming in good proportion.

It was very evident from these results that the soft ossein shavings, obtained after treatment with dilute acid as above, still contained considerable inorganic matter, which remained in part in the organic residue even after its complete disintegration in hot water.

Preparation No. 2. — The remaining substance of preparation No. 1, about 4 grams, was washed in 0.025 per cent hydrochloric acid re-

[1] The reader need hardly be reminded of the great difficulty in the way of absolute purification of residual tissue constituents, particularly when such products form a comparatively small proportion of the original structure. Products of the kind before us here, which are never dissolved, filtered, and precipitated, are very apt to accumulate dust particles, fragments of various extraneous matters, etc. The greatest precaution is insufficient to entirely prevent such adventitious admixture. In all of these preparations the greatest care was constantly taken to diminish such accidental adulteration, and before analysis was begun, each product was very thoroughly looked over for particles of foreign material.

[2] The ash was brick-red in color. The same color characterized the ash from all of these products — both from bone and cartilage. A fairly large proportion of iron was detectable in these inorganic residues.

[3] The methods of elementary analysis used throughout this work were those in general employment already described by us: This journal, 1901, v, p. 403.

peatedly for a week, until only slight quantities of phosphate could be detected in the washings. After dehydration, etc., this product still contained 46.25 per cent of ash.[1] The physical condition of the previously dried material was doubtless unfavorable to complete elimination of the saline matter in the very weak acid used.

This product was found to be entirely insoluble in cold dilute potassium hydroxide, even when as strong as 1 per cent. No biuret reaction could be obtained in the filtrate after the substance had been frequently stirred with the alkali for about a day.

In dilute hydrochloric acid — 0.2 per cent or less — the substance diminished in quantity by reason of the solvent action on the admixed phosphate, but no biuret reaction could be obtained with the acid extract even after it had been in contact with the substance for twenty-four hours.

The preparation itself gave the Millon's, xanthoproteic, and biuret reactions very distinctly. The composition of the ash-free substance was as follows:

C	H	N
49.71%	6.62%	16.11%

Preparation No. 3. — This was obtained from several pounds of shavings which had been made in 0.5 per cent hydrochloric acid[2] and preserved during their accumulation in 10 per cent alcohol. After the removal of the mucoid the shavings had been kept extracting in large excess of 0.25 per cent potassium hydroxide for four months, for complete elimination of traces of mucoid and nucleo-compounds.

When the alkali had been washed out, the ossein hash was kept in boiling water ten hours daily for thirteen days. At first the hot water became faintly alkaline each time it was renewed, because of

[1] The persistently high proportion of ash in these two preparations brought to mind the old question of possible chemical combination between some of the inorganic and organic substances of bone. (Consult the discussion of this matter by DRECHSEL in Hermann's Handbuch der Physiologie, 1883, v, (1), p. 609). Our later results, however, as will be seen, do not offer the same indications as those of the first two preparations. From our later data it appears that there are only mechanical obstacles to the ready removal of the inorganic matter, and that, when these are overcome by more thorough acid treatment, the amount of ash is not much above that found associated with the average proteid from other sources.

[2] Shavings for the preceding preparations were made from bones treated with 0.2 per cent HCl. The shavings had been kept in 25 per cent alcohol before extraction of the mucoid.

liberation of mechanically held alkali on disintegration of the tissue pieces. This alkali had persisted in spite of the previous thorough washing. Finally, however, the warmed mixture was entirely neutral. The boiling process was continued much longer than appeared to be necessary merely to make certain that all collagenous matter had been transformed into soluble material.

In order to remove more throughly inorganic matter from the substance remaining after the boiling process, the product was repeatedly washed for ten days in cold hydrochloric acid of a strength increasing at first from 0.05 per cent to 0.2 per cent, and later decreasing to 0.05 per cent. Much phosphate was taken out in this way. A slight biuret reaction was obtainable in the washings with the 0.2 per cent hydrochloric acid. This was not obtained with the 0.1 per cent acid at first, although as the phosphate content diminished the residual proteid became more susceptible to the action of the acid and slight solution in 0.1 per cent acid finally occurred.[1]

After purification in alcohol-ether, etc., 1.36 gram of substance remained. This preparation, in spite of the long-continued washing in acid just before dehydration, contained 5.85 per cent of ash. Samples of this substance gave the usual proteid color reactions and digested easily in artificial gastric juice. The digestive product was mostly proteose, after twenty-four hours at 40° C.

The analytic results for this preparation were as follows:

Carbon and Hydrogen. 0.1021 gm. substance gave 0.0576 gm. $H_2O = 6.31$ per cent H; 0.1030 gm. substance gave 0.1764 gm. $CO_2 = 46.71$ per cent C, and 0.0580 gm. $H_2O = 6.30$ per cent H.

Nitrogen. 0.1599 gm. substance gave 0.02413 gm. N = 15.09 per cent N.

Total Sulphur. 0.6440 gm. substance gave 0.0490 gm. $BaSO_4 = 1.05$ per cent S.

Ash. 0.1213 gm. substance gave 0.0071 gm. Ash = 5.85 per cent Ash; 0.2580 gm. substance gave 0.0151 gm. Ash = 5.85 per cent Ash.

Sulphur of the Ash. 0.2580 gm. substance left 0.0151 gm. Ash, which gave 0.0046 gm. $BaSO_4 = 0.16$ per cent S.[2]

[1] Note remarks on solubility, etc., of ligament elastin by RICHARDS and GIES: This journal, 1902, vii, p. 104.

[2] This amount of sulphur is not deducted from the quantity calculated for ash-free substance. The large amount of sulphur in the substance makes it probable that the SO_4 of the ash was derived by oxidation of organic sulphur. This applies equally well to all of our preparations, both from bone and cartilage.

PERCENTAGE COMPOSITION OF THE ASH-FREE SUBSTANCE.[1]

					Average.
C	....	49.61	....	....	49.61
H	6.70	6.69	....	....	6.70
N	....	....	16.03	....	16.03
S	....	....	....	1.11	1.11
O	....	....	....	....	26.55

Preparation No. 4. — This product was obtained from shavings made about six months previously from bones treated with 0.5 per cent hydrochloric acid. The shavings were washed once in 0.1 per cent hydrochloric acid and thereafter kept in acidified 25 per cent alcohol until several pounds of material had been obtained. During the six months after removal of the mucoid the ossein hash was repeatedly washed in 0.3 per cent potassium hydroxide. Finally, after the alkali had been removed as usual, hydration was effected in boiling water, repeatedly renewed and made faintly acid with acetic acid. From this point the process of treatment was identical with that for preparation No. 3.

A marked physical difference between this and the former products was observed. The residual material, although quite resistant to the action of the boiling water, was somewhat gelatinous in appearance. Though divided into minute flocks, these were somewhat adherent, and tended to collect at the top of the hot water in a semi-gelatinous layer. This was easily broken up into flocks on stirrring. The product was finally much diminished in bulk and appeared more soluble in dilute acids than any of the preceding preparations. We did not obtain sufficient for quantitative analysis. The residual substance gave the proteid color reactions. It appeared to be a transformation product resulting from the action of the acid in the boiling fluid during the process of hydrating the collagen, although, aside from differences in physical form and solubility, it was identical with the other products. It contained loosely-bound sulphur, was digestible, and did not yield reducing substance on decomposition with acid.

Preparation No. 5. — Ossein shavings were freshly prepared after treatment of the bones with 0.2 per cent hydrochloric acid. Several kilos of the material were made. While they were accumulating, the

[1] The substance was found to be entirely free from phosphorus in organic combination. Phosphate was the chief constituent of the ash.

shavings were kept in 0.1 per cent hydrochloric acid. This was frequently renewed. After elimination of the mucoid with lime-water, the shavings were washed free of alkali with very dilute acetic acid. The rest of the process was essentially the same as that for preparation No. 3.

The fluid poured off at first, after the hydration had been begun, was very faintly alkaline, showing, as in previous instances, that, in spite of the acid treatment, some of the lime-water was held unaffected in the tissue. This product appeared to be somewhat more soluble in 0.2 per cent hydrochloric acid than preparation No. 3. About one gram of purified substance was obtained.[1]

The ash of this preparation amounted to 5.88 per cent. The analytic data obtained for ash-free substance were:[2]

C	H	S
50.57	7.17	1.17

Preparation No. 6. — The results of the ash analysis of our previous preparations made it very evident that more attention was necessary to the removal of phosphates. Although treatment of the residual substance with dilute acid was effective in removing most of the phosphate held in it, it was impossible to use sufficiently strong acid for the purpose at that point because of the solvent and transforming action of the same on the remaining proteid. It seemed desirable, therefore, to give still more attention to the removal of inorganic matter from the shavings in the first place.

A large quantity of hashed ossein made with 0.5 per cent hydrochloric acid, from which the osseomucoid had been removed and which had been under 0.25 per cent potassium hydroxide for eight months, was washed free of alkali in water and then thoroughly stirred with 0.8 per cent hydrochloric acid at intervals for a day. Much phosphate was removed in this process. The hash was given similar treatment in 0.6 per cent hydrochloric acid, with the same result. A third washing was made in 0.4 per cent hydrochloric acid. Thereafter the hash was washed for several days in 0.2 per cent acid

[1] It should be kept in mind, of course, that the quantities of substance finally obtained do not represent fully the amounts of osseoalbumoid in the tissue. A considerable proportion is transformed into soluble products with the collagen in the hydration process, as well as lost mechanically in purifying.

[2] Our determinations of phosphorus of this and subsequent preparations showed that there is none present in organic combination.

until only traces of phosphate were being removed. At this point the washings did not yield a biuret reaction.

After the acid had been thoroughly washed out, leaving in a readily soluble form[1] such traces of phosphate as might still be in the tissue, the usual hydration process was carried out. The final residual substance had a somewhat gelatinous appearance, just as in the case of preparation No. 4. In this instance, also, the initial hydration was made in the presence of a trace of acid which had not been thoroughly washed out. Only about 0.6 gram of substance was obtained in the process.

The amount of ash in this preparation had been reduced by the improved method to 3.07 per cent. Analytic percentage data obtained for the ash-free substance were:

C	H
50.45	7.24

A microscopic study was made in this connection of the changes in the ossein during the heating process. Samples were taken each day during the ten days that the boiling was continued. Each sample was placed in 70 per cent alcohol after it had been washed in water.

At the end of the first day in the boiling water the fibrous structure of the material still remaining undissolved was but little modified, but much granular matter was present in the hydration fluid. The fibrous structure gradually disappeared, however, and long before the completion of the hydration process practically nothing but small collections of granular matter represented the original structures. An occasional fragment of what appeared to be an elastic fibre could be detected, however.[2]

Preparation No. 7. — Shavings, which had been made seven months previously from ossein obtained in 0.5 per cent hydrochloric acid, were kept in 0.25 per cent potassium hydroxide until ready for use in these experiments. After most of the alkali had been removed with water the hash was washed for several days in hydrochloric acid

[1] This method of concluding the preliminary extractive process with acid had the special advantage, over the previous methods, of transforming tri-basic earthy phosphate into acid modifications. The washing with alkali alone naturally had little or no extractive action on the earthy phosphates, but, on the contrary, tended to convert residual phosphates of calcium and magnesium into fixed forms.

[2] See foot-note, page 343.

increasing in strength to 0.2 per cent. When the acid appeared to be removed by subsequent washing in water the hydration process was begun. The fluid soon acquired an acid reaction, however. This reaction persisted in several of the first warm washings.

The product soon became quite gelatinous. It was very resistant to the further action of the boiling water. Eventually nearly all of the substance went into solution, although the renewed fluids remained neutral. At the end of a week's boiling, daily for about ten hours, too little remained for quantitative analysis.

Preparation No. 8. — The results obtained with preparations Nos. 4, 6, and 7 indicated that the presence of acid, however little it might be in the fluid during hydration, tended to effect transformation into somewhat gelatinous material. It was evident that this substance was not gelatin. At the same time it was clear that it was different from the residue obtained in the absence of acid or in the presence of alkali. That the difference was mainly physical was indicated by the fact that the analytic results for the semi-gelatinous form were essentially the same as for that obtained without the influence of acid in the hydration process. It seemed best to avoid this unnecessary complication, and in this preparation it was accomplished.

About 3 kilos of shavings were freshly prepared from bones treated with 0.5 per cent hydrochloric acid. After removal of the mucoid with lime-water, as usual, the shavings were kept in 0.3 per cent hydrochloric acid for three weeks to remove inorganic matter. The acid was frequently renewed. At the end of this time only a trace of phosphate reaction was obtainable in the acid washings. The acid was very thoroughly removed by repeated washing in cold and warm water. The boiling process in large volumes of frequently renewed water continued for 112 hours. The moist material was flocculent, granular, cream colored, and had no gelatinous qualities.

The usual treatment with acid before boiling in alcohol-ether was omitted. 16.6 grams of purified product were obtained. The ash amounted to only 2.08 per cent. It had the usual brick-red color.

The analytic results for this preparation were as follows :

Carbon and Hydrogen. 0.2032 gm. substance gave 0.3640 gm. CO_2 = 48.86 per cent C, and 0.1251 gm. H_2O = 6.89 per cent H ; 0.2035 gm. substance gave 0.3683 gm. CO_2 = 49.36 per cent C, and 0.1254 gm. H_2O = 6.90 per cent H.

Nitrogen. 0.4184 gm. substance gave 0.06573 gm. N = 15.71 per cent N ; 0.2420 gm. substance gave 0.03803 gm. N = 15.71 per cent N.

Total Sulphur. 0.5012 gm. substance gave 0.0406 gm. $BaSO_4$ = 1.12 per cent S; 0.5050 gm. substance gave 0.0421 gm. $BaSO_4$ = 1.15 per cent S.

Total Phosphorus. 0.4008 gm. substance gave 0.0078 gm. $Mg_2P_2O_7$ = 0.54 per cent P.

Phosphorus of the Ash. 0.0174 gm. Ash gave 0.0100 gm. $Mg_2P_2O_7$ = 0.33 per cent P.

Ash. 0.4850 gm. substance gave 0.0102 gm. Ash = 2.10 per cent Ash; 0.4838 gm. substance gave 0.0099 gm. Ash = 2.06 per cent Ash.

PERCENTAGE COMPOSITION OF THE ASH-FREE SUBSTANCE.

							Average.
C	49.90	50.41	....	....	....	....	50.16
H	7.04	7.04	....	....	....	....	7.04
N	....	....	16.04	16.04	....	....	16.04
S	....	....	....	....	1.14	1.17	1.16
O	....	....	....	....	....	....	25.60

This preparation, after purification and drying, was found to be entirely insoluble in water, 10 per cent sodium chloride, 0.2 per cent hydrochloric acid, and 0.5 per cent sodium carbonate; but slowly soluble in 10 per cent hydrochloric acid and 10 per cent potassium hydroxide. Solution was more rapid in the alkali than in the acid. In all of the reagents except water and sodium chloride, complete solution took place speedily on boiling. Albuminates were formed in this treatment and could be precipitated on neutralization. Part of the sulphur in the substance could be split off on heating with potassium hydroxide and detected as sulphide with lead acetate. The larger proportion of the sulphur was closely united, however.

The substance gave the typical proteid color reactions distinctly and digested in pepsin hydrochloric acid, with a formation of albuminate and proteoses. A small proportion of an albumid-like residue remained undissolved. This was soluble in dilute alkali and insoluble in dilute acid. Peptone could not be detected — probably only traces had been formed from the small quantity of substance used in the test.[1] On decomposition with 2 per cent hydrochloric acid the product failed to yield reducing substance.

Preparation No. 9. — This was made by essentially the same process

[1] Compare the similar results obtained with ligament elastin by RICHARDS and GIES: This journal, 1902, vii, p. 111.

as that for preparation No. 8. The original shavings, about 2 kilos, were washed in acid for about three weeks longer than those of the previous preparation, even after practically no more phosphate could be detected in the extracts. The acid was very completely washed out in cold and warm water before hydration was begun. The boiling process was discontinued at the end of eighty-two hours.

The physical properties of the product were identical with those of preparation No. 8.[1] Between 5 and 6 grams of purified substance were obtained. The ash amounted to only 2.76 per cent. It had the usual brick-red color.

This product was found to be identical, in qualitative chemical characteristics, with preparation No. 8. The results of its quantitative analysis are appended:

Carbon and Hydrogen. 0.1510 gm. substance gave 0.2710 gm. $CO_2 = 48.95$ per cent C, and 0.0944 gm. $H_2O = 6.99$ per cent H; 0.1520 gm. substance gave 0.2709 gm. $CO_2 = 48.61$ per cent C, and 0.0900 gm. $H_2O = 6.63$ per cent H.

Nitrogen. 0.2435 gm. substance gave 0.03847 gm. N = 15.80 per cent N; 0.2715 gm. substance gave 0.04317 gm. N = 15.90 per cent N.

Total Sulphur. 0.5042 gm. substance gave 0.0418 gm. $BaSO_4 = 1.14$ per cent S; 0.5050 gm. substance gave 0.0437 gm. $BaSO_4 = 1.19$ per cent S.

Ash. 0.4007 gm. substance gave 0.0108 gm. Ash = 2.69 per cent Ash; 0.4014 gm. substance gave 0.0114 gm. Ash = 2.84 per cent Ash.

PERCENTAGE COMPOSITION OF ASH-FREE SUBSTANCE.

							Average.
C	50.34	50.00	....	....	....	....	50.17
H	7.19	6.82	....	....	....	....	7.01
N	....	....	16.25	16.35	....	....	16.30
S	....	....	....	....	1.17	1.22	1.19
O	....	....	....	....	....	....	25.33

Conclusions from analytic data. — The summary on the next page shows at a glance the average results of all our elementary analyses. It also brings into comparison the figures for composition of typical preparations of keratin, elastin, collagen, and albumoid.

[1] Preparations Nos. 8 and 9 at this stage very closely resembled the similar products from cartilage to be described farther on.

SUMMARY OF ANALYTICAL RESULTS FOR PERCENTAGE COMPOSITION OF OSSEOALBUMOID.

Preparation.	Ash-free Substance.					Ash.
No.	C	H	N	S	O	
1	49.81	6.68	....	....	....	76.31
2	49.71	6.62	16.11	....	....	46.25
3	49.61	6.70	16.03	1.11	26.55	5.85
4	....	....	....	....	....	....
5	50.57	7.17	....	1.17	....	5.88
6	50.45	7.24	....	....	....	3.07
7	....	....	....	....	....	....
8	50.16	7.04	16.04	1.16	25.60	2.08
9	50.17	7.01	16.30	1.19	25.33	2.76
Average.						
1–7	50.03	6.88	16.07	1.14	25.88	
8–9	50.16	7.03	16.17	1.18	25.46	
1–9	50.07	6.92	16.12	1.16	25.73	
Albumoid[1] .	50.46	7.05	14.95	1.86	25.68	
Albumoid[2] .	53.12	6.80	16.62	0.79	22.67	
Collagen[3] . .	50.75	6.47	17.86	....	24.92	
Keratin[4] . .	49.45	6.52	16.81	4.02	23.20	
Elastin[5] . .	54.14	7.33	16.87	0.14	21.52	

[1] From cartilage. See page 357 of this paper.

[2] From the crystalline lens. MÖRNER: Zeitschrift für physiologische Chemie, 1894, xviii, p. 78.

[3] From gelatin. HOFMEISTER: *Ibid.*, 1879, ii, p. 322.

[4] From white hair. KÜHNE and CHITTENDEN: Zeitschrift für Biologie, 1890, xxvi, p. 291.

[5] From ligamentum nuchae. RICHARDS and GIES: This journal, 1902, vii, p. 104.

The chemical qualities of the albumoid product separated from bone in these experiments indicate that the substance is neither a collagen, a keratin, nor an elastin. This may also be seen from the analytic figures. Unlike the collagens, it does not yield gelatin. It is readily digestible, whereas the keratins are indigestible. It contains an abundance of loosely united sulphur; elastins contain only slight quantities of sulphur, — some of them, no loosely bound sulphur at all. The properties of our product, while somewhat different, as we have said, approach to a certain extent those of the elastins of ligamentum nuchæ[1] or the aorta.[2] They appear to be identical for the most part with those of the albumoid of cartilage.[3]

Since all the albumoids are residual tissue constituents of variable qualities and composition, though of typical resistance to the action of solvents,[4] it seems proper to classify the product we have obtained from bone as an elastin-like albumoid and to refer to it, therefore, as osseoalbumoid. We freely admit that, while our chemical knowledge of the albuminoids remains as slight as at present, such classification has the virtue of only temporary convenience.

No attempt has been made in these experiments to ascertain the exact location of osseoalbumoid in the tissue. It appears probable, however, that the substance is the same as that regarded as keratin by Broesike and which was found by him in the lining of the lacunæ and canaliculi. We are inclined to believe, also, that the elastic fibres of the bone, perhaps also elastic portions of blood-vessels in the Haversian canals, have contributed substance to our preparations.[5] It is possible, of course, that the residual matter prepared by the method we have employed is composed of more than one substance, although the harmony in our analyses, of preparations made by a changeable process, indicates that the products obtained are not admixed to any appreciable extent with variable constituents.

The proportionate amount of osseoalbumoid in bone is small. It

[1] Richards and Gies: *Loc. cit.*

[2] Schwarz: Zeitschrift für physiologische Chemie, 1894, xviii, p. 487.

[3] Mörner: Skandinavisches Archiv für Physiologie, 1889, i, p. 234. See also page 357 of this paper.

[4] Cohnheim: Chemie der Eiweisskörper, 1900, p. 299.

[5] Recent staining methods show that bone contains very little elastic material. See Abstract of Melnikow-Raswedenkow's paper, in American Medicine, 1901, ii, p. 466.

appeared somewhat greater, however, than the quantity of the corresponding constituent of cartilage.[1]

Chondroalbumoid.

The qualities of the albumoid obtained from bone were found to be so nearly the same as those ascribed to the albumoid in cartilage that a comparative study of the latter body appeared to be particularly desirable in this connection.

Historical. — It will be recalled that in his classical researches on the constituents of hyaline cartilage, Mörner[2] separated a product which he considered an albumoid. This body was a residual substance obtained from the tracheal cartilages of the ox after complete hydration of the collagenous elements in boiling water in a Papin's digestor at 110–120° C.

The substance obtained in this way was entirely insoluble in 1 per cent potassium hydroxide, but slightly soluble in 5 per cent solution of the same reagent. It was readily soluble in boiling 0.1 per cent alkali. It digested completely, with a formation of albuminate, proteose, and peptone. It contained considerable loosely united sulphur, but did not yield reducing substance on decomposition with acid.[3] Its resemblance to keratin and elastin in some respects, and its difference from them in others, made it necessary for Mörner to consider it a proteid of the indefinite albumoid type.

The quantities of albumoid obtained in Mörner's experiments were too small to offer favorable opportunity for elementary analysis. He transformed into albuminate such material as was available, however, for the sake of removing insoluble extraneous matter, and then determined the nitrogen content of the derived products. In two determinations the alkali albuminate made with boiling 0.1 per cent potassium hydroxide contained 15.87 per cent nitrogen; that made with boiling 0.5 per cent potassium hydroxide had 16.02 per cent. Neither of these results was for ash-free substance, the ash not having been determined. The nitrogen content, also not ash-free, of one preparation, made in boiling 0.5 per cent hydrochloric acid, was 15.43 per cent. Mörner concluded that the albumoid itself has a content of nitrogen ranging between 15 and 16 per cent.

[1] Further reference to osseoalbumoid is made on page 357.

[2] Mörner: *Loc. cit.*

[3] Compare with the results of our analysis of osseoalbumoid, page 353.

Nothing further has been done to determine the characters of chrondroalbumoid. When we recall that albuminates are products in which the proportion of nitrogen is usually different from its proportion in the substance from which the albuminates are derived, particularly when obtained with *boiling* reagents, it is obvious that Mörner's analytic results tell us very little about the composition of the original body.

The substance identified by Mörner was absent from the tracheal cartilages (the only ones examined) of calves. Mörner concludes, from this fact, that immature cartilage is essentially different from the mature form of the tissue in its lack of the albumoid constituent. This conclusion is based on only a few observations. If, however, it is found later to be correct, the fact that osseoalbumoid appears to be present in bone in greater proportion than in cartilage from the same animal would suggest that, in the development of bone from cartilage, the proportion of the albumoid constituent increases.

Method of preparation. — In these experiments we used the cartilaginous portion of the nasal septum of the ox. Several pounds of these pieces of typical cartilage, about ten inches long and three inches wide, were used. The outer membranes were removed, the pure cartilage put through a hashing machine, the resultant hash thoroughly washed in running water; mucoid, nucleo-proteid, etc., thoroughly eliminated in several extractions with dilute alkali after preliminary treatment with 0.1–0.2 per cent hydrochloric acid; and the alkali-free residue thoroughly hydrated in boiling water for several days under conditions identical with those for the preparation of osseoalbumoid. The final product was also extracted with 0.1 per cent sodium carbonate and 0.5 per cent hydrochloric acid in which the substance seemed to be entirely insoluble.

The physical appearance of the final products was practically identical with that of preparations Nos. 8 and 9 of the albumoid from bone. It accorded also with the appearance of the material described by Mörner.

Records of analysis. — After purification in boiling alcohol-ether, as usual, the following analytic results were obtained for the two preparations made by us:

Preparation A.

Carbon and Hydrogen. 0.1998 gm. substance gave 0.3542 gm. CO_2 = 48.35 per cent C, and 0.1200 gm. H_2O = 6.72 per cent H; 0.2008 gm. sub-

stance gave 0.3538 gm. CO_2 = 48.06 per cent C, and 0.1202 gm. H_2O = 6.70 per cent H.

Nitrogen. 0.1929 gm. substance gave 0.02786 gm. N = 14.44 per cent N; 0.2365 gm. substance gave 0.03396 gm. N = 14.36 per cent N.

Total Sulphur. 0.3028 gm. substance gave 0.0393 gm. $BaSO_4$ = 1.79 per cent S.

Total Phosphorus. 0.2821 gm. substance gave 0.0010 gm. $Mg_2P_2O_7$ = 0.10 per cent P.

Phosphorus of the Ash. 0.0295 gm. Ash gave 0.0012 gm. $Mg_2P_2O_7$ = 0.06 per cent P.

Ash. 0.1998 gm. substance gave 0.0076 gm. Ash = 3.80 per cent Ash; 0.2008 gm. substance gave 0.0070 gm. Ash = 3.44 per cent Ash.

Percentage Composition of the Ash-free Substance.

					Average.
C	50.16	49.87	....	....	50.02
H	6.99	6.95	....	....	6.97
N	....	....	14.98	14.90	14.94
S	....	....	....	...	1.85
O	....	....	....	....	26.22

Preparation B.

Carbon and Hydrogen. 0.2019 gm. substance gave 0.3644 gm. CO_2 = 49.22 per cent C, and 0.1254 gm. H_2O = 6.95 per cent H; 0.2027 gm. substance gave 0.3679 gm. CO_2 = 49.50 per cent C, and 0.1250 gm. H_2O = 6.90 per cent H.

Nitrogen. 0.4331 gm. substance gave 0.06276 gm. N = 14.49 per cent N; 0.4343 gm. substance gave 0.06307 gm. N = 14.52 per cent N.

Total Sulphur. 0.5028 gm. substance gave 0.0661 gm. $BaSO_4$ = 1.81 per cent S; 0.5034 gm. substance gave 0.0665 gm. $BaSO_4$ = 1.82 per cent S.

Ash. 0.4000 gm. substance gave 0.0120 gm. Ash = 3.02 per cent Ash; 0.4009 gm. substance gave 0.0121 gm. Ash = 3.02 per cent Ash.

Percentage Composition of the Ash-free Substance.

							Average.
C	50.76	51.04	....	....	....	....	50.90
H	7.17	7.12	....	....	....	....	7.14
N	....	....	14.94	14.97	....	....	14.96
S	....	....	....	....	1.86	1.87	1.86
O	....	....	....	....	....	....	25.14

These preparations possessed the same reactions as those summarized by us on page 354 from Mörner's paper, and also those referred to in some detail in connection with preparations Nos. 8 and 9 of our osseoalbumoid. The reactions for loosely bound sulphur were, however, very much stronger for the cartilage preparations than for those prepared from the femur. On the other hand, sulphur obtainable from chondroalbumoid, on boiling with 2 per cent hydrochloric acid, appeared to be less in comparative tests than for the bone products.

The following summary brings into contrast the analytic averages for the albumoid products from both sources :

PERCENTAGE COMPOSITION OF ALBUMOIDS FROM CARTILAGE AND BONE.

Elements.	Chondroalbumoid.			Osseoalbumoid.
	Preparation A.	Preparation B.	Average A–B.	Average. Preparations 8–9.
C	50.02	50.90	50.46	50.16
H	6.97	7.14	7.05	7.03
N	14.94	14.96	14.95	16.17
S	1.85	1.86	1.86	1.18
O	26.22	25.14	25.68	25.46

Conclusions from the data of analysis. — The properties of this substance are found to be those ascribed to it by Mörner. That it is not exactly the same as osseoalbumoid is indicated by its higher content of sulphur and its considerably lower content of nitrogen. The larger proportion of sulphur obtainable from it on cleavage with alkali has already been referred to.

These differences are not sufficient, however, to prevent the conclusion that the two substances are closely related members of the same class of proteids.

The relative amount of the substance in cartilage appears to be less, as we have already said, than the proportion of osseoalbumoid in bone.[1]

[1] For facts regarding location of albumoid in cartilage see MÖRNER'S paper, *Loc. cit.*

Summary of Conclusions.

1. Osseous tissue contains a residual proteid substance, obtainable after hydration of the collagen, which is neither keratin nor typical elastin, although it resembles the latter body.

This substance is present in bone in only comparatively small proportion, though apparently in greater relative quantity than the corresponding constituent of cartilage.

The average percentage elementary composition of the purest products was found to be as follows, calculated for ash-free substance:[1]

C	H	N	S	O
50.16	7.03	16.17	1.18	25.46

The analyzed products were free from organic phosphorus.

The substance appears to be very similar to some of the albumoids, particularly to that from cartilage. It has therefore been termed osseoalbumoid.

No attempts have been made to ascertain its location in the tissue, but it appears to be identical with the substance referred to erroneously by Broesike as keratin and found by him in the lining of the lacunæ and canaliculi. It is possible, also, that the elastic fibres of the bone have contributed substance to the preparations.

2. Further investigation of the qualities of chrondroalbumoid confirmed most of Mörner's conclusions regarding it.

In addition, its elementary composition has been determined, with the following percentage results for ash-free substance:

C	H	N	S	O
50.46	7.05	14.95	1.86	25.68

This product is likewise devoid of phosphorus in organic combination.

[1] Average of preparations Nos. 8 and 9, our purest products. See page 349.

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THE COMPOSITION OF YELLOW FIBROUS CONNECTIVE TISSUE.[1]

By G. W. VANDEGRIFT and WILLIAM J. GIES.

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Historical.

MOST of the animal tissues have been carefully analyzed and their general composition determined. We have not been able to find any record of such chemical study of ligament, however. Gorup-Besanez[2] mentions the fact that a few determinations of the composition of the middle coat of arteries, and several other forms of connective tissue containing elastic fibres, have been made, according to which the percentage of water varies between 57.5 per cent and 75.9 per cent. He doubtless refers to such incomplete analyses as those of the tunica intima and tunica media of the carotid artery, made by Schultze and quoted by Gautier,[3] as follows:

	Per cent.
Water	69.30
Elastin (including collagenous and cellular elements)	18.65
Other albuminoids	8.72
Extract in water-alcohol	2.27
Soluble salts	0.74
Insoluble salts	0.34

The functions of elastic tissues appear to be mainly of a mechanical nature, and there has been little to suggest that such forms of connective tissue as ligament contribute anything important in substance or effect to metabolism. Probably the seeming passivity, in the metabolic sense, of ligament and allied structures accounts for the lack of chemical attention they have received.

During Liebig's time, when elementary analysis was expected to throw much light on those transformations in the body which we now

[1] Reported, in part, before the American Association for the Advancement of Science, June, 1900: Proceedings, 1900, p. 123.

[2] Gorup-Besanez: Lehrbuch der physiologischen Chemie, 1878, p. 649.

[3] Gautier: Leçons de chimie biologique normale et pathologique, 1897, p. 297.

speak of as anabolic and catabolic, many of the tissues were given extended study.[1] Liebig, Scherer, Mulder, and many others, in those days, determined the elementary composition of muscle, blood, hair, cartilage, bone, tendon, and practically all of the other body parts (after desiccation), and gave empirical formulæ to these tissues just as they did to pure chemical substances. They deduced from these formulæ relationships and differences which were not particularly in harmony with observed functions, and which have not been borne out by subsequent research.

Scherer[2] determined the elementary composition of the dried middle coat of arteries. To this elastic tissue he ascribed the formula $C_{48}H_{76}N_{12}O_{16}$. Bergh[3] and Schwarz[4] have since made and analyzed several pure preparations of elastin from the aorta. The latter's studies of the composition and reactions of aorta elastin have led him to conclude that it is identical with the elastin of ligamentum nuchæ. The averages of the analytic percentage results obtained by these observers are here brought in contrast:

		C	H	N	S	O
Scherer.[5]	Tunica media	53.49	7.03	15.36	..	24.04
Schwarz.[6]	Purified aorta elastin . .	54.34	7.08	16.79	0.38	21.41
Bergh.	Purified aorta elastin . .	53.99	7.54	15.20	0.60	22.67

These results are sufficiently close in agreement to indicate chemically, as has been found histologically, that the tunica media of the main arteries is largely composed of elastin.

The earliest results of similar analysis which relate to ligament are, so far as we have been able to find, those obtained by Tilanus[7] and Müller[8] for ligamentum nuchæ, after extraction with water, alcohol, and ether by the first observer and with acetic acid, in addition, by the second. Tilanus gave his prepared tissue the formula $C_{52}H_{80}N_{14}O_{14}$. Numerous investigators have since analyzed elastin from the cervical

[1] Liebig: Die organische Chemie in ihrer Anwendung auf Physiologie und Pathologie, 1842, p. 320 *et seq.*

[2] Scherer: Annalen der Chemie und Pharmacie, 1841, xl, p. 1.

[3] Bergh: Zeitschrift für physiologische Chemie, 1898, xxv, p. 337.

[4] Schwarz: *Ibid.*, 1894, xviii, p. 487.

[5] Phosphorus and sulphur were not determined, but included (by difference) in the figures for oxygen.

[6] Compare with the analyses by Chittenden and Hart, p. 289.

[7] Tilanus: See Mulder, Versuch einer allgemeinen physiologischen Chemie, zweite Hälfte, 1844–51, p. 595.

[8] Müller: See Gorup-Besanez, *loc. cit.*, p. 140.

ligament, prepared by essentially the same process, but with more elaborate extractions. Comparison is made, in the following summary, of the latest analyses with Tilanus's and Müller's average results:

		C	H	N	S	O
Tilanus.[1]	Prepared ligament .	54.98	7.31	17.52	0.33	19.86
Müller.	Crude elastin . .	55.46	7.41	16.19	..	20.94
Chittenden and Hart.[2]	Pure elastin . . .	54.08	7.20	16.85	0.30	21.57

Analyses of Ligamentum Nuchæ.

In the analyses here to be described the results were obtained with ligamentum nuchæ, — a ligament composed in great part of yellow fibres and representing, perhaps better than any other part of the body, true elastic connective tissue.

Proportions of water, solids, organic and inorganic matter. — *Method of determination.* Perfectly fresh bloodless ligaments, taken from the animals immediately after their slaughter, were used. Within a few hours after removal from the body all adherent connective tissue was carefully cut off. The cleaned ligament was then divided into strips and very thin particles cut, from only the deeper portions of these, with scissors into weighed porcelain crucibles. This division of the tissue was made as minute as possible, and the process was carried out with the utmost rapidity to prevent loss of water by evaporation before the weight of tissue in use was determined. The weight of fresh tissue taken was determined by difference. The substance was then dried at 100–110° C. to constant weight, after which incineration was carefully conducted over a very low flame until all carbon was burned out and constant weight attained. No special difficulty was experienced in effecting complete combustion of the carbon over an ordinary Bunsen burner.

Analytic results. The tables on page 290 summarize the results of the general analyses of ligamentum nuchæ from the ox and calf.

Comparative results. — The data on page 290 show that the ligament of the full grown animal contains relatively less water and inorganic matter, and more solid substance and organic matter, than that of the calf, facts which are in entire agreement with comparative

[1] Phosphorus was not determined, but included in the figures for oxygen.

[2] Chittenden and Hart: Studies from the laboratory of physiological chemistry, Yale University, 1887–88, iii, p. 22. Compare with Schwarz's figures, p. 288.

Ox ligament.							
Number.	Ligament used.	Percentage of fresh tissue.				Percentage of solids.	
	Grams.	Water.	Solids.	Organic matter.	Inorganic matter.	Organic matter.	Inorganic matter.
1	5.47	59.34	40.66	40.26	0.40	99.02	0 98
2	4.34	60.34	39.66	39.28	0.38	99.06	0.94
3	7.89	58.58	41.42	40.86	0.56	98.65	1.35
4	8.96	58.46	41.54	41.11	0.43	98.96	1.04
5	7.64	56.36	43.64	43.18	0.46	98.94	1.06
6	4.49	57.37	42.63	42.13	0.50	98.83	1.17
7	4.22	56.32	43.68	43.17	0.51	98.85	1.15
8	3.22	55.39	44.61	44.17	0.44	99.01	0.99
9	3.29	58.10	41.90	41.45	0.45	98.93	1.07
10	3 94	56.42	43.58	43.05	0.53	98.79	1.21
11	3.92	56.55	43.45	42.96	0.49	98.89	1.11
Averages	5.22	57.57	42.43	41.96	0.47	98.90	1.10
Calf ligament.							
1	11.00	66.24	33.76	33.04	0.72	97.88	2.12
2	8.78	65.34	34.66	33.98	0.68	98.04	1.96
3	7.49	64.61	35.39	34.71	0.68	98.09	1.91
4	7.10	64.72	35.28	34.62	0.66	98.14	1.86
5	7.19	64.59	35.41	34.83	0.58	98.36	1.64
Averages	8.31	65.10	34.90	34.24	0.66	98.10	1.90

analytic results for other tissues of growing and mature animals. The summary on the opposite page contrasts the above average percentage figures with those for morphologically related parts:

	Ligament.		Vitreous humor.[1]	Costal cartilage.[2]	Bone with marrow.[3]	Adipose tissue; kidney fat.[4]
	Calf.	Ox.				
Fresh tissue.						
Water.	65.10	57.57	98.64	67.67	50.00	4.30
Solids.	34.90	42.43	1.36	32.33	50.00	95.70
Organic matter.	34.24	41.96	0.48	30.13	28.15	95.51
Inorganic matter.	0.66	0.47	0.88	2.20	21.85	0.19
Dry tissue.						
Organic matter.	98.10	98.90	35.29	93.20	56.30	99.80
Inorganic matter.	1.90	1.10	64.71	6.80	43.70	0.20

Inorganic matter. — The ash of ligamentum nuchæ contains chloride, phosphate, carbonate, and sulphate; also, sodium, potassium, calcium, magnesium and iron, the latter arising in all probability from minute quantities of blood held in the tissue capillaries.

Sulphate. — The sulphate reaction in our preliminary tests was decided enough to suggest unusual quantity. In numerous samples of ash obtained by burning in porcelain crucibles directly over gas flames we found 8.04 to 9.20 per cent of SO_3. Mörner[5] has lately called attention, in connection with the SO_3 content of bone ash, to the well known fact that, during incineration directly over an ordinary burner, sulphur is introduced in considerable proportion from the consumed gas. In ash made by incineration in platinum dishes over alcohol flames, however, we obtained the following results for SO_3, which were determined, in 0.2 to 0.6 gram portions after solution in hot dilute hydrochloric acid, by the usual barium chloride method:

[1] Representing jelly-like connective tissue. Analyses by LOHMEYER, source of material not specified. See GORUP-BESANEZ: *Loc. cit.*, p. 401.

[2] Human. Analyses by HOPPE-SEYLER. See KÜHNE: Lehrbuch der physiologischen Chemie, 1868, p. 387.

[3] Average of many analyses of various human bones before removal of marrow. HOPPE-SEYLER: Physiologische Chemie, 1881, p. 625.

[4] From the ox. ATWATER: Methods and results of investigations on the chemistry and economy of food, 1895, p. 34.

[5] C. TH. MÖRNER: Zeitschrift für physiologische Chemie, 1897, xxiii, p. 311.

Percentage of SO_3 in ligament ash.

	1	2	3	4	Averages.
A	5.58	5.66	5.61	....	5.62
B	5.80	5.71	5.46	5.61	5.64
C	5.71	5.50	5.79	5.66	5.67
				General average . .	5.64

The above results are significant when compared with the following percentage figures for content of sulphuric acid in the ash of the tissues and fluids specified :[1]

Bone [2]	0.02	Liver	0.92	Serum	2.10	Bile	6.39
Muscle [3]	0.30	Lungs	1.40	Spleen	2.54	Cartilage [4]	37.47
Brain	0.75	Blood	1.67	Milk	2.64		

The unusually large proportion of SO_3 found in ligament ash undoubtedly arises from an organic source. The ash of blood and lymph, it will be seen, contains much less in proportion, as does also that of all the other tissues except cartilage. Attention has lately been called to the fact that mucin is contained in ligament in appreciable quantity.[5] We shall presently show that its percentage amount is about half that in tendon.[6] Mucin contains ethereal sulphuric acid, in a radicle very similar to, if not identical with, chondroitin sulphuric acid.[7] This latter body, and chondromucoid containing it, doubtless contribute the surprisingly large proportion of SO_3 to cartilage ash.[8]

[1] Most of these are taken from SCHÄFER'S Text-book of Physiology, 1898, i, p. 77.

[2] C. TH. MÖRNER: *Loc. cit.*

[3] WEBER: Quoted from HOPPE-SEYLER, Physiologische Chemie, 1881, p. 651.

[4] Calculated from HOPPE-SEYLER'S analyses as given by KÜHNE, Lehrbuch der physiologischen Chemie, 1868, p. 387.

[5] RICHARDS and GIES: Proceedings of the American Physiological Society, This journal, 1900, iii, p. v; also, *Ibid.*, 1901, v, p. xi.

[6] The greatest amount thus far obtained from normal ox tendon was 1 per cent. CHITTENDEN and GIES: The journal of experimental medicine, 1896, i, p. 186.

[7] LEVENE: Zeitschrift für physiologische Chemie, 1901, xxxi, p. 395.

[8] Bone ash contains only a trace, which has also been attributed to constituent chondroitin sulphuric acid. See C. Th. MÖRNER: *Loc. cit.*; also, BIELFELD: Zeitschrift für physiologische Chemie, 1898, xxv, p. 350.

The unusual percentage of SO_3 in ligament ash must, it appears to us, be attributed, in much the greater part, to a similar source — that is, to the SO_3 radicle of the mucin, which, on burning, is transformed, in part at least, to sulphate.

Phosphate and chloride. — In view of the excessive amount of derived sulphate, determinations of the percentage quantity of other constituents in ligament ash could not be expected to give exact figures for proportionate content of inorganic matter in the fresh tissue. We have, however, determined phosphoric acid and chlorine, which appear to make up the bulk of the acid radicles. The former was determined by Mercier's modification of Neubauer's method,[1] in neutralized extracts of 0.5–0.8 gram of ash in 100 c.c., made by prolonged treatment with hot dilute hydrochloric acid. The latter was estimated by Mohr's method,[2] in aqueous extracts of 0.4–0.7 gram of ash in 100 c.c., made by continued heating on the water bath. The following percentage results were obtained:

		1	2	3	Average.
A.	P_2O_5	7.46	7.09	7.61	7.39
B.	Cl	29.16	28.91	28.79	28.95

These figures are all within the customary variations observed for other tissues. They suggest, of course, that chlorides are the predominant substances in the ash of ligament.[3]

Fat (ether-soluble matter). — Dormeyer's method[4] was used in these determinations. The percentage of water was ascertained for each sample dried to constant weight, and extraction of fat made from the pulverized dry material in quantities varying from 18 to 35 grams. The tissue used was taken from only the inner portions of the ligaments. The following percentage results were obtained:

	1	2	3	4	5	6	Average.
Fresh tissue.	1.26	0.94	1.03	1.45	0.89	1.17	1.12

The proteid constituents. — The chief organic substance in ligamentum nuchæ has long been known to be elastin. After Rollett's[5]

[1] Neubauer und Vogel: Analyse des Harns, zehnte Auflage, 1898, p. 731.

[2] *Ibid.*, p. 708.

[3] Bone contains only traces of chlorine (0.19% in the ash). Cartilage ash contains 3.70% of chlorine. See Halliburton in Schäfer's Text-book of Physiology, 1898, i, pp. 112 and 113.

[4] Dormeyer: Jahresbericht über die Fortschritte der Thier-Chemie, 1896, xxvi, p. 42.

[5] Rollett: Untersuchungen zur Naturlehre des Menschen und der Thiere (Moleschott), 1859, vi, p. 1. Also *Ibid.*, 1860, vii, p. 190.

researches on the structure of connective tissue, particularly tendon, it was assumed by various observers [1] that ligament contains representatives of the various proteids which Rollett identified. It was only recently, however, that particular attention was called to the fact that this representative of yellow fibrous tissue contains appreciable quantities of coagulable proteid, glucoproteid and extractives.[2] The quantities in which these substances are present make it probable that they are integral components of the tissue and not merely constituents of retained blood and lymph. Even after the finely divided tissue has been well washed in water, a process calculated to remove practically all lymph, these substances may still be separated from it in relatively large amount.

Coagulable proteid (*albumin, globulin*). The fresh cleaned tissue was cut into strips and these quickly torn into delicate shreds with forceps. 50–100 grams of the fibrous material were extracted, in each determination, with 200 c.c. of 1.25–5.0 per cent solution of sodium chloride, at room temperature for from three to four days. Powdered thymol prevented putrefactive changes. At the end of that time the extract was pressed through cloth, filtered, and the tissue thoroughly washed with water. The extract and washings were then heated to boiling. The coagulable proteids were completely precipitated on addition of a very small quantity of dilute acetic acid.[3] The precipitate was filtered on weighed papers, washed free from chloride with water, and the coagulated proteid determined gravimetrically after drying to constant weight at 100–110° C. The following percentage results were obtained in six determinations with samples from as many ox ligaments:

	1	2	3	4	5	6	Average.
Fresh tissue.	0.588	0.502	0.598	0.652	0.652	0.704	0.616

Mucin. — Rapidly shredded ligament, prepared as for the determinations of coagulable proteid, in portions of 100 grams, was extracted, with repeated shaking, in 250 to 300 c.c. half-saturated lime water for several days at room temperature. The glucoproteid was completely precipitated from the extract and washings on acidification with 0.2 per cent HCl. Its amount was determined, after filtering on weighed paper and washing free from soluble proteid and chloride,

[1] KÜHNE: *Loc. cit.*, p. 363.

[2] RICHARDS and GIES: *Loc. cit.*

[3] The amount of acid added was too slight to precipitate any mucin that may have been dissolved by the sodium chloride.

by drying at 110° C. and weighing. The following percentage results were obtained with ox ligament taken from as many animals:

	1	2	3	4	5	6	7	Average.
Fresh tissue.	0.565	0.429	0.539	0.510	0.490	0.574	0.569	0.525

Elastin. — Finely divided ox ligament from several animals, in quantities of 16 to 50 grams, after thorough extraction in 5 per cent sodium chloride solution was boiled in excess of water, with repeated renewal, until all collagenous fibres were removed by gelatinization and only very slight turbidity with tannic acid was obtainable in the cold concentrated filtrate. The undissolved residue was filtered on weighed papers, thoroughly washed free from traces of dissolved proteid and chloride, dried at 110° C. to constant weight and the percentage of elastin calculated from the weight obtained, with the following results:[1]

	1	2	3	4	Average.
Fresh tissue.	31.24	32.96	31.51	30.99	31.67

Collagen. — Eulenberg[2] observed long ago that ligamentum nuchæ yields gelatin on boiling. In these experiments the percentage content of collagen, in the form of gelatin, was determined gravimetrically. Weighed quantities, 20–40 grams, of finely divided fresh ox ligament were thoroughly extracted in half-saturated lime-water for several days at room temperature, for removal of albumin, globulin, mucin and extractives. Excess of calcium hydroxide was removed by washing in water. The tissue was then washed in alcohol and ether to remove fat, and finally boiled, in fresh portions of water, until only the merest turbidity could be obtained in small amounts of cold concentrated filtrate on addition of tannic acid. This process usually required six to ten hours. By this time all of the collagen was gelatinized and very little elastin hydrated. The filtrates were evaporated on the water bath in weighed crucibles, the residues dried at 100–110° C. to constant weight and gelatin determined, after subtraction of the ash obtained by burning the residue over a low flame, with the following percentage results:[3]

[1] This residue consists, strictly, of substances insoluble after such treatment. Only traces of non-elastin material could still be present, however — quantities too small to materially affect the results. Furthermore, a correspondingly small amount of elastin was probably lost by hydration.

[2] Eulenberg: See Schultze, Annalen der Chemie und Pharmacie, 1849, lxxi, p. 277.

[3] This method is, of course, open to the objection that possibly hydration pro-

	1	2	3	4	5	6	Average.
Fresh tissue.	7.61	6.77	7.38	6.99	7.13	7.52	7.23

Extractives. — Creatin and nuclein bases were detected qualitatively in aqueous extracts of large quantities of ligaments after removal of proteids and salts in the usual way, in confirmation of previous observations in this laboratory,[1] but no attempt was made to determine their quantity nor the character of the individual alloxuric bodies. In the summary below, extractives are included with the figures for "undetermined substance," which were obtained by difference.

Average composition. — The results of all our analyses are summarized in the following table, which gives the average percentage composition of fresh ligamentum nuchae and of the dry solid matter contained in it, and also the results of partial analysis of the ash:

Percentage composition.	Fresh ligament.		Dry ligament.		Ash.
	Calf.	Ox.	Calf.	Ox.	Ox.
Water.[2]	65.10	57.570			
Solids.	34.90	42.430			
Inorganic matter.	0.66	0.470	1.90	1.100	
SO_3.	. . .	0.026	. . .	0.062	5.64
P_2O_5.	. . .	0.035	. . .	0.081	7.39
Cl.	. . .	0.136	. . .	0.318	28.95
Organic matter.	34.24	41.960	98.10	98.900	
Fat (ether-soluble matter).	. . .	1.120	. . .	2.640	
Albumin, globulin.	. . .	0.616	. . .	1.452	
Mucin.	. . .	0.525	. . .	1.237	
Elastin.	. . .	31.670	. . .	74.641	
Collagen (gelatin).	. . .	7.230	. . .	17.040	
Extractives and undetermined substance.	. . .	0.799	. . .	1.883	

ducts of the elastin increased the quantity of gelatin. In reality, however, such increase is insignificant when the hydration is carefully conducted and is probably

[1] RICHARDS and GIES: *Loc. cit.*

[2] The quantity of water in "elastic tissue" given, from BEAUNIS' Physiologie

just about equal in amount to the loss of gelatin in the removal tests with tannic acid. EWALD and KÜHNE (Jahresbericht der Thier-Chemie, 1877, p. 281) found that collagen is not digested by the proteolytic enzyme of pancreatic juice unless it has been previously swollen by acid or hot water, whereas most other proteids (including those we have found in the ligament), are digested without such preliminary treatment. We might have determined collagen directly by this process, perhaps, but we believe the one employed, a modification of HOPPE-SEYLER'S method (Handbuch der physiologisch- und pathologisch-chemischen Analyse, 1893, p. 482), gave results quite as accurate as could be obtained by the former or any other.

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humaine, by HALLIBURTON (A Text-book of chemical physiology and pathology, 1891, p. 58) is 49.6%. The particular source of the tissue is not stated. This amount is lower than that for any of the connective tissues to which GORUP-BESANEZ referred (see page 287), and less than any others we have found recorded for particular forms of elastic tissue.

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THE CHEMICAL CONSTITUENTS OF TENDINOUS TISSUE.[1]

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IN a previous paper from this laboratory[2] the results were given of some analyses of yellow elastic tissue, represented by the ligamentum nuchæ of the ox and calf. So little attention has been given by chemists to structures such as tendon, which possess mainly mechanical functions, that it seemed to us desirable to investigate in a similar study the general composition of white fibrous connective tissue.

Historical.

Early in the last century, when it was assumed that elementary composition determined not only definite chemical relationships, but indicated similarities and differences in development as well as function, the tissues were carefully subjected to elementary analysis. Like a number of the other parts of the body, tendon, in the fresh condition, was looked upon as consisting of practically a single organic substance (collagen) holding water mechanically, and admixed with slight quantities of saline matter and other blood and lymph constituents.[3]

Scherer[4] analyzed several forms of gelatin-yielding fibrous tissues. On the next page we give the results of his elementary analysis of calf-tendon. The tissue was prepared for analysis by preliminary maceration and extraction in dilute saline solution. Subsequently the residue was washed in water and then in boiling alcohol and ether. To this residue, "collagen," Scherer ascribed the formula $C_{48}H_{82}N_{15}O_{18}$.

[1] Some of these results were given at the New York meeting of the American Association for the Advancement of Science, June, 1900: Proceedings, 1900, p. 123.

[2] Vandegrift and Gies: This journal, 1901, v, p. 287.

[3] See references to collagen content on page 230.

[4] Scherer: Annalen der Chemie und Pharmacie, 1841, xl, p. 46.

Marchand,[1] who pointed out a number of defects in Scherer's work, subjected dried tendons from the foot of the calf to similar analysis. The results given below for ash-free substance led him to ascribe to this "collagen" the formula $C_{40}H_{62}N_{12}O_{15}$. He also calculated its molecular weight from this formula, expressing it with the figures 5937.5. The composition of the ash-free hydrated tendon ("gelatin"), taken from the same source, was found by Marchand to accord very well with the average analytic results of similar products, from bone and other tissues, obtained by Mulder.[2] The latter observer gave the gelatin-yielding tissues (dry) the formula $C_{13}H_{20}N_4O_5$.

Winkler's[3] analysis of the tendon of the cow, after extraction in cold water and later in boiling alcohol and ether, led to similar results.

The following summary gives the analytic averages referred to above:[4]

		C	H	N	O
SCHERER.	Crude tendon collagen	50.51	7.16	18.37	23.96
MARCHAND.	Dry calf tendon	50.27	6.77	17.88	25.08
MARCHAND.	Crude tendon gelatin	50.02	6.82	18.00	25.16
MULDER.	Crude bone gelatin	50.37	6.33	17.95	25.35
WINKLER.	Crude tendon collagen	49.68	6.64	17.94	25.74
	Average	50.17	6.74	18.03	25.06

These close agreements in analytic figures naturally suggested to the earlier observers that the chief organic substance of bone, tendon, and related forms was the same in each; further, that "gelatin" and "collagen" were very nearly if not altogether isomeric.[5] In the light of modern chemical knowledge, however, these analytic harmonies emphasize the lack of information which elementary analysis of tissues furnished on the characters and qualities of the various constituents. Definite separation of the tissue-forming substances, however, and subsequent detailed analysis of them individually has increased our appreciation of the important parts the numerous constituents of the body play in the maintenance of its functions.

[1] MARCHAND: Lehrbuch der physiologischen Chemie, 1844, p. 166.

[2] MULDER: Versuch einer allgemeinen physiologischen Chemie, erste Hälfte, 1844–51, p. 333.

[3] WINKLER: Quoted by Mulder, *loc. cit*, zweite Hälfte, p. 583.

[4] The small amounts of phosphorus and sulphur detected in these substances at this time were attributed to inorganic impurity. Oxygen was calculated by difference, and the figures for it therefore include organic phosphorus and sulphur.

[5] HOFMEISTER has since shown, and it is now generally understood, that gelatin is the hydrate of collagen: Zeitschrift für physiologische Chemie, 1878–79, ii. p. 299.

Aside from the above elementary analyses, and a few others of similar character in close agreement with them,[1] practically nothing has been done to determine quantitatively the composition of tendinous tissue. Several observers have determined the proportion of ash.[2] Gorup-Besanez[3] states that a few determinations of water and solid matter in connective tissues, containing collaginous fibres in abundance, have been made, which show a variable content of water ranging between 57.5 and 78.9 per cent of the fresh tissue.[4] Beaunis, in the table presented by Halliburton,[5] gives the average proportion of water in "connective tissue" as 79.6 per cent; but this does not refer to tendon.[6]

Analyses of Tendo Achillis.

Material and methods of analysis. — In the work described in this paper the Achilles tendons of the ox and the calf were employed. The Achilles tendon is easily separated from extraneous matter. It is more completely collaginous and contains relatively less elastin than is found in any other tendinous tissue available for such work. It may be regarded as the best representative of white fibrous connective tissues.

This research followed so closely the plan of our previous study[7] that it is needless to describe in detail the methods of analysis. The details of procedure not mentioned here may be understood to correspond with those given by Vandegrift and Gies.

The main shaft of the tendon was used in each experiment. Occasionally small portions of the bifurcations were employed with parts of the former.[8] Only perfectly white tendons were analyzed. Any tendons showing bloody lines superficially or internally were rejected. Usually the tendons were rapidly cut into very thin cross sections of

[1] Gorup-Besanez: Lehrbuch der physiologischen Chemie, 1878, p. 142.

[2] See page 223. Also foot-note, page 225.

[3] Gorup-Besanez: *Loc. cit.*, p. 649.

[4] See Chevreul's results; given by Marchand: *Loc. cit.*, p. 164.

[5] Halliburton: Text-book of chemical physiology and pathology, 1891, p. 58.

[6] Results of analyses of various non-tendinous tissues containing collaginous fibres, such as the cornea, are not strictly comparable in this connection and are therefore not given here.

[7] Vandegrift and Gies: *Loc. cit.*

[8] See Cutter and Gies: This journal, 1901, vi, p. 157.

GENERAL COMPOSITION.

No.	Tendon used.	Percentage of fresh tissue.				Percentage of solids.	
	Grams.	Water.	Solid matter.			Organic matter.	Inorganic matter.
			Total.	Organic.	Inorganic.		
Ox Tendon.							
1	5.03	61.55	38.45	37.97	0.48	98.74	1.26
2	7.05	63.20	36.80	36.20	0.60	98.38	1.62
3	5.65	62.34	37.66	37.16	0.50	98.67	1.33
4	5.80	63.58	36.42	35.92	0.50	98.62	1.38
5	5.91	62.02	37.98	37.58	0.40	98.54	1.46
6	4.49	65.05	34.95	34.40	0.55	98.43	1.57
7	5.70	62.92	37.08	36.69	0.39	98.94	1.06
8	2.69	61.32	38.68	38.27	0.41	98.94	1.06
9	4.02	64.76	35.24	34.76	0.48	98.65	1.35
10	2.54	62.69	37.31	36.83	0.48	98.71	1.29
11	3.82	64.32	35.68	35.25	0.43	98.79	1.21
12	2.72	62.64	37.36	36.96	0.40	98.94	1.06
13	4.21	60.93	39.07	38.64	0.43	98.91	1.09
Aver.	4.59	62.87	37.13	36.66	0.47	98.71	1.29
Calf Tendon.							
1	2.21	65.39	34.61	33.98	0.63	98.18	1.82
2	3.96	66.54	33.46	32.89	0.57	98.30	1.70
3	5.17	68.75	31.25	30.60	0.65	97.91	2.09
4	4.32	68.32	31.68	31.06	0.62	98.04	1.96
5	4.12	67.23	32.77	32.33	0.44	98.68	1.32
6	2.68	68.84	31.16	30.42	0.74	97.63	2.37
Aver.	3.74	67.51	32.49	31.88	0.61	98.12	1.88

sufficient quantity for the determinations. Sometimes they were cut into strips with a knife and the strips finely divided with scissors. All preparations were conducted rapidly and with due regard to the usual precautions to prevent loss of moisture, etc.

Proportions of water, solids, organic and inorganic matter. — In these determinations the finely divided substance was dried at 100–110° C. to constant weight. Incineration was carefully conducted over a very low flame until all carbon was burned out and the ash was constant in weight.

The general summary on the opposite page gives the results of these determinations for the tendo Achillis from both the ox and the calf. It will be seen from the general averages that the tendon of the calf contains relatively more water and inorganic matter than that of the mature animal. The tissue of the full grown ox on the other hand contains larger proportions of solid substance and organic matter.

In his determinations of the composition of dry tendon from the foot of the calf, Marchand [1] also weighed the ash. In three separate determinations he found the ash to be 1.72, 1.82 and 1.89 per cent — an average of 1.81 per cent of the dry tissue.[2] These results accord very closely with our own, if it be assumed that the tendons of the calf which Marchand analyzed contained approximately the same amount of water found in these experiments — 67.5 per cent. At this rate, the fresh tendons analyzed by him contained 0.59 per cent of ash.[3]

The facts brought out by the figures in the table on the opposite page harmonize with comparative analytic data for other tissues of fully developed as well as immature animals. On the next page we present a summary giving percentage figures for the general composition of morphologically related parts. Attention may be called to the general similarity in the results for tendon and ligament. Costal cartilage is somewhat similar to these two in general composition, the analytic differences being mainly due to its larger content of water and inorganic matter.

Inorganic matter. — Ash in suitable quantity was prepared by gradual combustion in a nickel crucible over an alcohol burner and then by complete incineration over a very low flame in a platinum

[1] See page 220.

[2] See foot-note, page 225; also, summary on page 230.

[3] The ash of tendons containing ossa sesamoidea would naturally be much greater than any of the amounts here recorded for the normal tissue.

dish. The qualitative characters of the ash of the Achilles tendon are much the same as those of the inorganic matter in many other parts of the body. Solutions of the ash were strongly alkaline in reaction. We detected in it chloride, carbonate, sulphate, and phosphate. Of the basic elements sodium, calcium, magnesium, potassium, and iron were particularly prominent. It is probable that the iron came from traces of haemoglobin in the capillaries. Some of the

COMPARATIVE COMPOSITION.

	Tendon.		Ligament.[1]		Vitreous humor.[2]	Costal cartilage.[3]	Bone with marrow.[4]	Adipose tissue; kidney fat.[5]
	Calf.	Ox.	Calf.	Ox.				
Fresh tissue.								
Water	67.51	62.87	65.10	57.57	98.64	67.67	50.00	4.30
Solids	32.49	37.13	34.90	42.43	1.36	32.33	50.00	95.70
Organic	31.88	36.66	34.24	41.96	0.48	30.13	28.15	95.51
Inorganic	0.61	0.47	0.66	0.47	0.88	2.20	21.85	0.19
Dry tissue.								
Organic	98.12	98.71	98.10	98.90	35.29	93.20	56.30	99.80
Inorganic	1.88	1.29	1.90	1.10	64.71	6.80	43.70	0.20

[1] VANDEGRIFT and GIES: *Loc. cit.*

[2] Representing jelly-like connective tissue. Analyses by LOHMEYER, source of material not specified. See GORUP-BESANEZ: *Loc. cit.*, p. 401.

[3] Human. Analyses by HOPPE-SEYLER. See KÜHNE: Lehrbuch der physiologischen Chemie, 1868, p. 387.

[4] Average of many analyses of various human bones before removal of marrow. HOPPE-SEYLER: Physiologische Chemie, 1881, p. 625.

[5] From the ox. ATWATER: Methods and results of investigations on the chemistry and economy of food, 1895, p. 34.

carbonate doubtless arose from the proteid in the process of oxidation. Much of the sulphate came from the acid radicle of the tendon mucoid. The proportion of ash in tendon, as in ligament, is unusually small.

Schulz[1] has recently detected silicic acid in a number of the forms of connective tissue. The average amount of silicic acid in 1 kilo of

[1] SCHULZ: Archiv für die gesammte Physiologie, 1901, lxxxiv, p. 67.

dry ox tendon was found to be 0.1086 gram (0.01 per cent of the solid matter). In the same quantity of dry human tendon silicic acid amounts on an average to 0.0637 (0.006 per cent of the solid matter).[1]

Soluble and insoluble portions. Several direct determinations of the amount of insoluble matter in the ash were made. Ash which had been reheated in a platinum crucible was cooled in a desiccator. Quantities of this perfectly anhydrous material, from one to two grams in weight, were treated with 500 c.c. of distilled water per gram of substance. The mixture was repeatedly stirred for forty-eight hours, then filtered on weighed papers and the amount of insoluble substance directly determined gravimetrically in the customary way. The appended percentage results were obtained on three different preparations:

	1	2	3	Average.
Substance *insoluble* in cold water	27.1	27.4	26.6	27.0
Substance *soluble* in cold water	72.9	72.6	73.4	73.0

Similar determinations were made by us on samples of the ligament ash prepared by Vandegrift and Gies. 24.3 per cent of the same was found to be insoluble, 75.7 per cent soluble, in cold water. In Pickardt's[2] analyses of the ash of laryngeal cartilage 37.2 per cent was insoluble in water, 62.8 per cent soluble.

Sulphate. — The ash gave striking sulphate reactions with $BaCl_2$ in the presence of free HCl. In some preliminary experiments samples of ash which had been prepared quickly by incineration in a platinum dish over a Bunsen gas burner contained from 9.56 to 14.92 per cent of SO_3.[3] As these results were obviously affected by sulphur products in the gas, we next made several preparations of the ash in platinum dishes over alcohol burners. The following results for SO_3 content in ash prepared in this way were obtained by the usual $BaCl_2$ method,

[1] In these determinations SCHULZ also estimated the percentage of ash in the dry substance. In tendons of the calf it amounted to 3.19 per cent. In the older animals it was as low as 2.07 per cent. In human tendon it was as high as 3.88 per cent. The amount of silicic acid in the ash of the tendons from cattle ranged from 0.23 to 0.66 per cent. In the ash of human tendon it varied between 0.11 and 0.49 per cent. SCHULZ's results indicate that the older the animal is the larger is the percentage of silicic acid in its connective tissues.

[2] PICKARDT: Centralblatt für Physiologie, 1892, vi, p. 735.

[3] Compare with results for ligament ash, under similar conditions of preparation, given by VANDEGRIFT and GIES, *loc. cit.*, p. 291. See also, BIELFELD: Zeitschrift für physiologische Chemie, 1898, xxv, p. 352.

in 0.25–0.71 gram portions, after solution in hot dilute HCl and subsequent filtration:

PERCENTAGE OF SO_3 IN TENDON ASH.

	1	2	3	4	Average.
A	6.72	6.62	6.68	....	6.67
B	6.70	6.60	....	....	6.65
C	6.60	6.58	6.63	6.61	6.60
D	6.63	6.84	6.74	6.69	6.72
E	6.55	6.63	....	....	6.59
				General average . .	6.65

The relation of tendon ash to the ash of other tissues and various fluids, with respect to SO_3 content, may be seen at a glance in the following summary of SO_3 percentages [1]: —

Bone . .	0.02	Liver . .	0.92	Serum .	2.10	Ligament .	5.64
Muscle .	0.30	Lungs . .	1.40	Spleen .	2.54	Bile . . .	6.39
Brain . .	0.75	Blood . .	1.67	Milk . .	2.64	Cartilage .	37.47

There can be little doubt that most of the SO_3 in tendon ash arises from an organic source, just as in the case of bile, cartilage, and ligament. It could not have come from blood or lymph. Bile contains combined SO_3 in salts of taurocholic acid. Cartilage contains salts of chondroitin sulphuric acid, as well as chondromucoid.[2] Ligament contains mucoid[3] and possibly, also, chondroitin sulphuric acid.[4] Tendon contains considerable mucoid, as we shall see, but, according to Mörner,[5] no chondroitin sulphuric acid can be separated from the Achilles tendon. Tendo mucoid, however, contains a radicle similar to, if not identical with chondroitin sulphuric acid,[6] and it is probable

[1] VANDEGRIFT and GIES: *Loc. cit.*, p. 292.

[2] C. TH. MÖRNER: Skandinavisches Archiv für Physiologie, 1889, i, p. 210.

[3] RICHARDS and GIES: Proceedings of the American Physiological Society. This journal, 1900, iii, p. v; also, *Ibid.*, 1901, v, p. xi.

[4] KRAWKOW: Archiv für experimentelle Pathologie und Pharmakologie, 1897, xl, p. 195.

[5] C. TH. MÖRNER: Zeitschrift für physiologische Chemie, 1895, xx, p. 361.

[6] LEVENE: *Ibid.*, 1901, xxxi, p. 395.

that the SO_3 liberated during its combustion unites in part with the basic elements of the ash.[1]

Phosphate and chloride. — No extended quantitative analysis of the ash was made because of the large amount of derived sulphate in it. Figures for the percentage content of other constituents under the circumstances would afford only approximate values. Phosphate and chloride, the chief salts in the ash, were present in large proportion, as the following results for percentage content of P_2O_5 and Cl will indicate:

	1	2	3	4	Average
P_2O_5 . . .	8.38	8.53	8.30	8.16	8.34
Cl	31.73	30.99	31.26	31.52	31.37

The average quantity of chlorine in ligament ash was found by us to be 7.39 per cent. P_2O_5 was equal to 28.95 per cent of the ligament ash.

Fat (ether-soluble matter). — Although the Achilles tendon does not appear to hold as much admixed adipose tissue as ligamentum nuchæ, it seems to contain almost as much extractive substance. The following percentage results in this connection, calculated for fresh tissue in each case, were obtained by Dormeyer's method:

	1	2	3	4	5	6	7	Average
Fresh tissue . .	0.87	1.10	1.21	1.16	0.98	1.05	0.93	1.04

The proteid constituents. — It has been known for a long time that tendon consists mostly of collagen. As we have already indicated the earlier observers considered tendon to be almost pure collagen. Rollett's[2] researches on the structure and composition of connective tissues demonstrated the presence in tendon not only of such soluble proteids as might be constituents of contained lymph, but also of mucoid. Numerous histologists have shown the presence also of elastic fibres in tendinous tissue.

Coagulable proteid (albumin, globulin). — Rollett detected only traces of coagulable proteid in aqueous extracts of the Achilles tendon of the horse. Loebisch[3] called attention to the fact that

[1] Levene's result does not harmonize with Mörner's. The latter's method for the detection of chondroitin sulphuric acid in tendon should have revealed the presence of the acid substance in tendo mucoid identified by Levene. See Hawk and Gies: This journal, 1901, v, pp. 398–399.

[2] Rollett: Untersuchungen zur Naturlehre des Menschen und der Thiere (Moleschott), 1859, vi, p. i; also, *Ibid.*, 1860, vii, p. 190.

[3] Loebisch: Zeitschrift für physiologische Chemie, 1886, x, p. 43.

aqueous extracts of the same tendon of the ox contain slight quantities of coagulable proteid — "serum globulin" and an albumin coagulating at 78° C. Richards and Gies[1] recently observed that aqueous extracts of this tendon from the ox contain minute proportions of two coagulable proteids; one, a globulin, coagulating at 54°–57° C., the other, an albumin, coagulating at 73° C.

In this work we experienced great difficulty in making satisfactory quantitative estimations. The quantity of coagulum for 100–200 grams of tissue was always very slight. Frequently it was impossible to obtain the coagulum in a perfectly clear fluid. The results were the same in aqueous and in sodium chloride extracts. One or two indirect methods gave no more satisfactory results. Tendo mucoid is somewhat soluble in the aqueous and saline extracts of the tissue, and possibly the observed interference with perfect coagulation of the simple proteids was due to the presence of larger or smaller amounts of this glucoproteid.

The following percentage results were obtained in extracts from tissue which had been cut into narrow strips and then very finely divided with scissors: —

	1	2	3	4	5	6	7	Average
Fresh tissue .	0.231	0.184	0.191	0.274	0.177	0.219	0.262	0.220

It is possible that not only a small quantity of coagulable proteid was lost in each determination, but also that a small proportion of mucoid was admixed with the coagulum as a result of the addition of the dilute acid ordinarily employed to complete coagulation. We feel satisfied, however, that the above average amount is very nearly that contained in this tissue. Much of it doubtless is a part of contained lymph. The average quantity in ligamentum nuchæ is 0.616 per cent.

Mucoid.[2] — The proportion of mucoid in tendon is comparatively large. Halliburton states that the average amount for normal connective tissues is 0.521 per cent.[3] The amount in the human tendo Achillis he found varied under normal conditions between 0.298 and 0.770 per cent. Chittenden and Gies[4] obtained as much as 1 per cent of chemically pure mucoid from the tendo Achillis of the ox, al-

[1] RICHARDS and GIES: *Loc. cit.*

[2] See CUTTER and GIES: *Loc. cit.*, foot-note, p. 155.

[3] HALLIBURTON: *Loc. cit.*, p. 477.

[4] CHITTENDEN and GIES: Journal of experimental medicine, 1896, i, p. 186.

though their experiments were not designed for quantitative determinations. The amount in ligamentum nuchæ was found by us to average 0.525 per cent. Our percentage results for the Achilles tendon of the ox were the following:

	1	2	3	4	5	6	7	Average
Fresh tissue .	1.361	1.420	1.332	1.220	1.043	1.228	1.380	1.283

In these determinations we profited by the experience of Cutter and Gies that repeated treatment with excess of dilute alkali is necessary to extract completely mucoid from tendon.[1]

Halliburton[2] gives a record of determinations of mucoid in human tissues under abnormal conditions. In one case the Achilles tendon contained as much as 1.42 per cent. The tendons of the heart under similar conditions contained 1.65 per cent mucoid.

Elastin. — When tendon pieces are boiled in water they rapidly diminish in size and only a small quantity of elastin-like material is left behind. This residual material is not as resistant to the action of dilute acid and alkali as is the elastin of ligamentum nuchæ, although it appears to be true elastin.[3] The following results for percentage content were obtained in our quantitative determinations:

	1	2	3	4	5	Average
Fresh tissue	1.561	2.130	1.634	1.100	1.740	1.633

Münz[4] separated this substance, studied some of its reactions and decomposition products, and made a few analyses of it. He found its nitrogen content to vary between 14.31 and 14.48 per cent. The accuracy of these analytic results has been doubted, since the nitrogen content of all elastins has been found to be above 15 per cent. One of our own specially prepared samples of tendon elastin, after it had been extracted with alcohol and ether, gave the following percentage results on analysis: (a) Nitrogen — by the Kjeldahl method — 15.42, 15.49, 15.45; average, 15.45. (b) Sulphur — by the fusion method over alcohol burner — 0.48, 0.54; average, 0.52. (c) Ash — 1.32, 1.28; average, 1.28. These results agree fairly well with those for aorta elastin obtained by Bergh[5]: N, 15.20; S, 0.66; Ash, 0.51.

[1] Cutter and Gies: *Loc. cit.*, p. 161.

[2] Halliburton: Jahresbericht über die Fortschritte der Thier-Chemie, 1888, xviii, p. 324.

[3] Kühne: Lehrbuch der physiologischen Chemie, 1868, p. 356.

[4] Münz: Quoted by Gorup-Besanez, *loc. cit.*, pp. 143 and 645.

[5] Bergh: Zeitschrift für physiologische Chemie, 1898, xxv, p. 341.

Collagen. — The great bulk of the solid matter of tendon is collagen. We made five quantitative determinations by the indirect method,[1] with the following percentage results:

	1	2	3	4	5	Average
Fresh tissue	30.63	32.47	30.98	32.27	31.59	31.59

The proportion of collagen in the fresh tendo Achillis is almost exactly the same as that of elastin in ligamentum nuchae.

Recently, in testing his method for the determination of collagen in connective tissue containing little soluble proteid, Schepilewsky[2]

COMPOSITION OF TENDO ACHILLIS.

Constituents.	Fresh tissue.		Dry tissue.		Ash.
	Calf.	Ox.	Calf.	Ox.	Ox.
Water	67.51	62.870			
Solids	32.49	37.130			
Inorganic matter	0.61	0.470	1.88	1.266	
SO_3	....	0.031	....	0.084	6.65
P_2O_5	....	0.039	....	0.106	8.34
Cl	....	0.147	....	0.397	31.37
Organic matter	31.88	36.660	98.12	98.734	
Fat (ether-soluble matter) .	....	1.040	....	2.801	
Albumin, globulin	....	0.220	....	0.593	
Mucoid	....	1.283	....	3.455	
Elastin	....	1.633	....	4.398	
Collagen (gelatin)	....	31.588	....	85.074	
Extractives and undetermined substance . . .	....	0.896	....	2.413	

found 80.86 per cent of collagen in dry tendon. The particular tendon he used is not mentioned. In the dry Achilles tendons of the ox analyzed by us the collagen amounted on an average to 85.074 per cent.

[1] See VANDEGRIFT and GIES: *Loc. cit.*, foot-note, p. 295.

[2] SCHEPILEWSKY: Archiv für Hygiene, 1899, xxxiv, p. 351.

Crystalline extractives. — Our results for extractives were only qualitative. Creatin and nuclein bases could readily be detected. The proportion of extractive matter was small. Our results were similar to those previously obtained in this laboratory for ligament. In the table on the opposite page the extractives are included in "Extractives and undetermined substance," the figures for which were obtained by difference.

Average Composition. — The data of all our analyses are brought together in the summary on the opposite page, which gives the average percentage composition of fresh tendo Achillis and of the dry solid matter in it, together with the results of partial analysis of the ash.

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DO SPERMATOZOA CONTAIN ENZYME HAVING THE POWER OF CAUSING DEVELOPMENT OF MATURE OVA?

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[*From the Department of Physiology in the Marine Biological Laboratory at Wood's Holl, Mass.*[1]]

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OUR knowledge of the chemical properties of enzymes is very slight, and our understanding of the part they play in zymolysis anything but clear. Nevertheless, the great importance in biological events of these energy-transforming substances is generally recognized. The lack of precise information regarding the essential qualities of enzymes no doubt accounts for the current tendency to attribute indefinitely to ferment influence various processes of morphological or chemical character which are not satisfactorily comprehended through ordinary experimental means, or which, in some cases, have not even been subjected to such investigation.

A fundamental biological question has lately been put into this category. The process of segmentation in the fertilized egg has been ascribed in part, at least, to enzyme influence.

With the advice and many helpful suggestions of Professor Loeb, I have attempted to ascertain whether any experimental justification can be found for recent statements that the spermatozoön carries substance into the ovum which effects proliferation by zymolysis.

[1] I am indebted to the kindness of Professor Curtis for the use of the investigator's room at Wood's Holl, reserved for the Department of Physiology of Columbia University.

Historical.

Piéri,[1] after some observations on *Strongylocentrotus lividus* and *Echinus esculentus* in the Marine Laboratory at Roscoff, in August, 1897, reported that he had extracted soluble sperm enzyme having power to bring about segmentation of the ovum. "Ovulase," as he called it, was obtained by merely shaking the spermatozoa of these Echinoderms for a quarter of an hour in a flask with sea-water, or with distilled water. Microscopic examination of the filtrates showed that the spermatozoa which passed through the paper were without tails and immobile; "that is to say, dead."

The fresh mature ova, well washed in sea-water, were placed in shallow dishes (size not stated) with the extract, immediately, or within ten hours, after its preparation. Segmentation proceeded slowly and reached the morula stage in about ten hours, with the usual phenomena of karyokinesis. Microscopic examination showed that there had been no penetration by spermatozoa. The "ovulase" in distilled water was less effective than that obtained in sea-water; it produced only a few segmentations (greatest number not mentioned).

At the end of his paper Piéri himself mentions two "objections" to his conclusions which it appears to the present writer destroy their force: (1) Only the spermatozoa in the distilled water (which extract he has distinctly indicated possessed the lesser, if any, segmental power) were always killed by the shaking process. He suggests that the spermatozoa might be eliminated, and pure "ovulase" obtained with the aid of the centrifuge or porcelain filter. (2) Some of the main supply of eggs in sea-water, from which those tested were taken, segmented (to what stage is not stated), "in spite of the precautions taken."

Piéri gives few details of his work, and no direct judgment can be passed on his methods. What proportion of the eggs developed? The few divisions caused by the distilled water extract can hardly be emphasized, for Piéri found that distilled water alone caused control eggs to become clear and fragmentary. Is it possible, in microscopic examination of myriads of such minute bodies as spermatozoa, to be certain that each individual can be seen? Is the apparent lack of

[1] Piéri: Archives de zoologie expérimentale et générale, 1899, vii; Notes et revue, ix, p. xxix.

motility in those actually observed conclusive evidence of the death of all? Besides, not all of the fluid in use can be examined by means of the microscope. Further, what effect did boiling have on "ovulase"? Was it destroyed at that temperature, as all ferments are? What means were taken to kill the spermatozoa which may have been present in the sea-water used to wash the eggs? These important points Piéri has not considered.

Shortly after Piéri's communication, Dubois[1] presented a brief note of a similar character. Dubois arrived at the conclusion that natural fertilization comes about through the action of a fecundative ferment. He claims that he was able to separate such a body, "d'une zymase fécundante," from the testicles of *Echinus esculentus*, but no experiments showing its qualities were reported by him. Dubois named the ferment (?) "spermase" and credited it with the power of modifying a hypothetical substance pre-existent in the ovum, which he called "ovulose." As long as experimental evidence of the truth of such a conclusion is wanting, it must continue to remain an unsatisfying speculation.

Winkler's[2] experiments were made on *Sphaerechinus granularis* and *Arbacia pustulosa*. Every precaution was taken to prevent the action of live spermatozoa. Winkler made extracts of spermatozoa by shaking them for about half an hour with distilled water (quantities not stated). In order to prevent destructive action on the part of the distilled water, a precaution Piéri had not observed, Winkler added to the extract, before using it on the test ova, a sufficient quantity of evaporated sea-water to make the concentration of the extract the same as that of sea-water ("ca. 4%"). Another kind of extract of sperm was made in the fluid obtained by evaporating 400 c.c. of sea-water to one fourth its volume.[3] The filtered extract was finally treated with enough distilled water to lower its concentration to that of normal sea-water.

[1] DUBOIS: Comptes rendus hebdomadaire des séances de la Société de Biologie, 1900, lii, p. 197. The author has not had access to the original paper and relies upon the review made of it by Winkler. (Ref. below.)

[2] WINKLER: Nachrichten von der königliche Gesellschaft der Wissenschaften zu Göttingen. Mathematisch-physikalische Klasse, 1900, p. 187.

[3] Winkler states that the sea-water he used contained "ca. 4%" of saline matter and that by evaporating 400 c.c. to 100 c.c. he obtained a solution of "ca. 20%." The author fails to see how anything but a 16% solution was obtained if the process was conducted as described. Loeb's experiments have shown how necessary exact knowledge of concentration is in such work.

In both kinds of extract the eggs showed some tendency to segment, but only a few divided.[1] Sometimes with the same extract the eggs of one individual "reacted," while the eggs of another did not. Finally, it is decidedly significant that the proliferation went at most only to the 4-cell stage, and that then separation of the cells occurred from the absence of retaining membrane, and "abnormal" forms resulted. In the control experiments these manifestations were not apparent.

Winkler does not claim that the slight changes he observed were due to an enzyme. He states that he did not determine the effect of heat on the power of his extracts. The nature of the active substance, he says, is completely unknown. It might be reasonable to assume that dissolved nucleoproteid had stimulated proliferation, but it seems much more probable that the initial segmentations Winkler observed were really due to increased concentration and the consequent osmotic conditions, not to ferment action or extractive influences. Errors in making up the saline solutions might of themselves have accounted for all that was observed. A concentration very little above that of normal sea-water would produce the results.[2] Further, it is well known that the eggs of sea-urchins are prone to divide into a few cells if they are allowed to remain undisturbed in normal sea-water for about a day.[3]

Winkler's results are hardly positive enough, therefore, to permit of the deduction he draws; they might, in fact, be used to show how unwarranted were Piéri's conclusions.

Experimental.

General methods of procedure. — The investigations recently done under Professor Loeb's supervision in this connection were conducted with *Arbacia punctulata*. In a few experiments, as will be pointed out, the testes of *Strongylocentrotus purpuratus* were used. Males and females were kept together in a tank in running sea-water until they were needed. Immediately before they were used all extraneous matter was carefully washed off in an abundance of *fresh* water, which killed any adherent spermatozoa. The various instruments employed in the work were repeatedly washed in the same way.

[1] "Nur ein nicht sehr grosser Theil."

[2] Loeb: This journal, 1900, iii, pp. 436 and 437.

[3] Loeb: *Loc. cit.*

The sea-water in these experiments was collected in a large stoppered bottle on one day for use upon the next. This insured the use of the same water for each set of experiments and the corresponding controls. Gemmill[1] has shown experimentally that if free spermatozoa are kept in sea-water (in "*dilute* mixture") for five hours they lose their ability to impregnate the ovum. Consequently our method rendered inert any spermatozoa which may have been alive in the water at the time of collection and made boiling unnecessary. Moreover, Loeb[2] has lately called attention to the fact that sea-urchins have practically died out in the immediate neighborhood of Wood's Holl, and that for this reason, even at the height of the spawning season, there is little or no danger that the supply of sea-water used in this laboratory contains any live spermatozoa of this animal.

In procuring testes or ovaries the oral surface of the animal was cut away and the alimentary and vascular membranes carefully torn out. After thorough flushing in sea-water to eliminate body fluid and dissolved matter such as digestive enzyme, etc., the glands were transferred to perfectly clean vessels for appropriate treatment without delay.

The ovaries, from which the eggs used as indicators were taken, were transferred directly to a shallow dish with just enough sea-water to cover them. In most cases the eggs from one animal were sufficient for a connected series of observations. As a rule the ovaries were full of eggs and mere shaking sufficed to liberate the latter into the surrounding fluid, where a comparatively thick layer quickly formed. A few drops of this sediment, containing thousands of eggs, were sufficient for each individual test. The ovaries were never taken from the animal until all other preparations had been completed, so that the eggs were perfectly fresh when employed.

Only such unfertilized eggs as were found to be normal and mature were used. In each of the series of experiments to be described some of the ova were either fertilized directly with spermatozoa or were first subjected for an hour or two to the influence of solutions of higher osmotic pressure than sea-water (mixtures of 88 c.c. sea-water + 12 c.c. $\frac{2.0}{8}$ *n* KCl were usually made up for the purpose) and then were placed in sea-water to test their capacity for parthenogenetic division. In many experiments both methods were used.

[1] GEMMILL: Journal of anatomy and physiology, 1900, xxxiv, p. 170.

[2] LOEB: *Loc. cit.*, p. 450.

Under these test conditions the eggs employed were always found to develop into swimming larvæ within twenty-four hours. These facts are not specially noted in the records given below because of their uniformity throughout. The "control" tests mentioned with each series refer to the eggs which had been placed only in normal sea-water for comparison with ova treated by special processes.

In each of the following series of experiments the volume of sea-water in each test was, as a rule, 100 c.c. (Note exceptions farther on.) It was increased only by the addition of portions of extract as specified under each series and by the few drops of sea-water carrying the eggs, in pipette, from the main supply. The sea-water was contained in small bowls of uniform size, making the depth of the fluid (about an inch) practically the same for all of the experiments. Throughout each series the bowls were kept covered with glass plates. The air space above the fluid was about an inch in depth, thus insuring abundant supply of oxygen. Occasionally, as will be noted, eggs were placed in quantities of the extract alone, held in smaller vessels. These were also kept covered. The temperature of the room varied between 18–20° C. The amount of evaporation, as indicated by sensible condensation on the under side of the cover-plates, was comparatively slight during twenty-four hours, so that no material concentration occurred during the interval.

The extracts of the spermatozoa were made directly from the testes. It was not thought necessary to attempt separation of the non-spermatic tissue elements. The testes were always thoroughly ground to a thick paste in a mortar with dry sand which had been heated above 100° C. for from fifteen to twenty minutes. Water and saline extracts were used within a few hours. Fluids containing preservatives, however, were given more time for extraction, as will be noted below. The extractions were made in bottles to permit of frequent and vigorous shaking. Clear filtrates were obtained in each case without special difficulty.

In each series of experiments carefully measured quantities of extract were added to sea-water, and the mixtures stirred to prevent inequalities of concentration. The eggs were distributed after the mixtures of sea-water and extract had been made. The experiments were begun in the morning. At intervals of an hour or two until late at night, samples of eggs were quickly removed with pipettes from the bowls to watch glasses for observation under the microscope. Hundreds were examined carefully each time. None were

ever returned to the main supplies. The eggs in each series were always under observation for from at least twenty to twenty-four hours, seldom longer than that, and unless otherwise stated the "results" recorded below are for periods of that length.

Experimental Data.

Our experiments are described here briefly, though in some detail, so that whatever value they may possess may be accurately estimated. The first series of extracts were made with spring water.

Fresh water extract. — *Fresh testes.*— I. The glands from one animal were extracted in 15 c.c. H_2O for 1 hr., 30 mins. Three tests were made as follows: —

(1) Control (2) Extract — 4 c.c. (3) Fresh H_2O — 4 c.c.[1]

Result: No segmentation.

II. The glands from one animal were extracted in 15 c.c. H_2O for 3 hrs.

(1) Control (2) Extract — 2 c.c. (3) Fresh H_2O — 2 c.c.

Result: No segmentation.

III. Glands from two animals in 10 c.c. H_2O for 4 hrs.

A. Control. B. Extract: (a) 1 c.c. (unfiltered), (b) 4 c.c., (c) 0.05 c.c., (d) eggs in 3 c.c. + equal volume of $\frac{1.0}{8}$ *n* NaCl. C. Some of (d) into sea-water after 2 hrs.

Result: Irregular parthenogenetic forms in a very small proportion of (a), (b), and (c) after 4 hrs. A few groups of 8 and one or two of 16 cells from individual eggs, in 24 hrs., in (b). None beyond the 4-cell stage in (a) and (c). A few parthenogenetic in C as far as the 8-cell stage. No morulæ in any. No segmentations in the control.

The results of the third series encouraged the belief that enzyme action was demonstrable, although we did not lose sight of the fact that perhaps increased concentrations, induced by unobserved circumstances, or other unknown conditions, would account for the proliferations noted. In the fourth and fifth series the effects of fresh were compared with those of boiled extract.

IV. Five sets of testes extracted in 60 c.c. H_2O for 3 hrs. One half was boiled in an Erlenmeyer flask 10–15 mins. An appreciable concentra-

[1] It will be understood from what was stated on page 58 that this abbreviated reference to the three tests means that besides being under normal conditions (in 100 c.c. sea-water alone), eggs were subjected to the influence of both 4 c.c. of extract in 100 c.c. of sea water and 4 c.c. of fresh H_2O in the same large quantity of sea-water. This system will be adopted throughout for brevity's sake.

tion resulted, but of course no approximation to the specific gravity of sea-water was effected.

A. Control. B. Fresh extract: (a) 10 c.c., (b) eggs in 8 c.c. extract alone. C. Boiled extract: (c) 10 c.c., (d) eggs in 8 c.c. extract alone. D. Samples of B and C in 100 c.c. sea-water after 1 hr., 30 mins.

Result: During the first 12 hrs. there was no segmentation in any of B and C. An occasional kidney-shaped cell was found in the control and D after 5 hrs. At the end of 24 hrs. there were a few 4 to 8 cell divisions in the eggs of (a) and (c) which had been transferred to sea-water. Only a few 2 to 4 cell groups were found in the control at the end of the same period.

V. Testicles from 15 animals extracted in 85 c.c. H_2O for 3 hrs. One half was boiled as in the preceding series.

A. Control. B. Fresh extract: (a) 20 c.c., (b) 10 c.c., (c) eggs in 10 c.c. extract alone. C. Boiled extract: (d) 10 c.c., (e) 8 c.c. D. Eggs in B and C transferred to normal sea-water after 1 hr.

Result: Not a single segmentation could be detected. A very few of the eggs of (d) and (e) which had been transferred to sea-water were kidney-shaped as though in an initial parthenogenetic stage.

The results of the first five series were indecisive, but, where positive, they strongly suggested initial osmotic parthenogenesis, caused probably by conditions beyond control, rather than zymolytic influences. On the assumption that the concentration of the extracts was somewhat lower than sea-water in spite of the salts and proteids dissolved from the testes, and that variations in effects occurred as a consequence, the sixth series was arranged to overcome this difficulty.

VI. Fourteen sets of glands were extracted in 35 c.c. H_2O for 3 hrs. Just before the filtered extract was used it was mixed with an equal volume of normal NaCl, making approximately a $\frac{4}{8}\,n$ NaCl mixture (sea-water is equivalent to about $\frac{5}{8}\,n$ NaCl).

A. Control. B. Extract: 20 c.c., 10 c.c., 1 c.c., eggs in 10 c.c. extract alone. C. Eggs in each of B transferred to 100 c.c. sea-water at the end of 2 hrs.

Result: No divisions or irregular forms.

The generally negative results of the preceding experiments made it seem desirable to resort to other means before abandoning the study of fresh water extracts. Various enzymes are more easily extracted after the containing cells have been dried and thoroughly broken up. This expedient was tried, therefore.

Dry testes. — The glands from each animal were macerated and spread out separately in a thin layer on watch glasses. These were placed in desiccators over concentrated sulphuric acid or calcium chloride. Drying was accomplished within eighteen hours. When desired for use the dry substance was scraped into a mortar, and ground up thoroughly with sand and extracted as in the previous experiments.

VII. The dry substance of four sets of glands was extracted in 30 c.c. H_2O for 3 hrs.

A. Control. B. Extract: (a) 5 c.c. (unfiltered), (b) 10 c.c., (c) 5 c.c., (d) 1 c.c., (e) eggs in extract + equal volume $\frac{1}{8}$ *n* NaCl.

Result: Within 12 hrs. no change. At the end of 24 hrs. a very few were in initial parthenogenetic stages, 2 to 4 cell groups, in all except (a). They could be found only after careful search and there were as many in the control as in any of the others.

VIII. Eight sets of dried testes in 25 c.c. H_2O for 4 hrs. Filtrate mixed with an equal quantity of $\frac{1}{8}$ *n* NaCl before using.

A. Control. B. Extract: 7 c.c., eggs in extract alone. C. Some of the eggs in B were transferred to 100 c.c. sea-water after 1 hr., 45 mins.

Result: No segmentations or parthenogenetic forms in any.

It seemed necessary to conclude at this point that fresh water extracts of spermatozoa do not contain substance of zymolytic power or else that the conditions attending their use are unfavorable to such manifestation. Enzymes which are soluble in water are also soluble in solutions of electrolytes, so that attempts were next made with the latter.

Salt water extract. — A common method of extracting enzymes includes treatment of the tissue with ordinary salt solution. Sea-water itself furnishes such a dilute solution, but is not so favorable to rapid destruction of spermatozoa as fresh water or stronger salt solution. Since spermatozoa pass through ordinary filter paper, however often they may be subjected to filtration, it was necessary in using fresh testes to give particular attention to killing the spermatozoa by mechanical means. Prolonged grinding in a mortar with fine sand, as had been done previously, followed by continuous shaking for several hours, accomplished this.

Fresh testes. IX. Twelve sets of glands were extracted in 50 c.c. sea-water for 4 hrs.

A. Controls (2). B. Extract: 20 c.c., 10 c.c., 5 c.c., 1 c.c., 0.25 c.c.
Result: Not a single division could be found.

The very greatest care is necessary, in this connection, in the use of solutions of electrolytes, because of the ready osmo-parthenogenetic response the eggs make to slightly increased concentration. There is little reason for believing that an enzyme is present in spermatozoa which is insoluble in dilute, but soluble in strong salt solution. Therefore it seemed unnecessary to try the effect of more concentrated extractive. The tenth series shows the result of an effort to make the best of saline extraction of fresh testes, however, in a way somewhat different than that of the preceding.

X. Eight sets of testes in 40 c.c. $\frac{5}{8}$ *n* NaCl for 2 hrs. One half was warmed to 35–40 C. 15–20 minutes.
A. Controls (2). B. Extract (unwarmed): (a) 5 c.c., (b) eggs in 5 c.c. extract alone. C. Extract (warmed): (c) 5 c.c., (d) eggs in 5 c.c. extract alone. D. Some eggs of B and C in 100 c.c. normal sea-water after 2 hrs.
Result: No segmentation within 6 hrs. In 12–24 hrs. a very few 2-cell groups were found with difficulty in (a), (b), and (c) and in one of the controls.

Dry testes. The preliminary process of drying was also resorted to in this connection.

XI. Dry material from three animals was extracted in 5 c.c. sea-water for 2 hrs.
A. Control. B. Extract: 2 c.c. (unfiltered), 1 c.c., 0.25 c.c.
Result: Not a sign of segmentation.

Do the extracts possess poisonous qualities? — One condition that may appear to be against the action of an enzyme in the extracts used in these experiments is the possible presence of poisonous substances in the extract. This question now required a definite answer. We had varied the quantities of extract considerably, between all reasonable extremes, in the belief that the most favorable amount might be indicated, but it will be observed from the foregoing account of results that no such relation was suggested. The eggs which had been subjected to the extracts alone, and those placed in sea-water with the greater proportions of extract, usually showed abnormalities after a few hours, such as the development of enclosing membrane or transparent periphery (thicker and not comparable to the "vitelline" membrane after fertilization), swelling, disintegration, discol-

oration, agglomeration of pigment, etc., but none of these changes were constant so far as their relation to observed conditions could be determined. The sperm extracts contained salts and dissolved proteids, of course, and it would be reasonable to assume that these bodies were present in larger proportion, in some of these experiments at least, than they ever are under normal conditions of fecundation.

This important matter was definitely tested several times. The following results of two experiments are cited to show the facts in the case:

A. Five sets of fresh testes were ground in the usual way and extracted for 2 hrs. in 30 c.c. fresh water. An equal quantity of $\frac{1}{8}$ *n* NaCl was added to the filtrate. The eggs were placed in this mixture and samples transferred at intervals of an hour to 100 c.c. sea-water, to which fresh spermatozoa had been added. Results of examination at the end of 24 hours, the numerals indicating the number of hours the eggs were kept in the extract: (1) Swimming gastrulæ. (2) Blastulæ (none alive). (3) A few dead blastulæ, mostly morulæ. (4) Many unsegmented, none beyond the 32-cell stage. (5) About the same as those after the 4-hr. treatment. (6) Very few went so far as the 32-cell stage, many were in the 4 to 8 cell groups. There were no segmentations in the eggs kept for 24 hrs. in the extract.

B. Six sets of fresh glands were extracted in 30 c.c. sea-water, 3 hrs. Eggs from one animal were placed in the filtered extract and also into an equal quantity of sea-water (as control). At intervals eggs were withdrawn from each supply and transferred to 100 c.c. sea-water containing perfectly fresh spermatozoa. Results at the end of 36 hours from the time of the first transferral, the numerals again indicating the number of hours the eggs were under the direct influence of the extract or the normal sea-water: (1) Plutei in each. (2) Advanced gastrulæ in each. (3) Gastrulæ in each. (4) Many gastrulæ in the control; hardly any live ones, mostly morulæ, among those treated with the extract. (7) A large number of blastulæ were present in the control, but no divisions beyond the 32-cell stage could be found among the eggs which had been in the extract; most of the ova were unsegmented. There were no proliferations in the eggs retained in the extract itself. In the earlier tests the proportion of unsegmented cells was uniformly greater in the control than in the other series, whereas the living larvæ were relatively more numerous in the latter. The extract seemed at first to stimulate, and later to inhibit karyokinesis. Possibly, however, the accumulation of bacteria in the bowls containing extract was responsible for the latter effect.

It is clear, from the foregoing, that the dissolved substances of our extracts have not prevented the eggs from segmenting. From this

we may safely conclude that they doubtless would not interfere with zymolysis if such were demonstrable.

The results of all the preceding series seemed to point in the same general direction and to indicate no mitotic action. Before accepting this negative conclusion, however, we proceeded to employ various other familiar methods for the separation of enzymes in the hope of eventually extracting and demonstrating the presence of such a substance.

Extract of spermatozoa which had been treated with, and preserved in alcohol. — Enzymes may readily be extracted from tissues hardened in alcohol. In fact they are frequently isolated by such preliminary treatment, which brings about disintegration of the cellular protoplasm as well as coagulation of soluble proteid, and thus diminishes the proportion of undesirable extraneous material in the final extract. Through the kindness of Professor Loeb, I was enabled to make extracts of the spermatozoa of *Strongylocentrotus purpuratus*, which had been preserved in an excess of 95% alcohol. The testes were taken from animals collected on the Pacific Coast about a year ago, while Professor Loeb was engaged there in his classical researches on artificial parthenogenesis.

In these experiments, with Arbacia as well as Strongylocentrotus, the alcoholic sperm mixture was filtered. Both the solid and fluid portions were transferred to shallow dishes and dried in the air. The liquid soon evaporated and left an oily residue which dissolved to a milky fluid when mixed with water.

Strongylocentrotus purpuratus. XII. Three grams of the dry sperm residue were thoroughly ground with sand and 30 c.c. fresh H_2O. After an hour an equal volume of $\frac{1}{8}^{0}$ *n* NaCl was added. Extraction in this mixture was continued an hour.

A. Control. B. Extract: 17 c.c., 7 c.c., and eggs in 8 c.c. of extract alone. C. Some of the eggs in each of B were transferred to 100 c.c. sea-water after 3 hrs.

Result: Not the slightest trace of segmentation.

XIII. Two grams of the finely divided dry substance were extracted in 40 c.c. sea-water for 3 hrs.

A. Control. B. Extract: (a) 12 c.c., (b) eggs in 10 c.c. extract alone. C. Eggs from B transferred to 100 c.c. normal sea-water after 2 hrs., 15 mins.

Result: Only a few forms in initial parthenogenesis in the control and in (a). These were found only after very careful search. Entirely negative results in the others.

It did not seem very likely that the alcoholic filtrate would contain a mitotic enzyme, if such a substance could not be extracted from the portion insoluble in alcohol. Yet, since some enzymes are soluble in diluted alcohol, the following experiments were made in order to ascertain definitely.

XIV. Half the residue of evaporated alcoholic extract was dissolved in 40 c.c. sea-water and filtered.

A. Control. B. Extract: (a) 15 c.c., (b) 5 c.c., (c) eggs in 20 c.c. of the extract alone. C. Eggs from (c) were transferred to 100 c.c. sea-water after 1 hr., 30 mins.

Result: Within 6 hrs. no perceptible effect. At the end of 18 hrs. a number of irregular parthenogenetic forms and some groups of 4 and 8 cells in C. No traces of segmentation in any of the others.

XV. The result in the preceding series seemed to be due to increased concentration caused by the accumulated salts of the original alcoholic extract. If this assumption were correct, dilution of the extract should prevent the effect noticed above. Only a fourth of the residue was next dissolved in 50 c.c. sea-water.

A. Control. B. Extract: (a) 20 c.c., (b) eggs in 20 c.c. extract alone. C. Samples of B were transferred to 100 c.c. sea-water after 2 hrs.

Result: Only a very few irregular shapes in the control and the transferred eggs of (b). One 4-cell group was found among thousands in the control; none among the others even after prolonged search.

XVI. A third experiment was made with the alcoholic residue. The solution was made more concentrated again. The remaining portion (one fourth) of the evaporated extract was dissolved in 15 c.c. sea-water.

A. Control. B. Extract: (a) 8 c.c., (b) eggs in 5 c.c. extract alone. C. Samples of each of B transferred to 100 c.c. sea-water after 3 hrs.

Result: Parthenogenetic groups of small cells in the transferred eggs of (b), but nothing of the sort in any other.

The results of the last three series emphasize the necessity of preventing material change in the composition of the sea-water and suggest how easy it might be, in cases of slightly increased concentration to mistake ion parthenogenesis for enzyme proliferation.

Arbacia. Twenty-one sets of testes were treated with 500 c.c. 95% alcohol. After remaining in contact with the latter for two days the solid substance was collected on a filter.

XVII. The dry solid matter was thoroughly extracted in 100 c.c. sea-water for 12 hrs.

A. Control. B. Extract: (a) 25 c.c., (b) 15 c.c., (c) 10 c.c., (d) 5 c.c., (e) 1 c.c., (f) 0.5 c.c. C. Samples of B transferred to 100 c.c. sea-water after 2 hrs.

Result: A very small percentage of 2-cell groups was found in the control, in (b) and among those of (d) which had been transferred to normal sea-water. One 2-cell segmentation had been found among the normal eggs immediately after they had been taken from the ovaries.

XVIII. In 24 hours the alcoholic filtrate (500 c.c.) had evaporated to 30 c.c. Practically all the alcohol had disappeared. The residue was made up to 100 c.c. with sea-water and filtered.

A. Control. B. Extract: 25 c.c., 15 c.c., 10 c.c., 5 c.c., 1 c.c. C. Samples of B transferred to 100 c.c. sea-water after 2 hrs.

Result: An occasional 2 to 4 cell group in practically all including the control — less than 2 per 100.

Glycerine extract. — Glycerine in water seems to be one of the best of enzyme extractors. Extracts of fresh Arbacia sperm were made by the previous general process in mixtures of equal parts of glycerine and water. It has been assumed, of course, that the glycerine in such extracts would exert specific deleterious effects and naturally careful control experiments were made to ascertain its influence in the quantities used in this series. These preliminary control tests determined the influence of glycerine under three general conditions: (*a*) its direct effect on the eggs, (*b*) its influence on normal fecundation, (*c*) its action on artificial parthenogenesis.

An abundant supply of equal parts of glycerine and sea-water was made for use in all these experiments. Normal eggs were found to remain unsegmented in all proportions of this glycerine solution with sea-water, although a few irregular parthenogenetic forms were produced by 15 c.c. in 100 c.c. normal sea-water. Quantities of this glycerine solution greater than 5 c.c. in 100 c.c. of sea-water prevented the normal segmentation by spermatozoa, but many swimming larvæ formed in the presence of 2 c.c. of the glycerine solution per 100 c.c. sea-water. Even 15 c.c. of the glycerine solution in 100 c.c. of sea-water did not, however, entirely prevent proliferation in ova which had previously been kept for 2 hrs. in 88 c c. sea-water + 12 c.c. $\frac{2.9}{8}$ *n* KCl, yet none of the segmentations under these conditions went beyond the 8 to 16 cell stage. With smaller quantities, swimming larvæ were obtained.

With these facts established the result of the following experiments are not without significance.

XIX. Seventeen sets of testes in 75 c.c. of the above glycerine solution for 48 hrs.

A. Control. B. Extract: (a) 15 c.c., (b) 5 c.c., (c) 2 c.c. C. Samples of each of B transferred to 100 c.c. sea-water after 1 hr.

Result: Here and there a kidney-shaped cell was found among those of (a) which had been transferred to normal sea-water. No distinct segmentations.

XX. Same glycerine extract after having been shaken with the tissue 24 hrs. longer.

A. Controls (2). B. Extract: 5 c.c., 2 c.c., 0.5 c.c., 0.25 c.c. C. Some of each of B transferred to 100 c.c. sea-water after 1 hr.

Result: Not the slightest suggestion of segmentation.

XXI. Twenty sets of testes were extracted in 80 c.c. of the glycerine solution four days. The filtrate was poured into a litre of 95% alcohol. A bulky, though light, white flocculent precipitate formed at once. After 24 hrs. this precipitate was filtered off, treated with 25 c.c. of sea-water for several hours and the filtrate used in the following experiment:

A. Control. B. Extract: (a) 10 c.c., (b) 5 c.c., (c) 2 c.c., (d) 1 c.c., (e) 0.25 c.c. C. Samples of each lot of B transferred to normal sea-water after 2 hrs.

Result: One or two irregular parthenogenetic forms were found in (c) and among those of (a) which had been transferred to normal sea-water. The number of such was less than 5 per 1000.

Ether extract. — Substances which cause the death of the cell or which appreciably lessen its vitality are known to favor solution of enzyme into the surrounding medium. Small quantities of alcohol or ether effect such results. Mathews[1] has recently shown that exposure of the unfertilized eggs of Arbacia to a saturated solution of ether in sea-water for ten to fifteen minutes leads to karyokinetic division of nearly all the eggs. In the use of ether in these experiments the greatest care was taken, therefore, to ascertain the influence of ether in the small quantities employed.

A solution for general use in this connection was made by mixing sea-water and ether in the proportion of 100 c.c. of the former and 7 c.c. of the latter. This amount seemed sufficient for any extractive usefulness ether might possess here. Intimate solution resulted. The odor of ether from the solution was still quite distinct at the conclusion of the experiments, though not strong at

[1] MATHEWS: This journal, 1900, iv, p. 345.

any time. In three control experiments, similar to those outlined under the head of glycerine extract, it was found that as much as 15 c.c. of this ether solution failed to effect parthenogenesis, although after eighteen hours a few 2-cell groups and irregular forms suggesting an initial stage of mitosis were found. As these were also present in the control, however, no importance could be attached to the result. After the usual treatment with sea-water plus $\frac{2}{8}^{0}$ *n* KCl, swimming larvæ developed when the eggs were transferred to 100 c.c. of sea-water containing as much as 25 c.c. of the ether solution. The same result was obtained, with as much ether solution present, when spermatozoa were added to the eggs in 100 c.c. of sea-water.

XXII. Ten sets of fresh testes were extracted in 60 c.c. of the ether solution for 3 days.

A. Control. B. Extract: 25 c.c., 15 c.c., 5 c.c., 1 c.c., 0.25 c.c. C. Some of each lot of eggs in B transferred to 100 c.c. normal sea-water after 2 hrs.

Result: During the first 12 hrs. no changes were manifested. At the end of 24 hrs., however, all, including the control, had a few 2 to 4 cell groups. The effect was not at all striking; it required careful search to find any signs of proliferation.

XXIII. The same extract, after having been 24 hrs. longer in contact with the tissue, was again employed.

A. Control. B. Extract: 4 c.c., 2 c.c., 0.5 c.c. C. Eggs from each of B placed in 100 c.c. normal sea-water after 1 hr., 30 mins.

Result: No sign of segmentation.

Alcohol extract.— Mathews[1] has also shown that alcohol affects Arbacia eggs much as ether does. He found that when the ova are placed in sea-water containing 4 to 5 parts of alcohol and are left there for from ten to fifteen minutes, they segment into several cells when they are replaced in sea-water. In these experiments, care was taken, therefore, to determine precisely the influence of the smaller quantities of alcohol employed.

A general supply of 10% alcohol in sea-water was kept for the experiments. Quantities not over 25 c.c. of this dilute alcohol, added to 100 c.c. of sea-water, were without mitotic influence. As much as 15 c.c. in 100 c.c. of sea-water interfered to no appreciable extent either with normal fertilization or osmotic parthenogenesis, as swimming larvæ developed within the usual period in both cases.

XXIV. Testes from 12 animals in 60 c.c dilute alcohol solution 48 hrs.

A. Controls (2). B. Extract: (a) 25 c.c., (b) 15 c.c., (c) 5 c.c.,

[1] MATHEWS: *Loc. cit.*, p. 346.

(d) 2 c.c., (e) 0.5 c.c. C. Some of each of B in 100 c.c. normal sea-water after 1 hr., 30 mins.

Result: No appreciable effect in any during the first 12 hrs. At the end of 24 hrs., however, several 2, 3 and 4 cell groups were found in both controls and also in each of those transferred to sea-water. The eggs of (d) which had been put into sea-water had a relatively larger proportion that showed initial division, although the actual number was in reality small — less than 10 in 1,000.

XXV. Some of the filtrate used in the preceding series was taken to repeat a part of the experiment just described.

A. Control. B. Extract: 2 c.c. C. Eggs from B into 100 c.c. sea-water after 1 hr., 30 mins.

Result: No divisions at any time within 24 hrs.

XXVI. Seven sets of testes in 10% alcohol 4 days.

A. Control. B. Extract: (a) 15 c.c., (b) 8 c.c., (c) 2 c.c. C. Some of the eggs of each of B in 100 c.c. normal sea-water after 2 hrs.

Result: Negative during the first twelve hours. At the end of 24 hrs. there were a very few 2 and 4 cell groups in the control and among those of (a) which had been transferred. No effect in any of the others.

Alkaline extract. — Many enzymes show their greatest activity in media which are either acid or alkaline. Fluids of either reaction are also especially efficient in transforming zymogens into enzymes. If the latter cannot be extracted from spermatozoa, as the preceding results may be taken to indicate, might not zymogens be detected?

Loeb[1] found, in his experiments on Echinoderms and Annelids that the addition of a small quantity of acid or alkali caused the unfertilized eggs to segment much more quickly than when they were left in normal sea-water. NaOH seemed less effective than KOH, but some development occurred in the presence of as little as 2 c.c. $\frac{n}{10}$ NaOH in 100 c.c. sea-water. Great care had to be exercised here, therefore. Proportionately smaller amounts were used as a safeguard.

A saline solution was made for this series containing 8 c.c. of $\frac{n}{10}$ NaOH for every 100 c.c. $\frac{1}{8}$ *n* NaCl. This solution was faintly though distinctly alkaline and could hardly be considered destructive to any enzymes in the cells. In control experiments similar to those conducted previously to ascertain the influence of foreign substances it was found that as much as 25 c.c. of this

[1] Loeb: This journal, 1901, iv, p. 438; also *Ibid.*, 1900, iii, p. 136.

solution when added to eggs in 100 c.c. of sea-water caused only a few initial segmentations and that comparatively slight influence was exerted either on osmotic parthenogenesis or spermatic proliferation by the same quantity.

XXVII. Twenty sets of testes in 100 c.c. alkaline solution 24 hrs.

A. Controls (2). B. Extract: 25 c.c., 10 c.c., 5 c.c., 1 c.c.

C. Some of each of B in 100 c.c. normal sea-water after 1 hr.

Result: Not a single division.

Extract made in fluid of alternate reaction. — XXVIII. With a view of aiding still further the transformation of any zymogen not affected by previous extractions, twelve sets of testes were macerated in the usual way and allowed to remain in the mortar, covered with a glass plate, for 12 hours. The normal alkaline reaction of the fresh tissue became faintly acid to litmus during that interval. 25 c.c. of fresh water was added, the mixture neutralized and then made faintly alkaline with $\frac{n}{10}$ NaOH and repeatedly shaken up in this mixture for about 6 hours. Finally it was neutralized with very dilute HCl and the filtrate mixed with one-third its volume of $2\,n$ NaCl to bring the concentration of the extract close to that of ordinary sea-water.

A. Controls (2). B. Extract: (a) 20 c.c., (b) 10 c.c., (c) 1 c.c.

C. Samples of B in 100 c.c. normal sea-water after 1 hr., 30 mins.

Result: No effect during the first twelve hours. At the end of 24 hrs. only an occasional 2-cell division could be found in (c) and among those of (a) which had been transferred.

The persistently negative results of the preceding experiments, in which the existence of neither an enzyme nor a zymogen could be indicated, gradually developed the idea that possibly an enzyme is formed from material in the egg, or in the sperm, or in both, on contact of the two living elements. If such were really the case it would seem that extracts of the eggs which had been normally fertilized might, under appropriate conditions, possess the power of inducing segmentation in unfertilized ova.

Extracts of fertilized eggs.—The general experimental procedure by which this matter was investigated was essentially the same in some respects as for the preceding series. The fresh full ovaries were broken up in sea-water in shallow dishes. Only sufficient ova were kept in each dish to form a single layer at the bottom. The glandular tissue, with such eggs as remained entangled in it, was withdrawn. A minute quantity of fresh spermatic fluid was thrown into 100 c.c. of sea-water and a few drops of this mixture transferred to the dishes containing the eggs. Within a few hours practically all of the eggs were developing and some spermatozoa in excess were in active motion among them.

When the eggs were desired for extraction the fluid containing them was

thrown into a large funnel, the outlet of which was closed with a stopper. The eggs quickly converged to the neck and soon settled to the bottom of the tube in a thick layer, with a clear supernatant fluid. Practically all of this could be eliminated by decantation, leaving a thick mass of eggs in only a small quantity of fluid. The whole process of collection could be completed in two hours. The segmented eggs were finally thoroughly ground with sand and appropriately extracted.

Glycerine extract. — XXIX. Eggs from 15 females, many of which had developed to the 16-cell stage, were ground, in small quantities, with 30 c.c. sea-water and 30 c.c. pure glycerine. They were repeatedly shaken in this mixture. At the end of 24 hours the eggs were considerably swelled and distorted, but were little disintegrated, in spite of the grinding. The latter process was repeated. More of the eggs were broken up, but many were held intact by the fertilization membrane. The extraction process was continued 36 hours longer, by which time at least half of the eggs were still unbroken, though distended. A clear filtrate was obtained.

A. Controls (2). B. Extract: (a) 12 c.c., (b) 8 c.c., (c) 4 c.c., (d) 1 c.c., (e) 0.25 c.c. C. Some eggs in each of B were transferred to 100 c.c. normal sea-water after 2 hours.

Result: No segmented cells were found in any except (d). After 12 hours 3 or 4 irregular 2 to 4 cell groups could be found among thousands after diligent search.[1]

Saline extract.— XXX. Eggs from 20 females. Development was allowed to continue until the more advanced had reached the morula stage, when only a very few remained unsegmented and the majority were at or beyond the 8-cell proliferation. They were ground up in 40 c.c. of fresh water, to which 40 c.c. of $\frac{1}{8}^{0}$ *n* NaCl was added later. Extraction was continued 36 hours. At the end of that time many groups of cells remained tightly held together in the enclosing membrane; thorough grinding had not sufficed to disintegrate them as completely as was desired.

A. Controls (2). B. Extract: (a) 35 c.c., (b) 20 c.c., (c) 10 c.c., (d) 5 c.c., (e) 1 c.c. C. Some of the eggs of each of B transferred to 100 c.c. of sea-water after 2 hrs.

Result: Negative at first. After 12 hrs. occasional irregular forms in initial cleavage were found among thousands in one of the controls, in (b), (c), (d), and among those of (a), (b), (c), and (e), which had been transferred to normal sea-water — just such forms as are sometimes found among normal unfertilized Arbacia eggs which have been kept undisturbed in sea-water for about 24 hours.

Alcoholic extract. — XXXI. Eggs from 18 sets of ovaries, after segmenta-

[1] The extracts of the fertilized eggs were no more destructive to the test-eggs than the sperm extracts had been. See page 63.

tion had proceeded in many to the blastula stage, were ground in 20 c.c. of sea-water and extracted in this fluid plus 20 c.c. of 20% alcohol. Extraction was continued for 48 hours. The alcohol favored complete disintegration, for before 24 hours practically all of the cells were reduced to granules.

A. Controls (2). B. Extract: (a) 15 c.c., (b) 8 c.c., (c) 5 c.c., (d) 1 c.c. C. Some of each of B transferred to 100 c.c. normal sea-water after 2 hrs.

Result: After 12 hrs. a small number of cells in irregular initial segmentation were found among those of one of the controls, also in (d) and among those of (a) which had been transferred to sea-water. The number was less than 10 in 1,000.

Discussion of Results.

The chief feature of the results we have obtained is their negative character. Occasionally segmentations were noted, but these were few and rarely went beyond the 2-cell stage. Further, when the test-eggs segmented those of the controls did also. These few divisions could not have been due to spermatozoa, since not a single group was surrounded with the fertilization or so-called "vitelline" membrane, whose absence, Loeb[1] has indicated, practically proves non-spermatic influence. Thousands of eggs in the control and extract series were carefully examined in each experiment and yet only a trifling proportion showed initial segmentation; excepting very few, none of these went as far as the 8-cell stage; and no morula or swimming larva was ever seen.

The conditions of the experiments were made as nearly normal as possible and every precaution was taken to guard against evaporation. Special ion parthenogenesis was entirely excluded, therefore. All of the eggs were ascertained to be ripe and susceptible to segmentation influences. Sufficient variety of extraction process was employed to guard against failures in withdrawal method and the many experiments excluded accidental sources of error. It seems necessary to conclude, therefore, that the occasional segmentations in initial stages that were observed were only such as have repeatedly been seen in ripe unfertilized Arbacia eggs which have been exposed to sea-water for from twelve to twenty-four hours.[2]

I have not exhausted the means commonly used for enzyme extraction. The time at my disposal for this work, and the facilities of

[1] Loeb: This journal, 1901, iv, p. 454.

[2] Loeb: *Ibid.*, 1899, iii, p. 136; 1900, iii, pp. 436 and 437.

this laboratory, have not favored the trial of every known method nor attempts to devise new ones. It may be that sperm enzyme is as intimately connected with the structural elements of the cell, and as resistant to extraction processes, as Fischer has found the inverting ferment of *Monilia candida* to be. Buchner's experience with zymase has not been overlooked, nor the suggestions it offers ignored. However, unless the hypothetical sperm enzyme were very different from most of the others, the numerous methods employed would have succeeded in bringing it to light, if any enzyme action can be exerted by substance in fluids surrounding the ova.

It should be recalled in this connection that Loeb[1] has recently made a series of experiments with various foreign enzymes to determine proliferative power on unfertilized Arbacia eggs, but with negative results. He states that "the only enzyme that caused the egg to segment at all was papain," but he could not be certain that this was not due to some accidental constituent of the sample of enzyme used. "The other enzymes were absolutely without effect." Two years ago Mathews, in some unpublished experiments cited by Loeb,[2] tried the effect of rennin on unfertilized eggs of the sea-urchin. The eggs were placed in sea-water solutions of rennet tablets for a while and then transferred to normal sea-water, when segmentation into a comparatively small number of cells resulted. The effect closely resembled those previously described by Morgan,[3] and Mathews concluded that the results noted had been produced not by the enzyme, but by the salts in the tablets increasing the concentration of the water.

Negative results rarely justify sweeping deductions. The outcome of these experiments, negative in detail, rather emphasizes possibilities which have not yet been specially considered. It may be that either too much extract was employed in each series for positive results to occur or else possibly not enough was taken. Such possibility led to the wide variations of quantity and condition in these experiments, but as no differences were noted between the effects of the largest as contrasted with the smallest proportions of extract, the results afford no conclusive answer in this connection.

Again, since enzymes are indiffusible, or, at most, are only very

[1] LOEB: This journal, 1901, iv, p. 456.

[2] LOEB: *Ibid.*, 1900, iii, p. 437.

[3] MORGAN: Archiv für Entwickelungsmechanik der Organismen, 1899, viii, p. 448.

slightly diffusible, it is possible that, in experiments of the kind conducted by Loeb, Mathews, Piéri, Winkler, and myself, enzyme which may be contained in the extract does not or cannot enter the substance of the ovum. It might be assumed that mere contact with enzyme in such solution would not cause segmentation and that, even if the peripheral portions of the cytoplasm should be directly affected by such immersion, the general effect would be entirely different if contact, or diffusion, occurred within the substance farther toward the nucleus. Further, may not the morphological character of the spermatozoön, specially adapted as it is for great motility and penetration, imply that segmentation by indiffusible enzyme, contained in fluid surrounding the ovum, is no more possible in artificial than in normal fecundation. If it be ever found that enzymes, or zymogens, are causative influences in natural fertilization, I venture to predict, in view of the results of these experiments, that their action will also be shown to depend on their direct delivery to points *within* the ovum.

The results of this work do not warrant any additions to current speculations on the mechanism of fertilization, but a recent suggestion may seem to be connected with these results and therefore should be considered here.

Loeb,[1] referring to his experiments with Echinoderms and Annelids, has expressed the view that "the spermatozoön can no longer be considered *the cause* or *the stimulus* for the process of development, but merely an agency *which accelerates a process that is able to start without it*, only much more slowly." Accordingly it may be assumed that "the spermatozoön carries a catalytic substance into the egg." Loeb considered that enzymes and ions may be among these "catalytic substances."

If ions are to be reckoned among the agents of proliferation, why it may be asked, did they not make active the sperm extracts used in these experiments? But what is the proportion of dissociated electrolyte in the spermatozoön and in such extracts, it may be inquired in return? The composition of the ash does not furnish an accurate idea of the amount in the spermatozoön of salts pre-existent as salts and *dissociable* in extracts. Arbacia spermatozoa have not been analyzed in this connection nor the amount of *dissociated* electrolytes in these extracts determined. We know little of the relative proportions of the various constituents of spermatozoa and ova. As

[1] Loeb: This journal, 1901, iv, p. 456.

we have no knowledge of the absolute or relative quantity of free *ions* entering or acting within the ovum, we therefore know nothing of the influence or sufficiency in this connection of the methods used in these experiments. Further, the ions which become active in the ovum may be originally a part of the molecules of the proteid compounds of the ovum or of the sperm, or of both, until the sperm mingles with the protoplasm of the ovum and forms new and probably simpler combinations. These experiments were neither intended for, nor were their conditions suited to an investigation of these particular problems. The results therefore cannot be interpreted as having any bearing on them.

It may not be amiss to state, before concluding, that Vigier's [1] assumptions that unfertilized eggs of Arbacia develop into swimming larvæ in normal sea-water were invariably contradicted by my numerous experiments. Vigier says he was unable to repeat Loeb's results on artificial parthenogenesis. I have often used Loeb's methods with success in order to determine the responsive character of the eggs used in the extract series.[2] Swimming larvæ can be produced and reared to the pluteus stage with ease.

Summary of Conclusions.

The positive experimental results of Piéri should be attributed to the action of spermatozoa which had not been removed from the extracts.

Winkler's uncertain results were doubtless the effects of osmotic influences.

Extracts of the spermatozoa of Arbacia, which have been made by the ordinary methods for the preparation of enzyme solutions, and used in the proportions and under the conditions of these experiments, do not possess any power of causing proliferation of the ripe ovum.

No evidence could be furnished of the existence of a zymogen in spermatozoa.

Extracts of fertilized eggs in the earlier stages of development seem likewise to be devoid of any segmental activity.

The extracts did not produce the typical peripheral "vitelline" membrane always formed immediately in Arbacia eggs, on fusion of the male and female elements.

[1] See Loeb's criticism: This journal, 1901, iv, p. 454.

[2] See references in this connection on p. 57.

These negative results cannot be put forward as proof that there are no enzymes in spermatozoa which function during the normal process of fertilization. They do not show that enzyme action is impossible after, or at the time of union of the spermatozoön with the ovum within the latter, although the results of Series XXIX–XXXI might be interpreted as suggesting that enzymes are not thus elaborated.

In conclusion I wish to thank Professor Loeb not only for the suggestions which led me to undertake these experiments, but also for much kindness and encouragement.

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ON THE NATURE OF THE PROCESS OF FERTILIZATION.[1]

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Since the time of Leeuwenhoek and his pupils (1677) it has been known that the fluid secreted by the male generative organs contains spermatozoa. The earlier observers noted the active movement of these innumerable minute bodies in the fresh fluid and assumed them to be parasitic animalcules, "sperm animals." A century later, about 1786, Spallanzani demonstrated that the fertilizing power of the semen is possessed by the spermatozoa and not by the liquid portion, since the semen loses its potency when the spermatozoa are separated from it by filtration. Kölliker, in 1841, proved that the spermatozoa are formed from the cells of the testis and, therefore, are not parasites as the earliest observers had assumed, but, like the ova, are derived directly from the parent-body. In 1865, Schweigger-Seidel and La Valette St. George showed that the spermatozoön, like the ovum, is a peculiarly-modified single cell of extraordinary minuteness, containing a nucleus and cytoplasm, and on the whole morphologically equivalent to other cells. In 1875, O. Hertwig established the fact that normal fertilization of the ovum is brought about by immediate union with but one spermatozoön.

Although Leeuwenhoek had assumed that the spermatozoa must penetrate the ova in order to effect proliferation, nearly two centuries passed before the fusion process was actually observed. It was first described in detail by Fol in 1879. "In every known case an essential phenomenon of fertilization is the union of a sperm-nucleus, of paternal origin, with an egg-nucleus, of ma-

[1] The substance of this paper was given by the author at a recent meeting of the Society of Physiological Chemists New York City.

ternal origin, to form the primary nucleus of the embryo."

The exact nature of the process which causes proliferation of the fertilized egg is not yet understood. During the past few years important additions to the facts bearing on this question have been made by Loeb, whose well known studies of the mechanics of life phenomena have not only added greatly to our exact knowledge of biological events, but, also, have shown the important influence which the modern physico-chemical theories may have upon our understanding of animal functions.

Loeb had come to the conclusion, as a result of numerous and varied experiments, that "something in the constitution of the sea-water prevented the unfertilized eggs of marine animals from developing parthenogenetically." It had been known for some time that the unfertilized ova of arthropods, echinoderms and worms segment into a few cells (2-4) when left for a comparatively long time in sea-water, but this was generally considered a pathological phenomenon. In his earlier experiments Loeb kept *unfertilized* eggs of a common species of sea-urchin for two hours in sea-water whose osmotic pressure was slightly increased by the addition of various electrolytes. When the eggs were returned to normal sea-water they soon began to segment, and blastulæ, gastrulæ and plutei, which appeared to be normal in every respect, rapidly developed. In brief, the general effect in the production of the embryo was apparently the same as that ordinarily caused by spermatozoa. These same results have been obtained by Loeb with the eggs of other animals and have been verified repeatedly by other observers, including the author.

In one of his first communications of the results of the work just referred to, Loeb says: "From these experiments it follows that the unfertilized egg of the sea-urchin contains all the essential elements for the production of a perfect pluteus. The only reason that prevents the sea-urchin from developing parthenogenetically under normal conditions is the constitution of the sea-water. The latter either lacks the presence of a sufficient amount of the ions that are necessary for the mechanics of cell division (Mg, K, OH or others) or it contains too large a quantity of ions that are unfavorable to this process (Ca, Na or others), or both. All the spermatozoön *needs* to carry into the egg for the process

of fertilization are ions to supplement the lack of the one or counteract the effects of the other class of ions in the sea-water or both. The spermatozoön *may*, however, carry in addition a number of enzymes or other material. The ions and not the nucleins in the spermatozoön are essential to the process of fertilization. . . . I consider it possible that only the ions of the blood prevent the parthenogenetic origin of embryos in mammalians and I think it further not impossible that a transitory change in the ions of the blood may also allow complete parthenogenesis in mammalians."

At a somewhat later period in his work on marine animals, Loeb stated: "The spermatozoön not only starts the development of non-parthenogenetic eggs, but it is also the bearer of the hereditary qualities of the male. From our experiments it becomes evident that these two functions of the spermatozoön are not necessarily bound together, for nobody would assume for an instant that the hereditary qualities that are carried by the spermatozoön could be imparted to the egg by a change in the inorganic constituents of the sea-water. We have learned to attribute the different activities of a cell to different enzymes. We must in future consider the possible or probable separation of the fertilizing qualities of the spermatozoön from the transmission of hereditary qualities through the same. . . . The bulk of our protoplasm consists of proteid. . . . The proteids are characterized by two qualities which are of the utmost importance in the analysis of life phenomena. The proteids change their state very easily, and readily take up or lose water. . . . The agencies which affect these two variable qualities of the protoplasm most powerfully are, first of all, certain enzymes. . . . Almost equally powerful are ions in certain concentrations. . . . The third agency is temperature. In our experiments it was evidently the second factor which affected the condition of the colloids." The latter sentence refers, naturally, to the colloids of the ovum.

Subsequent experiments on sea-urchins enabled Loeb to give a more definite answer to the question of the nature of the process of fertilization. He found that an increase in the osmotic pressure of the sea-water through the addition of cane sugar or urea can produce parthenogenesis. *"This proves conclusively,"* says Loeb,

"*that the development of the unfertilized egg is produced through an increase in the concentration of the surrounding solution. As it is immaterial whether the increase in the osmotic pressure is brought about by electrolytes or non-conductors, there can be no doubt that the essential feature in this increase in the osmotic pressure of the surrounding solution is a loss of a certain amount of water on the part of the egg.* . . . . A consequence of the loss of water on the part of the egg is an increase in its osmotic pressure. The osmotic pressure inside the egg is furnished chiefly or almost exclusively by electrolytes. It is thus not impossible that the ions in the egg, if their concentration is raised, bring about that change which causes the egg to develop. If we assume that the spermatozoön starts the development of the egg in the same way as in the case of artificial parthenogenesis, it follows that the spermatozoön must possess more salts or a higher osmotic pressure than the eggs. . . . But there is no reason why the spermatozoön should not bring about the same effects that we produce by reducing the amount of water in the egg, in some different way. . . . It seems as if the liquefaction of the nuclear membrane and other constituents of the nucleus were a prerequisite for cell division." Possibly this liquefaction is accomplished by enzymes.

In his last paper, after many additional experiments on marine fauna, Loeb stated that "the bridge between the phenomena of natural and artificial parthenogenesis is formed by those animals in which physical factors decide whether or not their eggs develop parthenogenetically. In plant lice parthenogenesis is the rule only as long as the temperature is high or the plant has plenty of water. If we lower the temperature or let the plant dry out, sexual reproduction occurs. The drying-out of the plant causes the tissues of the lice to lose water. The same factor, loss of water, makes the artificial parthenogenesis of echinoderms and chætopterus possible. In plant lice the effect is of the same kind, only in the opposite direction."

Further on in the same communication, Loeb adds: "The general opinion concerning the rôle of the spermatozoön in the process of fertilization is that it acts as a *stimulus*, and that as such it starts the development of the egg. . . . If we consider the fact that the eggs show at least a beginning of segmentation under 'normal' con-

ditions, the act of fertilization assumes a different aspect. The spermatozoön can no longer be considered *the cause* or *the stimulus* for the process of development, but merely an agency which *accelerates a process that is able to start without it,* only much more slowly. Substances that accelerate chemical or physical processes which would occur without them are called catalyzers (Ostwald). According to this definition we may assume that the *spermatozoön carries a catalytic substance* into the egg, *which accelerates the process that would start anyhow but much more slowly.* . . . It would be wrong to say that *the K-ions are the stimulus* that causes the developmental process. *They merely act as catalyzers, accelerating a process that would otherwise proceed too slowly.* The loss of water on the part of the egg-cell must have a similar effect, but possibly a less direct one. It may be that the loss of water alters the chemical processes in the egg in such a way as to give rise to the formation of a substance which acts catalytically. . . . The introduction of the catalytic substances which accelerate the processes of development saves the life of the egg. This may be made intelligible on the following assumption. Two kinds of processes are going on in the mature egg after it has left the ovary. The one leads to the formation of substances which kill the egg; the other leads to the formation of substances which allow growth and cell division and are not poisonous. We may use as an illustration Pasteur's well-known experiments on the behavior of yeast cells in the presence and absence of atmospheric oxygen. In the presence of oxygen the yeast cells multiply on a sugar solution, while the zymase effect is comparatively small. In the absence of oxygen the multiplication of cells is limited or may stop, while the zymase effect becomes more prominent. The products of alcoholic fermentation are comparatively harmless for the yeast cell, and for this reason an increase in the fermentative activity of the cell does not cause the death of the yeast. I imagine that matters are similar in the mature egg-cell after it has left the ovary, with this difference, perhaps, that the substances formed (by fermentation?) in the egg-cell are more poisonous for the egg than the alcohol and the other products of fermentation are for the yeast. The process that causes the death of the egg-cell and the one that causes cell division are

at least partly antagonistic. They are both inhibited by a low temperature, so that in this case death does not occur, although no cell division is possible. If we succeed in finding a substance which accelerates the process of cell division at the normal temperature, this will at the same time lead to a suppression or a reduction of the antagonistic process that shortens life. In the case of the egg of chætopterus a trace of K-ions acts as such a catalytic substance; possibly a trace of H-ions; and perhaps certain substances that are formed when the egg loses a certain amount of water. For the echinoderm egg we know at present only the last factor. In addition there are the catalytic substances carried or produced by the spermatozoön (ions? enzymes?). But there are certainly other catalytic substances, as is proved by tumors and galls, in which the variety of structures corresponds to an almost equal variety of parasites. We do not need to assume a specific parasite for each kind of tumor. Teratomata may be explained on the basis of the parthenogenetic tendency of the mammalian egg in connection with some chemical change that furnishes the catalytic substance. But it is not impossible that even in benign tumors, such as a teratoma, the catalytic substance may be due to parasitic organisms.] It is very important to realize that the introduction of catalytic substances into the egg does not prolong its life unless the egg has reached a critical point determined by two sets of conditions. The one is the maturity of the egg, the other the change of conditions connected with the egg leaving the ovary. . . . . The fact that there is an age limit for the development of carcinoma may be a similar phenomenon. The catalytic substances which are given off by the cancer parasite may not be able to bring about cell division in the epithelial cells unless the latter have reached a critical point, which is at least partly determined by the age of the individual."

Among the catalytic substances which Loeb has constantly had in mind in his brilliant observations in this connection are enzymes, as has already been indicated. With the advice and many helpful suggestions of Professor Loeb, the writer, working in Prof. Loeb's laboratory at Wood's Holl, recently attempted to ascertain whether any experimental justification can be found for the assumption that the spermatozoön

carries substance into the ovum which effects proliferation by zymolysis.

Pieri appears to have been the first to give this question experimental examination. Several years ago (1897) he reported that he had extracted soluble enzyme from the testicles of two varieties of sea-urchin, which had the power to bring about segmentation of ova of the same varieties. The enzyme, which he called "ovulase," was obtained, he said, by merely shaking the testicles in distilled water or sea-water. As he himself was not sure that all spermatozoa were killed in the extraction process, it seems certain that his results were due not to "ovulase," but to live spermatozoa.

Dubois, in 1900, arrived at the conclusion that natural fertilization comes about through the action of a fecundative ferment. He claims that he was able to separate such a body, "*d' une zymase fecundante,*" from the testicles of a variety of sea-urchin, but, unfortunately, no experiments showing its qualities or method of preparation were detailed by him. Dubois named the ferment (?) "spermase" and credited it with the power of modifying a hypothetical substance pre-existent in the ovum, which he called "ovulose."

Winkler, a little more than a year ago, reported the results of experiments similar to those of Pieri. Great care was taken to destroy the spermatozoa in the extracts and Pieri's work was much improved. The influence of the extracts was practically negative. Sometimes with the same extract the eggs of one individual "reacted," whereas the eggs of another did not. The proliferation never went beyond the 4-cell stage. It is well known that the unfertilized eggs of the sea-urchin are prone to divide into a few cells if they are allowed to remain undisturbed in normal sea-water for about a day—the usual length of Winkler's experiments. Winkler's results are hardly positive enough for the deduction that fecundative enzyme was obtained; they might, in fact, be used to show how unwarranted were Pieri's conclusions.

Shortly after Winkler's paper appeared, Cremer published a very brief note giving a general statement regarding some unfinished experiments by himself and Hofer. They worked with the testicles of trout and used the Hahn-Buchner pressure method for obtaining sperm extract. They found that none of the expressed fluids

from the trout spermatozoa possessed any segmental activity on mature trout ova. No description of the experiments nor methods used in testing the extracts were given by these observers in their preliminary note.

Loeb recently made a series of experiments with various non-spermatic enzymes to determine proliferative power on the unfertilized eggs of the sea-urchin, but with negative results. He states that "the only enzyme that caused the egg to segment at all was papain," but he could not be certain that this was not due to some accidental constituent of the sample of the enzyme used. "The other enzymes were absolutely without effect."

Two years ago Mathews, in some unpublished experiments cited by Loeb, tried the effect of rennin (rennet tablets) on unfertilized eggs of the sea-urchin. Segmentation into a comparatively small number of cells resulted. Mathews concluded, however, that the results noted had been produced not by the enzyme, but by the salts in the tablets increasing the concentration of the water.

Up to the time, then, that the author's work was begun it seemed possible that enzyme action might be a causative influence in normal segmentation of the ovum after introduction of spermatozoön, but no definite experimental evidence had been presented to support the theory.

Regarding the writer's work a multitude of details may be passed over and the essential facts regarding methods of procedure, etc., stated in the following brief account:

Because of the ease with which large quantities of the spermatozoa and ova of the common sea-urchin can be obtained, we used the sexual organs of this marine animal, which has furnished the material for many classical studies of cell development. The normal conditions under which fertilization and proliferation of the ova of the sea-urchin occur can be easily maintained in sea-water in the laboratory. Many of the usual methods of enzyme extraction were employed on the testicles. The eggs, always normal and mature, were kept in ordinary sea-water to which various quantities of sperm extract were added. Careful examination of the eggs was made at frequent intervals during twenty-four hours. Concentration of the sea-water was entirely prevented. The results of twenty-eight series of three to thirteen twenty-four-hour ex-

periments were entirely negative—that is, no proliferation resulted and every extract was devoid of segmental power. Control experiments were made with each series, which showed that normal conditions prevailed and that the eggs would have segmented had the extract possessed proliferative power. It was also ascertained in control experiments that the extracts were devoid of toxic property.

The persistently negative results of these experiments, in which the existence of neither an enzyme nor a zymogen could be indicated, gradually led me to believe that possibly an enzyme is formed from material in the egg, or in the sperm, or in both, on contact of the two living elements. If such were really the case it would seem that extracts of the eggs which had been normally fertilized might, under appropriate conditions, possess the power of inducing segmentation of unfertilized ova.

A large number of eggs in sea-water were accordingly treated with a drop of spermatic fluid and allowed to develop in the normal manner to various stages—in one experiment as far as the blastula stage—when the fluid was separated by decantation, the cell-groups thoroughly ground in a mortar with sand and extracted in several of the usual ways for the isolation of enzymes. None of these extracts had any power of causing fresh mature eggs to segment.

Entirely negative results rarely justify sweeping deductions. Since enzymes are indiffusible, or, at most, are only very slightly diffusible, it is possible that in experiments of the kind conducted by Loeb, Mathews, Winkler, Pieri, Cremer and myself, enzymes which may be contained in the extract does not and cannot enter the substance of the ovum, yet it may be that direct absorption of such enzyme in solution could take place through the micropyle. It may be that sperm enzyme, if such really exists, is as intimately connected with the structural elements of the cell, and as resistant to extraction processes, as Fischer has found the inverting ferment of the mould *Monilia candida* to be. But even if it is extractable, it might be assumed, with reason, that mere contact of the ovum with enzyme in solution would not cause segmentation and that, even if the peripheral portions of the cytoplasm should be directly affected by such immersion, the general effect would be entirely different if contact, or diffusion, occurred within the sub-

stance farther toward the nucleus. Possibly the morphological character of the spermatozoön, specially adapted as it is for great motility and penetration, should imply that segmentation by indiffusible enzyme contained in fluid surrounding the ovum is no more possible in artificial than it is a part of normal fecundation. If it is ever found that spermatic enzyme, or zymogens, are causative influences in natural fertilization, I venture to predict, in view of the results of our experiments, that their action will also be shown to depend on their direct delivery to points *within* the ovum.

If ions are to be reckoned among the agents of proliferation, why, it may be asked, did they not make active the sperm extracts used in these experiments? Unfortunately, we know nothing at present of the proportion of *dissociated* electrolytes in the spermatozoön and in such extracts. The composition of the ash does not furnish an accurate idea of the amount in the spermatozoön of salts pre-existent as salts and *dissociable* in extracts, although the comparatively large quantity of ash in spermatozoa, as found by Hammarsten and others, may suggest proportionately large quantity of dissociable electrolyte. We know little of the relative proportion of the various constituents of spermatozoa and ova, and we have no knowledge of the absolute or relative quantity of free *ions* entering or acting within the ovum. The ions which become active in the ovum may be originally a part of the molecules of the proteid compounds of the ovum, or of the sperm, or of both until the spermatozoön mingles with the protoplasm of the ovum and forms new and probably simpler combinations. The writer's experiments were neither intended for, nor were their conditions suited to an investigation of this particular phase of the fertilization problem. The results cannot, therefore, be interpreted as having any bearing on them.

Summing up briefly, the chief experimental results of our work are:

1. Extracts of the spermatozoa of the sea-urchin, which have been made by the ordinary methods for the preparation of enzyme solutions, do not possess any power of causing proliferation of the ripe ovum.

2. No evidence could be furnished of the existence of a zymogen in spermatozoa.

3. Extracts of fertilized eggs, in the earlier

stages of development, were likewise entirely devoid of segmental activity.

4. Enzyme seems to be excluded from the catalytic substances which Loeb and others have thought may influence the initial divisions of the ovum after the introduction of the spermatozoön, although it is possible that the conditions of these and previous experiments were unfavorable to the manifestation of activity on the part of fecundative ferment. It seems more probable, however, that Loeb's theory of the influence of spermatic *ions* in fertilization affords the true explanation of the phenomena in question.

Free use in the preparation of this paper has been made of facts and statements in the following publications:

Wilson. The Cell in Development and Inheritance, 1898.

Loeb. Papers in the American Journal of Physiology on Artificial Parthenogenesis: 1899, iii, p. 135; 1900, iii, p. 434, and iv, p. 178; 1901, iv, p. 424.

Gies. Do Spermatozoa Contain Enzyme Having the Power of Causing Development of Mature Ova? American Journal of Physiology, 1901, vi, p. 53.

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11

NOTES ON THE "PROTAGON" OF THE BRAIN.[1]

By W. W. LESEM and WILLIAM J. GIES.

SEVERAL years ago Chittenden and Frissell[2] made a study of the distribution of phosphorus-containing substances in the brain. The results obtained by them seemed to "indicate that protagon contains but a small proportion of the total phosphorus of the brain and that other phosphorized organic bodies, such as lecithins, are present, preformed in the tissue, in relatively large proportion." They concluded that "the dry solid matter of the brain contains as much or even more lecithin than protagon." Chittenden and Frissell also observed that, "contrary to previous statements, protagon tends to undergo cleavage by long-continued heating at 45° C. in 85 per cent alcohol, a certain amount of an alcohol-soluble (at 0° C.) body richer in phosphorus than protagon, being split off while the residual protagon obtained by recrystallization at 0° C. contained a somewhat diminished percentage of phosphorus.

Shortly after the publication of the brief note containing the above deductions, Dr. Gies repeated and extended the experiments begun by Dr. Frissell. The general conclusions of this second series of experiments were practically the same as those previously reported, but as the work was unavoidably interrupted, no further reference was made to them. Recently, however, new experiments on protagon have been performed by Mr. Lesem and Dr. Gies. The results of these experiments, to which we shall refer farther on, make it seem desirable to give here some of the related data of the earlier experiments in which the work of Chittenden and Frissell was repeated.

[1] This work was begun by Dr. Gies under Professor Chittenden's supervision, in the Sheffield Laboratory of Physiological Chemistry at Yale University. It was completed by Mr. Lesem and Dr. Gies in the Laboratory of Physiological Chemistry at Columbia University.

[2] Chittenden: Proceedings of the American Physiological Society, Science, 1897, v. (N. S.), p. 901.

I. On the General Distribution of Phosphorus-Containing Substances in the Brain.

The brains employed in the experiments by Chittenden and Frissell were taken from sheep. Although the brains were used within twenty-four hours after the death of the animals, it seemed possible that, even within that short period, bacterial changes might have had some influence on the results.[1] In repeating the first series of experiments, this difficulty was obviated by the adoption of the following procedure, which is the same as that used by Chittenden and Frissell,[2] except in the steps taken at the beginning to prevent possible alterations through the influence of bacteria.

First experiment. — In this experiment glass-stoppered bottles of convenient size, containing about 750 c.c. of 85 per cent alcohol, were accurately weighed and removed to the slaughter house without loss of fluid. The sheep were killed in the usual way. The greater portion of blood disappeared from the brain in a minute or two, when the head was opened with a cleaver and the entire brain quickly removed. Superficial blood and lymph were taken off promptly with a clean dry cloth. While the brains were still at practically the normal body temperature, they were rapidly slashed with a scalpel and at once transferred to the bottled alcohol. Two whole brains were deposited in each of three bottles. Special care was taken to prevent any loss of alcohol by evaporation or by spilling.

It would seem that this prompt treatment with alcohol prevented such post-mortem changes as exposure for several hours to the air, a lowered temperature, etc., might induce. We do not mean to suggest, however, that the alcohol itself has no transforming power on the phosphorized constituents. Such influence, if exerted, would doubtless have been no greater, nor any different, at this point than later on.

The quantities of tissue in each bottle were 152.99, 172.19, and 148.89 gms.

Preliminary cold extracts. — The tissue remained in the original alcohol about four hours, when the filtrate was collected and the tissue very thoroughly macerated in a mortar. The finely divided material was next transferred to 750 c.c. of 85 per cent alcohol, and kept under it over

[1] The results of the following experiments show, however, that no appreciable changes of such character could have been effected.

[2] The methods employed by Chittenden and Frissell could not be described in the very brief abstract of the preliminary report of their work. For that reason we give the methods here in some detail.

night, after which the filtrate was again separated. These two cold extracts were combined.

Extracts at 45° C. — Extraction was next made in 85 per cent alcohol (1½ litres for each pair of brains) for ten hours at 45° C., and the filtrate again collected. After standing in 2 litres of 85 per cent alcohol, at room temperature over night, the alcohol-tissue mixture was warmed to 45° C. and held at that temperature for twelve hours, after which the filtrate was again obtained. The residual tissue was once more kept in 2 litres of 85 per cent alcohol over night and further extracted in the same fluid at 45° C. for fourteen hours, when the filtrate was preserved as before. After each of these filtrations, the solid substance was washed with a little warm alcohol (85 per cent), and the washings added to the appropriate filtrate.

Extraction in boiling alcohol. — At this point the tissue remained in 1 litre of 85 per cent alcohol over night, when the mixture was boiled on a water bath for a half hour. After filtering, the tissue was also extracted in boiling 95 per cent alcohol for the same length of time. These two hot alcoholic extracts were combined.

Tissue residue. — The residual tissue was finally washed with cold 95 per cent alcohol, then with absolute alcohol, and dried to constant weight at 80° C.

Treatment of the extracts. — The extracts obtained at room temperature and in boiling alcohol were separately evaporated in silver crucibles almost to dryness, and the total phosphorus content determined directly. The cold extract of our first preparation, however, was separated into protagon and filtrate therefrom by the method referred to below.

The three extracts obtained at 45° C., in the second and third preparations, were separately reduced to 0° C. with the aid of common freezing mixture, and held at that point for six hours. A heavy flocculent precipitate containing much crystalline cholesterin, protagon, etc., quickly separated from the first of each series of three extracts. The precipitate was considerably less in the second extract, and only a very faint turbidity was formed in the third. Each precipitate was quickly filtered, at a temperature slightly below 0° C., on funnels surrounded by freezing mixture. The precipitates were washed once with cold 85 per cent alcohol, and then with cold ether until free from cholesterin. The alcohol washings were added to the same filtrates. The filtrates were combined and evaporated for the determination of phosphorus. The ether washings were given the same treatment. The protagon products were dried at a low temperature on the filter papers. Phosphorus was determined in the mixture of protagon and filter papers, the latter having been free from that element.

Phosphorus was always determined by the usual fusion method.

Analytic results. — The following table gives our analytic results for phosphorus in the various solids and fluids separated by the above method:

TABLE I.

Extracts, etc.	Phosphorus content.						
	I.	II.	III.	II.	III.	II.	III.
	Grams.			Percentage of total solid matter.		Percentage of total phosphorus.	
A. Cold extracts (2) . . .	0.1423	0.1348	0.1841	0.35	0.43	26.32	32.33
a. Protagon	**0.0432**						
b. Filtrate from protagon	0.0991						
B. Extracts at 45° C. (3) . .	....	0.2599	0.2887	0.68	0.67	51.14	50.38
a. Protagons	....	**0.0874**	**0.1008**	**0.23**	**0.23**	**17.30**	**17.30**
b. Filtrates from protagons	....	0.1370	0.1401	0.36	0.33	27.07	24.81
c. Ether washings of protagons	....	0.0355	0.0478	0.09	0.11	6.77	8.27
C. Extracts in boiling alcohol	....	0.0047	0.0054	0.01	0.01	0.75	0.75
D. Tissue residue	....	0.1098	0.0939	0.29	0.22	21.80	16.54
Total phosphorus . .	....	0.5092	0.5721	1.33	1.33		
Weight of fresh tissue . .	148.89	152.99	172.19				
Weight of tissue residue . .	....	15.25	17.08				
Estimated solids in fresh tissue (25 per cent)	....	38.25	43.05				
Estimated weight of extracted matter	....	23.00	25.97				

That the preliminary cold extracts contained a comparatively small amount of protagon seems to be indicated by the results for our first preparation. Protagon is only slightly soluble in 85 per cent alcohol at 0° C., and is practically insoluble in ether at the same temperature. Thus of 2 grams of protagon, 0.03 to 0.04 gram dissolved in 500 c.c. of 85 per cent alcohol at 0° C. The same quantity of ethereal filtrate from 3.6 grams of protagon, at the same temperature, contained nothing yielding a phosphorus reaction after fusion with alkali. It is possible that the presence of the other constituents of the alcoholic extract may increase or decrease this solubility. It is hardly probable, however, that more than an insignificant portion

of the protagon remains unprecipitated on lowering to zero the temperature of alcoholic extracts such as the above.

Second experiment. — We decided to repeat the experiment again, but with less tissue. The results of our previous experiment had been obtained for the whole brain. We now endeavored to ascertain whether the above data apply equally to all portions of the brain or whether there are wide phosphorus variations for the parts. This was accomplished indirectly without materially altering the conditions of the previous experiment. For the purpose indicated we took amounts of tissue equivalent in weight to a whole brain, but made up of different parts of two brains.

The method of treatment at the slaughter house, transportation in weighed alcohol, extraction in 85 per cent alcohol at room temperature, at 45° C., etc., separation of protagon, etc., were the same in this as in the first experiment. Samples of the fresh tissue were used for determinations of solids and phosphorus.

At the slaughter house the brains were carefully sectioned transversely into halves just before their deposition in the alcohol. The halves were combined as indicated in the next table. The preliminary extracts in cold alcohol were united with those obtained at 45° C., and the protagon was removed from the mixture. Four extractions of each sample of tissue were made at 45° C. One litre of 85 per cent alcohol per brain was used each time. The washing of the protagons with ether was omitted.

Table II, on page 188, gives the essential results of this experiment.

Only insignificant differences are to be observed between the results of the first two experiments. The analytic data are, therefore, essentially the same for the anterior and posterior halves of the brain. The similarity of the results of this series to those of the preceding is especially evident from the directly comparable data given in Table III on page 188.

The results of the first and second experiments show that the greater portion of the phosphorus of the brain is contained in substances not precipitable as protagon. The bulk of the phosphorus in the preliminary cold extract (Exp. 1), and in the filtrates from the protagons (Exps. 1 and 2), is doubtless contained in substances as readily soluble in alcohol as lecithin. Some phosphate was also present. Probably most of the phosphorus of the ether washings (Exp. 1) was contained in substance which was soluble in the

alcohol (and in the ether), but which adhered to the precipitate until it was treated with ether.

TABLE II.

Extracts, etc.	Phosphorus content.			
	A. Ant. half of 1. Post. half of 2. Grams.	*B.* Ant. half of 2. Post. half of 3. Grams.	*C.* Ant. half of 3. Ant. half of 4. Grams.	*D.* Post. half of 4. Post. half of 5. Grams.
I. Ext. at room temp. and at 45° C. .	0.2512	0.2152	0.2446	0.2353
a. Protagons (4) .	**0.0953**	**0.0728**	**0.0900**	**0.0869**
b. Filtrates from protagons . .	0.1559	0.1424	0.1546	0.1484
II. Extracts in boiling alcohol . . .	0.0013	0.0013	0.0015	0.0016
III. Tissue residue . .	0.0635	0.0432	0 0517	0.0467
Total phosphorus: *a.* Total in all parts	0.3160	0.2597	0.2978	0.2836
b. As determined directly . . .	....	0.2694	....	0.3038
Weight of fresh tissue .	84.86	72.07	86.44	82.23

Whether these soluble substances exist "preformed" in the brain, as Chittenden and Frissell and others believe, or are decomposition products resulting from the use of the reagents, as some infer, is not made clear by these experiments. The former view seems more probable.

TABLE III.

Exp.	Brain.	Weight of fresh tissue. Gms.	Phosphorus content.				
			Protagon. Gm.	Filtrate. Gm.	Hot extract Gm.	Tissue residue. Gm.	Total. Gm.
First	One half of III.	**86.10**	0.0743 [1]	0.1621 [2]	0.0027	0 0469	0 2860
Second	C.	**86 44**	0.0900	0.1546	0.0015	0 0517	0.2978

[1] Including ether washings. [2] Including cold extract.

The results of the next experiment lead to essentially the same conclusions as those drawn from the preceding.

Third experiment. — The methods of this experiment were, in general, the same as those of the first and second. The following differences of treatment are to be noted. The divisions of the brains were made longitudinally instead of transversely. The alcoholic filtrates (2), obtained at 0° C. after separation of the protagon, were evaporated almost to dryness on a water bath at 35°–40° C. The residues thus resulting were thoroughly extracted several times with a moderate excess of cold ether. The extracts were filtered and evaporated to dryness. The residue left after treatment with ether was extracted with boiling 95 per cent alcohol. So little seemed to dissolve that the alcoholic extracts were evaporated with the ethereal. The substance remaining after the extraction with alcohol, mostly inorganic matter, was next treated with water. All of it dissolved very readily. This solution was then evaporated to dryness. Phosphorus was determined in the substance from each of these extracts and in the protagon, with the results tabulated below:

TABLE IV.

Extracts, etc.	Phosphorus content.		
	A. Same lateral halves of brains 1 and 2. Grams.	*B.* Opposite lateral halves of brains 1 and 3. Grams.	*C.* Opposite lateral halves of brains 4 and 5. Grams.
I. Protagons (2)	**0.0576**	**0.0701**	**0.0667**
II. Filtrates	0.1725	0.1841	0.2037
a. Substance soluble in alcohol and ether	0.1531	0.1603	0.1750
b. Residual substance soluble in water	0.0194	0.0238	0.0287
Total phosphorus . . .	0.2301	0.2542	0.2704
Weight of fresh tissue	89.46	104.50	102.50

II. On the Question of the Chemical Individuality of Protagon.

Twenty years ago Gamgee expressed himself on this subject as follows: "There is no subject in physiological chemistry concerning which it is more difficult to give a statement, which would be accepted

as correct by those who have devoted their attention to it, than the chemistry of the complex phosphorized fats which exist in the nervous tissue."[1] The same may be said perhaps with equal force to-day, in spite of the careful work done in the mean time to solve the problems connected with the chemical constituents of the brain.

Soon after Liebreich[2] separated from the brain the substance he called protagon, Thudichum[3] and others denied the existence of such a substance. Thus, Diaconow,[4] working as did Liebreich, in Hoppe-Seyler's laboratory, obtained results which led him to conclude that protagon is a mixture of lecithin and cerebrin. The later researches of Gamgee and Blankenhorn,[5] however, furnished data which were generally accepted as amply confirming the original conclusions of Liebreich. The subsequent work of Baumstark,[6] Kossel and Freytag,[7] and Ruppel,[8] particularly, further emphasized the growing confidence in the existence and importance of protagon as a brain constituent. Until recently the matter seemed to be settled in the general conviction that protagon is a chemical individual, in spite of Thudichum's claims to the contrary. As late as 1899 Hammarsten[9] indicated, as follows, the prevalent feeling toward the non-concurrent conclusions in which Thudichum has persisted: "Thudichum claims to have isolated from the brain a number of phosphorus-containing substances which he divides into three main groups: kephalins, myelins, and lecithins. Thus far, however, his results have not been confirmed by any other investigators."

The work of Kossel and Freytag may be regarded as an approach to Thudichum's position with reference to the composite nature of protagon. Kossel and Freytag discovered that protagon contains sulphur. Variations among their several products, in spite of great care in preparation, also led them to believe in the existence of several protagons. Further than this, they found that protagons

[1] GAMGEE: A text-book of the physiological chemistry of the animal body, 1880, i, p. 425.

[2] LIEBREICH: Annalen der Chemie und Pharmacie, 1865, cxxxiv, p. 29.

[3] THUDICHUM: Chemisches Centralblatt, 1875, p. 408.

[4] DIACONOW: Centralblatt für die medicinischen Wissenschaften, 1868, p. 97.

[5] GAMGEE UND BLANKENHORN: Zeitschrift für physiologische Chemie, 1879, iii, p. 260.

[6] BAUMSTARK: *Ibid*, 1885, ix, p. 145.

[7] KOSSEL UND FREYTAG: *Ibid*, 1893, xvii, p. 431.

[8] RUPPEL: Zeitschrift für Biologie, 1895, xxxi, p. 86.

[9] HAMMARSTEN: Lehrbuch der physiologischen Chemie, 1899, p. 366.

readily yield several substances similar to or identical with some described by Thudichum,[1] and which he still contends are among the fourteen (!) different bodies contained in the protagon mixture. The subsequent work of Chittenden and Frissell also gave indications of facts in harmony with the earliest results of Diaconow and his view that protagon is a mixture. Lately, Wörner and Thierfelder[2] attacked the problem by improved methods, and obtained results which seem to show that protagon is not an individual substance, or else that it is a remarkable labile body, physically and chemically.

Below we give the results of our repetitions of the experiments of Chittenden and Frissell bearing on the matter in question.

Fourth experiment. — A sample of protagon which had been prepared by Dr. Frissell from sheep brains by the usual method — precipitation from warm alcoholic extract at 0° C. and thorough washing in ether at 0° C. — was placed at our disposal for this experiment.

We further purified the protagon by recrystallizing it once from alcohol. 25 gms. of the product was kept in 1500 c.c. of 85 per cent alcohol at 40° C. for twelve hours and the mixture repeatedly stirred. At the end of that time only about half of the substance had dissolved.

First product and filtrate. — The mixture was filtered and the protagon separated from the extract by the usual cooling process, etc. The filtrate from the protagon was evaporated to dryness.

Second product and filtrate. — That portion of the original protagon which remained undissolved was again subjected to treatment in the same amount of alcohol. Most of the substance dissolved at the end of twelve hours. The second portions of protagon and evaporated filtrates were obtained as before from the filtered extract.

Third product and filtrate. — The protagon still remaining undissolved after the second extraction with alcohol was again placed in the same amount of warm alcohol for a similar period. Protagon was separated from the extract and the filtrate from it evaporated to dryness as before.[3]

Insoluble portion. — A fairly large proportion of the original protagon remained insoluble under these conditions.

Alcohol-ether washings. — Each successive residual portion of protagon referred to above was washed with warm alcohol and the washings added to

[1] THUDICHUM: Die chemische Konstitution des Gehirns des Menschen und der Tiere, 1901, pp. 54–57; 328.

[2] WÖRNER UND THIERFELDER: Zeitschrift für physiologische Chemie, 1900, xxx, p. 542.

[3] The crystalline appearance of these various protagon products was practically the same.

the filtrates. All of the samples of freshly precipitated protagon were washed first with a small quantity of cold 85 per cent alcohol and later with moderate excess of cold ether. The alcoholic and ethereal washings of the freshly precipitated protagon were combined and evaporated.

Treatment of the products. — The portions of protagon, and the substance in the filtrates and washings, were carefully determined quantitatively. Phosphorus was also estimated in each by the usual fusion method.

The following summary gives our data in this connection : —

TABLE V.

Protagon, etc.	Weight in grams.[1]	Percentage of phosphorus.
A. Freshly precipitated protagon :		
a. From first extract	10.834	1.23
b. From second extract . . .	7.599	0.89
c. From third extract	1.729 (**20.162**)	0.57
B. Insoluble protagon (residue) . .	**2.009**	0.12
C. Substance in filtrates from the freshly precipitated protagon:		
a. Of first extract	0.785	2.59
b. Of second extract	0.678	1.31
c. Of third extract	0.250 (**1.713**)	0.85
D. Alcohol-ether washings of the freshly precipitated products	**0.282**	2.02
Total substance recovered . .	24.17	
Total substance taken	24.34	1.16

[1] The weights are for substance dried in vacuo over H_2SO_4 to constant weight.

Fifth experiment. — We repeated the preceding experiment with two freshly prepared samples of protagon made by us from two different quantities of sheep brains. These samples of protagon were prepared by the usual method and were twice recrystallized. Twelve gms. of each was used. Two treatments were made with 1½ litres of 85 per cent alcohol at 45° C., etc., as in the fourth experiment, with the results tabulated on page 193 :

Among the points to be noted in Tables V and VI is the decreasing percentage content of phosphorus in each successive protagon and in the final insoluble residue. Also, the unusually high though diminishing proportion of phosphorus in the substance of the filtrates obtained each time protagon was separated at 0° C.

Our method of fractional separation was that customarily employed in the purification of protagon. Here it was merely repeated more frequently than usual. Instead of obtaining purer protagons in the process, however, it appears that, with each successive precipitation, the substance itself changed in composition and, also, that variously composed products were liberated into the filtrates from the protagons at the same time. The final residue was wax-like and quite different from the snow-white protagon of the first extracts. We are certain that our products were "pure" at the start.

TABLE VI.

Protagon, etc.	I.		II.	
	Weight in grams.	Percentage of phosphorus.	Weight in grams.	Percentage of phosphorus.
A. Freshly precipitated protagon:[1]				
a. From first extract . . .	5.945	1.21	3.659	1.19
b. From second extract . .	2.680 (**8.625**)	1.01	2.009 (**5.668**)	1.11
B. Insoluble protagon (residue) .	**0.655**	0.91	**3.892**	1.18
C. Substance in filtrates from the freshly precipitated protagon:				
a. Of first extract	1.613	2.22	1.321	1.80
b. Of second extract . . .	0.983 (**2.596**)	1.30	0.981 (**2.302**)	1.45
Total substance recovered[2] .	11.876	....	11.862	
Total substance taken . . .	12.150	1.26	12.150	1.23

[1] The precipitates were washed only with cold alcohol.
[2] See note 1 in the preceding table.

The data of the last two experiments are in close agreement with the similar facts found by Chittenden and Frissell. They are in harmony with corresponding data recently published by Thudichum.[1]

These results were obtained by applying the usual purification method. They show, we think, that protagon is either a mixture of bodies, or else a substance decomposing quite readily under the conditions of such experiments. If the latter conclusion appears to

[1] THUDICHUM: Die chemische Konstitution des Gehirns des Menschen und der Tiere, 1901, pp. 84–85.

be more probable than the former, it must then be admitted that thus far no standard of purity for protagon has been raised which is not open to the objection that it is based on methods involving unavoidable decomposition.

Elementary composition of protagon. — It seemed desirable at this point to ascertain the general elementary composition of several of the protagon products prepared in the preceding experiments. The summary below gives our results for four representative preparations:

TABLE VII.

Elements.	Percentage composition of protagons.[1]											
	Fourth experiment.						Fifth experiment.					
	a.			*b*			I.			II.		
C	65.98	66.24	**66.11**	66.63	66.46	**66.55**	65.87	65.77	**65.82**	65.54	65.70	**65.62**
H	10.83	10.97	**10.90**	10.72	10.60	**10.66**	10.73	10.47	**10.60**	10.77	10.91	**10.84**
N	2.09	1.95	**2.02**	2.22	2.16	**2.19**	1.97	1.99	**1.98**	2.05	2.00	**2.03**
P	..	..	**1.23**	..	..	**0.89**	1.25	1.26	**1.26**	1.21	1.25	**1.23**
S	..	..	**0.77**	..	..	**0.72**	..	..	**0.67**	..	..	**0.72**
O[2]	..	..	**18.97**	..	..	**18.99**	..	..	**19.67**	..	..	**19.56**

[1] The methods of analysis employed were those already described by us: HAWK and GIES: This journal, 1901, v, p. 403.

[2] The amount of ash varied between 2 and 3 per cent. It consisted very largely of phosphate derived during the incineration process.

The results for elementary composition are in fairly close accord with those of previous observers.[1] Since all of our samples were made by practically the same method as that employed in most of the earlier investigations, however, this harmony proves nothing more than that the materials analyzed by all of us were of essentially the same character. The minor variations suggest that the products may be fairly uniform mixtures, but Kossel and Freytag's conclusion that several protagons exist might also be drawn from them. In fact, much to our surprise, these results accord as well as many analytic

[1] See the summary lately given by NOLL: Zeitschrift für physiologische Chemie, 1899, xxvii, p. 376.

series given for what are undoubtedly individual substances. Our data in this connection, considered by themselves, would seem to harmonize with the older view of the integrity of protagon. In the light of our other results, however, they illustrate the fact that uniformity in composition frequently hides chemical differences. In this case general uniformity seems to give no assurance of chemical individuality.

Application of the methods of Wörner and Thierfelder. — We have repeated some of the recent preliminary experiments of Wörner and Thierfelder without, however, anticipating any of the steps which it may be the intention of these investigators to take in furtherance of their work.

Wörner and Thierfelder used material from human brains. We used purified protagon from sheep brains. The agreement between their results and ours is, therefore, all the more significant. Our data in this connection will be given only briefly.

We made use of freshly prepared protagon, as well as some of the preparations already referred to. Our protagon products dissolved almost entirely in moderate quantities of solutions of equal parts of alcohol and chloroform, or alcohol and benzol, at 45° C. The latter solution appeared to exert solvent action less rapidly than the other. The crystals obtained from such fluids, after gradual evaporation at 40°–45° C., varied somewhat with changes in the composition of the solvent and in the concentration of the solution.

The residue left behind at this point, on treatment of the protagon with a moderate quantity of the solution, resembled that remaining in Experiments 4 and 5 preceding. It consisted of globular forms and amorphous substance. On cooling the filtrate from the melted matter, a bulky precipitate of snow-white "cerebron" spheres was deposited. The filtrate from the cerebron, on evaporation, yielded microscopic needles. The filtrate from these crystals contained other organic matter which, however, furnished only a slight amount of crystalline substance on further evaporation or on longer standing. These experiments were repeated several times with similar outcome.

Of these varous products the cerebron was the only one we attempted to separate in any quantity for further examination. In all the ordinary tests tried on the several preparations of purified cerebron, we found that our products gave the reactions already attributed to the substance by Wörner and Thierfelder. All the crystals figured for it by these investigators were observed in the

various fluids. The typical transformation of the cerebron balls in 85 per cent alcohol at 50° C. into needles, minute plates, etc., was also brought about several times. We were unable to make any elementary analyses of the cerebron, but verified the statement that on decomposition with acid a reducing substance may be detected among its cleavage products.

In view of these results, also, it appears necessary to conclude that protagon is not merely an unstable substance, but a mixture of bodies.[1] It is not at all likely that these various products arise by decomposition from such mild treatment. Further study of cerebron and its related products, also of the new substance very recently isolated by Ulpiani and Lelli,[2] and called by them, "paranukleo-protagon," may throw more light on the protagon question.

III. Summary of General Conclusions.

(1) The protagon of the brain is a mixture of substances, not a chemical individual.

(2) The mixture called protagon does not contain the bulk of the phosphorized organic substance of the brain.

[1] See very recent paper by Koch: Zeitschrift für physiologische Chemie, 1902, xxxvi, p. 140.

[2] Ulpiani und Lelli: Chemisches Centralblatt, 1902, ii, p. 292.

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Reprinted from the Archives of Neurology and Psychopathology, 1899, ii, p. 1.

ON THE NUCLEOPROTEID OF THE BRAIN (CEREBRONUCLEOPROTEID).

By P. A Levene.

[From the Pathological Institute of the New York State Hospitals and the Department of Physiological Chemistry of Columbia University.]

Different as the activity of a nerve cell may be from that of any other cell, there are still many features common to all, and the main point of similarity is that the source of its specific peculiar energy is the substance of the cell itself, that its work is being performed at the expense of its own body. From this follows the second point of similarity, that the nerve cell cannot work forever, or any indefinite time without repairing its own substance, its own body. How does it accomplish this task? Is there in the cell a peculiar organ for that purpose or a peculiar chemical agent that is in charge of that function? Cytologists have long ago observed that when a cell is divided into two parts, so that the nucleus is left in one of them, this last part is able to recuperate from the loss and continue its life, while the other part has a life of very short duration, and during the brief time it remains alive it does not digest nor does it assimilate food, while the first part continues to do so as well as any normal cell. Thus the cytologists have come to the conclusion that the nucleus controls the chief functions of the cell, viz., those of repair, growth, reproduction.

Further, it is a long-established fact that the predominating difference between the nucleus and the plasma of a cell is the amount of chromatin substance in them. It can be justly said the nucleus is the seat of the chromatin. Thus again biologists have come to the conclusion that the chromatin is the most important substance for the life of the cell and that most functions are connected with some changes in that substance.

If this be true, we should naturally expect to find this substance to be, first, of a very complex nature, and second, of such a nature that it can undergo different and manifold changes.

So it actually is. The chromatins belong to the class of compounds known as nucleoproteids, the most complex compounds in living matter, and probably in nature. The study of these substances in different conditions of the cell, in state of rest and activity, or better, in the state where repair predominates or dissimilation prevails, is the means of finding a clue to the solution of the problem of how the organism repairs its waste, and how we can successfully aid the organism in the most important of its tasks, when this power of restitution is for some reason or other diminished. We must remark, however, that our knowledge of the composition of these substances is not quite as extensive as is desirable, that the study of them does not date back much further than twenty years, and that least attention has been paid to the study of the nucleoproteids (or the proteids generally) of the brain.

It was my aim to fill this gap in the study of the brain. But before reporting my results I shall recall in a few words some of the characteristics of the nucleocompounds and the main points of difference between the individual compounds of this group.

The chief characteristics are that they contain phosphorus, possess the properties of acids, and are mostly met with in combination with proteids.

The points of distinction are, first, the presence or absence of the xanthin bases in the molecule of these compounds; the character of the bases, if present; the amount of phosphorus and of proteid in the molecule, and finally the character of the proteid.

Those compounds that contain a relatively higher percentage of P and whose acidity is but little neutralized by proteids, possess a comparatively higher affinity for certain basic anilin dyes. On account of this peculiarity the substance causing it was named chromatin by the microscopists.

In order to understand the chemical changes accompanying and probably responsible for the workings of the brain it is of great interest to study the chemical changes of its chromatin in different normal and pathological conditions of the organism.

In the nerve cell chromatin is located, in distinction from many other cells, not only in the nucleus, but also in the cytoplasm (Nissl's granules), and thus naturally the question arises

whether the chromatin of the latter is the same substance as is met with in the nucleus, or is it different in its nature; in other words, is there only one nucleoproteid in the nerve tissue or more than one?

Method of Obtaining the Nucleoproteid. — As far as I know, the study of the nucleocompounds of the brain is limited to two researches, both of them quite old, dating back to the time when our knowledge of the nature of these substances and their classification was very unsatisfactory. Thus, Halliburton extracted the brain tissue with H_2O and precipitated from the extract with acetic acid a proteid containing 0.3 per cent. of phosphorus. Von Jacsch treated a few human brains with pepsin-hydrochloric acid and from the residue extracted a nuclein — the nature of which he did not describe with much detail. There are a few more works dealing in a very unsatisfactory way with the general nature of the proteids of the brain, but none of them described the nucleocompounds.

The method that in my experience gave the most satisfactory results was the following:

The brains from freshly killed calves were immediately placed in alcohol-free ether and thus brought to the laboratory. After stripping the membranes, the brains were finely divided in a chopping machine and treated with large quantities of 4 per cent. AmCl solution and on addition of chloroform left in well-stoppered bottles for twenty-four hours. The supernatant fluid was then decanted, and the extraction repeated with distilled water, two, three and even four times, until the extracts ceased yielding an appreciable precipitate on addition of acetic acid.

The decanted fluid was then strained through gauze and filtered repeatedly till the filtrate was perfectly clear. I found later that the filtration is greatly accelerated, and the loss of material minimized if the strained liquid is left for several hours in separating funnels with ether. The small particles of brain tissue were then collected on the surface, and the liquid below was perfectly clear. The filtration was thereby rendered easy. The greater part of the material I worked with, however, was obtained by simple filtration without previous treatment with ether.

The perfectly clear filtrates were then treated with acetic acid, 0.5 c.c. of the acid to each 100 c.c. of the liquid, and thus a precipitate of the crude nucleoproteid was obtained.

This freshly precipitated proteid is insoluble in dilute acetic acid, also insoluble in dilute hydrochloric acid, but is soluble in glacial acetic acid, in weak alkalies, as one per cent. sodium carbonate, and 0.5 per cent. ammonium hydrate.

It is enough, however, to let the precipitate stand over night in acidulated H_2O to lower its solubility to a very great extent, so that only a very small part of the precipitate will dissolve in weak alkalies.

The usual method of purifying nucleoproteids is to redissolve them in dilute alkalies and to reprecipitate by acids. Our proteid, for which I would suggest the name *cerebronucleoproteid*, could not well be purified by this method, owing to the rapid loss of solubility.

There are also some objections to repeated treatment with alkalies in the fact that these might alter to a certain extent the original constitution of the proteid. For these reasons I attempted to purify the substance by repeatedly washing the precipitate first with acidulated H_2O, then with distilled water, until the latter failed to give the biuret reaction and was free from chlorine.

There still remained the possibility that the proteid thus purified might contain some other proteids, likewise rendered insoluble by prolonged treatment with acidulated water. To ascertain whether this was the case, and also to ascertain whether the usual method of purification affects the proteids, I endeavored to redissolve and reprecipitate some of the substance.

In doing this I encountered great difficulty in filtering the fluid, as the insoluble matter immediately clogs the filter paper. Even a constant change of the filter does not help much. In order to overcome this difficulty I recurred again to ether. The substance was treated with ether in a separatory funnel and left in it a few hours. It was then separated and filtered. The substances after they were thus purified were treated with cold alcohol, then boiled with 95 per cent. alcohol, then absolute alcohol and finally with ether, until extraction was nearly complete. We found it next to impossible even after continuous extraction during several

weeks to get the product in such a condition that the evaporated alcohol or ether would leave absolutely no residue.

Of the second product there was only sufficient for an estimation of the phosphorus, but a complete analysis was made of the first product.

PREPARATION I.

1. 0.1675 gr. of the substance gave on combustion 0.2845 gr. of CO_2; C = 42.44 per cent. and 0.0987 gr. of H_2O; H = 5.99 per cent.
2. 0.2133 gr. of the substance gave 0.3615 gr. of CO_2; C=42.28 per cent.; and 0.1126 gr. of H_2O; H = 5.82 per cent.
3. 0.1415 gr. digested after Kjeldahl = 0.0219 gr. of N = 15.46 per cent.
4. 0.458 gr. fused with NaOH and KNO_3 (S-free) = 0.043 gr. of $BaSO_4$, S = 1.28 per cent.
5. 0.3166 gr. fused with NaOH and KNO_3 = 0.0065 gr. of $Mg_2P_2O_7$; P = 0.573 per cent.
6. 0.4665 gr. fused with NaOH and KNO_3 = 0.0092 gr. $Mg_2P_2O_7$; P = 0.557 per cent.

PREPARATION II.

0.4897 gr. of the substance fused with NaOH + KNO_3 = 0.0078 gr. of $Mg_2P_2O_7$; P = 0.45 per cent.

	C.	H.	N.	S.	P.	O.
1	42.44	5.99	.....	.....	.....	.....
2	42.28	5.82	.....	.....	.....	.....
3	.....	.....	15.46	.....	.....	.....
4	.....	.....	.....	1.28	.....	.....
5	.....	.....	.....	.....	0.57	.....
6	.....	.....	.....	.....	0.56	.....
Average	42.36	5.90	15.46	1.28	0.56	34.44

Ash = 0.5 per cent.

Apparently the first method of purification affects the proteid less than the second method, but in either case the nucleoproteid contains very little phosphorus, probably less than any other true nucleoproteid; in fact it resembles in this respect the pseudo- or para-nucleoproteids, or as Hammarsten calls them, nucleoalbumins. It was of course important to ascertain to which of the

two main groups of the nucleocompounds our substance belongs, since the physiological rôle of the two is quite different.

For this purpose about 60 gr. of the substance was heated in a flask with a return condenser with 2 per cent. H_2SO_4 for about ten hours. It was then filtered, the greater part of the acid neutralized by means of $Ba(OH)_2$, filtered, the filtrate concentrated and treated in the usual way for nuclein bases (xanthin bases).

In the xanthin fraction but a very slight precipitate of the xanthin silver salt was obtained—so little that the attempt to obtain a xanthin reaction after the silver was eliminated, was without success. The hypoxanthin fraction consisted mostly of guanin and adenin, no hypoxanthin being found.

Thus, it was established that our substance is a true nucleoproteid, and that two bases take part in the formation of its molecule.

Cerebronuclein. —The next task was to ascertain the cause of the low percentage of P in the nucleoproteid. This might be due to two different causes; either the nuclein itself might contain little P, or other substances might be bound to a nuclein with a high content of P, thus giving rise to an unusually complex substance.

A considerable amount of the proteid purified by the first method, but not extracted with alcohol and ether, was digested with pepsin-hydrochloric acid, for a week. The digestive fluid was then changed every two days, 0.2 per cent. HCl being employed, and care being taken to have free HCl always present in the fluid. After that, the soluble products of digestion were separated by repeated treatment with acidulated water and decantation until the wash water gave no biuret reaction, and contained no chlorine. The insoluble residue was then extracted with alcohol and ether until the latter ceased extracting, which took place after several weeks' continuous treatment. About 2.5 gr. of the pure air-dry substance was thus obtained. A small portion of it was then extracted with HCl water in order to ascertain whether it contained inorganic P; the result was negative.

The percentage of P was then estimated and 0.275 gr. of the substance fused with NaOH and KNO_3 gave 0.0140 gr. of $Mg_2P_2O_7$; P = 1.42 per cent.

In comparison with other nucleins the phosphorus is seen to be rather low.

Cerebronucleic Acid. — It is known that nucleins are compounds of nucleic acid and proteids. The nuclein of the brain is exceptionally poor in P, and we are confronted again by the two possibilities that were met with in connection with the nucleoproteid itself. The low percentage of P in the nuclein might be due to the peculiar nucleic acid or to the different amounts of proteid combined with an acid having a comparatively high content of P.

The investigation in this direction is not completed at present, as we found great difficulty in obtaining a sufficient quantity of the substance. The method of obtaining the nucleic acid that gave the most satisfactory results is the following :

The purified nucleoproteid, not extracted with alcohol and ether, was dissolved in 2 per cent. NaOH, while being slightly warmed on a water-bath. While still warm the fluid was neutralized with acetic acid, cooled and filtered. This was found necessary for the reason that that part of the proteid which was precipitated on neutralization (alkali albuminate) was again soluble in an excess of acetic acid. The filtrate was rendered strongly acid by means of acetic acid, and was then left for twenty-four hours and filtered ; to the filtrate alcohol containing 0.3 per cent. HCl was added until the fluid became very opalescent. After standing twenty-four to forty-eight hours, the precipitate was washed with acidulated alcohol, then with pure alcohol and ether, dried and weighed. The acetic solution of this proteid precipitated albumoses and proteids from their solutions.

Seventy grams of the proteid treated with 300 c.c. of 2 per cent. NaOH gave less than 100 mgr. of the nucleic acid. 0.0875 gr. of this substance fused with NaOH and KNO_3 gave 0.0105 gr. of $Mg_2P_2O_7$; P = 3.35 per cent.

This P estimation can be accepted for the present only as more or less approximate to the true percentage of P in the nucleic acid.

From all these results it may be inferred that the nucleocompound of the brain is a true nucleoproteid, that it differs from other nucleoproteids by its low percentage of P, by the nature of its xanthin bases, and by the considerably high amount of proteids bound to its nuclein.

The next aim was to investigate whether the residue of brain tissue after extraction of this nucleoproteid contained another nucleocompound different in nature from the cerebronucleoproteid.

For that purpose the residue just mentioned was extracted during different lengths of time with dilute alkalies of different strengths. It was found that 0.5 per cent. ammonium hydrate will extract in twenty-four hours a considerable quantity of a proteid which can be precipitated by acetic acid, and that this is a nucleoproteid. It was also found that twenty-four hours treatment of the proteid with 0.5 per cent. ammonia solution will not split off any noticeable quantity of nucleic acid. Hence, 0.5 per cent. of ammonia could be applied for the extraction of the residual nucleocompounds. It remained to ascertain whether the latter was different in nature from the cerebronucleoproteid. The estimation of P in it, however, argued against such a supposition. Thus, 0.5800 gr. of the purified substance gave on fusion 0.0105 gr. of $Mg_2P_2O_7$ or 0.5 per cent. of P.

We attempted also to obtain the nuclein of that residual substance, but as the quantity of the latter in our possession was rather small, we digested the residue of forty brains with pepsin hydrochloric acid with the same precautions as mentioned above. After the digestion and purification was completed, the residue was extracted with cold and boiling alcohol for several weeks, then with ether until the myelin was nearly extracted. In order to ascertain whether this residue contained an appreciable amount of nucleocompound, a P estimation was made.

0.4325 gr. of the substance gave 0.0140 gr. of $Mg_2P_2O_7$, or P = 0.896 per cent.

Thus, the presence of a considerable quantity of nuclein in the brain residue was demonstrated. However, the prolonged treatment with boiling alcohol rendered the nuclein insoluble to such un extent that but little of it could be extracted by means of dilute alkalies. Thirty grams of the residue was treated for five hours with 0.25 per cent. NaOH and filtered directly into dilute HCl solution; a white flocculent precipitate was formed, but in a quantity insufficient for further analysis.

No marked difference between the residue and the cerebronucleoproteid could be found in the character of their xanthin bases.

Here, again, guanin was found to predominate, the other bases not being sufficient in quantity to be identified.

These results do not bear out the supposition of the existence of more than one nucleoproteid in the nerve cell.

From these results it may also be inferred that the nature of the chromatin of the cytoplasm does not differ from that of the nucleus. However, this question can be fully elucidated only by a comparative chemical study of the nerve tissue under different physiological and pathological conditions, *i. e.*, in conditions when the chromatin nearly disappears from the nucleus and is located only in the cytoplasm and *vice versa*.

I wish to acknowledge my indebtedness to Professor Chittenden for his valuable suggestions and for the privileges accorded to me in the Laboratory of Physiological Chemistry of Columbia University.*

May 20, 1899.

REFERENCES.

Halliburton. Journal of Physiology, Vol. XV., 1893.
Hammarsten. Zeitschr. f. physiol. Chem., Vol. XIX.
Von Jacsch. Pflüger's Arch., Vol. XIII., p. 469.

* See Preface, page 7.

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THE MUCIN OF WHITE FIBROUS CONNECTIVE TISSUE.*

By R. H. Chittenden and William J. Gies.

(Contribution from the Sheffield Biological Laboratory of Yale University.)

All of the bodies belonging to the group of mucins and mucoids are possessed of considerable physiological interest, owing especially to their peculiar compound nature and the illustration which they afford of a possible intimate union between the proteid group and carbohydrate radicles. That there are a number, possibly a large number, of closely related bodies belonging to the mucins and mucoids there can be no question. Thanks to the labors of Hammarsten † and his pupils, many of these bodies have been subjected to careful and thorough investigation, and much light has been thrown upon their relationships and differences. There is still, however, much to be ascertained regarding these bodies, and any additional facts broadening or substantiating our present knowledge are to be welcomed as contributing toward a more complete understanding of their genetic relationships. The union of carbohydrate groups with proteid molecules is probably more common than has hitherto been supposed, as witness the peculiar gluco-nucleoproteid recently described by Hammarsten ‡ as a constituent of the pancreas and other glands, and the identification by Kossel § of a peculiar carbohydrate group as a cleavage product of certain forms of nucleic acid. Presumably in these compound proteids of the mucin type the character of the proteid radicle as well as of the carbohydrate radicle is subject to variation, and it is easy to conceive of differences in the nature and

* A preliminary report of this research was made by Professor Chittenden before the American Physiological Society in Philadelphia, in December, 1895, and an abstract was published in Science, January 24, 1896; iii (N. S.), p. 109. No reference was made to the fact that the report was presented for *both* authors.

† Pflüger's Archiv f. Physiol., Band xxxvi; Zeitschr. f. physiol. Chem., Band x and xii.

‡ Zeitschr. f. physiol. Chem., Band xix.

§ Du Bois-Reymond's Archiv f. Physiol., Physiol. Abtheil, 1891.

properties of the mucins dependent upon variations in the amount and character of both the carbohydrate and proteid groups. The ready formation of acidalbumin, or syntonin, albumoses and peptone when mucins are decomposed by the action of superheated water or boiling dilute acids, affords ample evidence of the presence of true proteid radicles in the bodies of this class, although we do not know definitely the exact nature of the proteid groups present in the original molecule. On the other hand, the simultaneous formation of reducing bodies whenever mucins are broken down by the action of dilute acids, and the separation of a dextrin-like body (the animal gum of Landwehr *) by cleavage with superheated water, clearly indicate the existence of some form of carbohydrate matter in the mucin molecule.

Of the true mucins present in the tissues of the higher animals, the mucin of the submaxillary gland and the corresponding body present in or between the fibers of ordinary connective tissue are the most important from a physiological standpoint. The former is a product of the metabolic activity of secretory cells which are among the most active of the secreting cells of the body, while the latter is a product of a tissue whose activity is certainly of a low order. That these two mucins, though closely related, are unlike, is clearly indicated by their divergence in chemical composition as well as by their general reactions and properties.

Loebisch,† whose careful study of the mucin from tendons constitutes the chief source of our knowledge regarding the chemical composition of this body, ascribes to tendon mucin the formula $C_{160}H_{256}N_{32}SO_{80}$, with a molecular weight of 3,936. Such a formula calls for the presence of 0.81 per cent. of sulphur and this amount was found by Loebisch in the three preparations of mucin from ox tendons analyzed by him. In a recent examination of mucin prepared from this same source we have obtained quite different results as regards the content of sulphur, and this fact has led us to make a careful study of the composition of this form of connective-tissue mucin. Our results in the main have afforded a close substantiation of the conclusions arrived at by Loebisch,

*Zeitschr. f. physiol. Chem., Band viii and ix. Also Pflüger's Archiv. f. Physiol., Band xxix and xl.

† Zeitschr. f. physiol. Chem., Band x, p. 40.

with the single exception of the sulphur, for which we can find no adequate explanation. Further, some additional facts have been found which are perhaps worthy of note.

The first sample of mucin studied was prepared from the Achilles tendons of oxen by the following method, analogous to the method described by Loebisch : The fresh tendons were freed as carefully as possible from all adherent tissues, then cut into very thin transverse sections with a razor, washed thoroughly with distilled water, frequently renewed for twenty-four hours, in order to remove all blood and soluble albuminous matter, and finally pressed as dry as possible. The resultant material weighed 1,200 grammes. In order to extract the mucin, the tissue was placed in 2.4 liters of half saturated lime water, where it was allowed to remain for forty-eight hours with frequent agitation. At the end of this period the pale-yellowish fluid was strained through a cloth filter and finally filtered through paper. The clear fluid was then treated with an excess of 0.2 per cent. hydrochloric acid — a little more than a liter — by which a heavy flocculent precipitate resulted, quickly settling to the bottom of the cylinder, leaving a nearly clear supernatant fluid.

The residue of tendon tissue was again extracted for forty-eight hours with 2.4 liters of half saturated lime water, and the resultant solution precipitated with an excess of 0.2 per cent. hydrochloric acid. The precipitate so formed was nearly as heavy as the first, thus showing that extraction of the mucin by weak lime water is a slow and gradual process.

The precipitated mucin, separated from the acid fluid by subsidence and decantation of the supernatant liquid, was washed thoroughly with 0.2 per cent. hydrochloric acid, by whipping up the precipitate with the fluid and then allowing it to subside, this operation being repeated with fresh quantities of acid until the latter failed to give any proteid reaction. In this manner it was hoped to remove all adherent albuminous matter extracted from the tissue by the lime water. The two portions of mucin were then united and washed by decantation with distilled water until the acid was entirely removed. As the fluid became less and less acid, more time was required for the precipitate to settle, as the latter tended to swell in the water and was more inclined to float on the surface of the fluid.

The mucin was next dissolved in half saturated lime water, of which a large volume was required, the solution filtered through paper, and the mucin reprecipitated by the addition of an excess of 0.2 per cent. hydrochloric acid, a small quantity of stronger hydrochloric acid being likewise added to induce a good flocculent separation of the substance. The precipitate was again washed by decantation with 0.2 per cent. hydrochloric acid, and lastly with water, until the acid was entirely removed. Whenever it was necessary for the precipitate to stand for some time with water, the mixture was kept as cool as possible, and a little alcoholic solution of thymol added to guard against putrefactive changes. When the acid was wholly removed from the precipitate the water was replaced by weak alcohol, and finally by ninety-five per cent. alcohol, repeatedly renewed, until the substance was thoroughly dehydrated, after which the precipitate was collected on a filter and allowed to drain. It was then boiled with alcohol-ether (a mixture of equal parts absolute alcohol and ether) in a suitable flask connected with an inverted Liebig's condenser for many days — *i. e.*, with renewed quantities of alcohol-ether until the latter gave no residue on evaporation. As Loebisch has shown, this is quite an important part of the process of purification, since a certain amount of foreign extractive matter adheres tenaciously to the mucin, and can be removed only by long-continued extraction with the above mixture. When this process was completed the mucin was thrown upon a filter, washed thoroughly with ether, and finally dried over sulphuric acid. When quite dry it presented the appearance of a perfectly white powder, light and fluffy. The yield amounted to twelve grammes of the dry product, and assuming that the entire amount of mucin had been extracted from the tendons, and disregarding the loss incidental to purification, this quantity would imply the presence in the fresh tendons of one per cent. of mucin.

The composition of the product, dried at 110° C. until of constant weight, was as follows: *

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*The nitrogen was determined by both the absolute and the Kjeldahl method, while carbon and hydrogen were determined by combustion in oxygen gas in an open tube, the products of combustion passing over a layer of cupric oxide, chromate of lead, and metallic copper.

PREPARATION NO. 1.

I. 0.2670 gramme of substance gave 0.4781 gramme of CO_2 = 48.84 per cent. C, and 0.1585 gramme of H_2O = 6.60 per cent. H.

II. 0.2277 gramme of substance gave 0.4082 gramme of CO_2 = 48.89 per cent. C, and 0.1329 gramme of H_2O = 6.48 per cent. H.

III. 0.1975 gramme of substance gave 0.3548 gramme of CO_2 = 48.99 per cent. C.

IV. 0.2363 gramme of substance gave 0.1417 gramme of H_2O = 6.66 per cent. H.

V. 0.2426 gramme of substance gave, by the Kjeldahl method, 0.02865 gramme of nitrogen = 11.81 per cent. N.

VI. 0.2754 gramme of substance gave, by the Kjeldahl method, 0.03246 gramme of nitrogen = 11.79 per cent. N.

VII. 0.2784 gramme of substance gave, by the absolute method, 27.63 c.c. of nitrogen at 13.3° C., and 764.7 mm. pressure = 11.96 per cent. N.

VIII. 0.3345 gramme of substance gave, by the absolute method, 33.3 c.c. of nitrogen at 13.2° C., and 754.5 mm. pressure = 11.84 per cent. N.

IX. 0.5373 gramme of substance gave, by fusion with NaOH + KNO_3, 0.0943 gramme of $BaSO_4$ = 2.41 per cent. S; after deducting sulphur of ash = 2.36 per cent. S.

X. 0.4969 gramme of substance gave, by fusion with NaOH + KNO_3, 0.0856 gramme of $BaSO_4$ = 2.37 per cent. S; after deducting sulphur of ash = 2.32 per cent. S.

XI. 0.2943 gramme of substance gave 0.0023 gramme of ash = 0.78 per cent. ash.

XII. Ash from 0.2943 gramme of substance gave 0.00112 gramme of $BaSO_4$ = 0.05 cent. S.

PERCENTAGE COMPOSITION OF THE ASH-FREE SUBSTANCE.

											Average.
C.	49.22	49.27	49.37	.....	.....	.....	.....	.....	.....	.....	49.29
H.	6.65	6.54	.....	6.71	.....	.....	.....	.....	.....	.....	6.63
N.	.....	.....	.....	.....	11.90	11.88	12.05	11.93	.....	.....	11.94
S.	.....	.....	.....	.....	.....	.....	.....	.....	2.36	2.32	2.34
O.	.....	.....	.....	.....	.....	.....	.....	.. ...	.....	.....	29.80

The second preparation of mucin was made in a somewhat different manner. The fresh tendons, freed as far as possible from foreign tissue, were cut into thin transverse sections, washed with water somewhat, then soaked for thirty-six hours in about four liters of ten per cent. salt solution, with vigorous agitation from time to time, after which the saline solution was decanted and the tissue washed with water until the chloride was entirely removed. The salt solution on dilution with water gave a distinct turbidity, indicating the presence of a globulin. The application of heat likewise produced a precipitate, as did also the addition of dilute acetic and hydrochloric acids. It is thus evident that the salt solution removes at the outset quite an appreciable amount of pro-

teid matter, with perhaps some mucin. The moist tissue, pressed as dry as possible, weighed 1,700 grammes. It was then extracted with 3.4 liters of half saturated lime water for forty-eight hours, two such extractions being made. From these extracts the mucin was precipitated by the addition of 0.2 per cent. hydrochloric acid, the second extract apparently yielding as heavy a precipitate as the first. The combined precipitates were washed repeatedly by decantation with 0.2 per cent. hydrochloric acid, lastly with water. The mucin was next dissolved in a little 0.5 per cent. sodium carbonate, the solution filtered, made nearly neutral by the addition of a little ten per cent. hydrochloric acid, so as to avoid undue dilution, and then precipitated by 0.2 per cent. hydrochloric acid. The precipitate was again washed thoroughly with 0.2 per cent. hydrochloric acid, and lastly with water, until the acid was entirely removed. It was then transferred to ninety-five per cent. alcohol, frequently renewed, and finally boiled with alcohol-ether as long as anything could be extracted. Dried over sulphuric acid, the product came out quite white, but not so bulky as the preceding preparation, and weighed a little over fifteen grammes — an amount equal to about 0.9 per cent. of the moist tissue.

As already stated, mucin is not readily extracted from tendons by lime water; at least four cubic centimeters of half saturated lime water are required for every gramme of tissue in order to insure a complete extraction. Thus, after the second extraction of the above 1,700 grammes of tissue, a third extraction was made, using again three litres of half saturated lime water. This solution, on treatment with hydrochloric acid, gave a precipitate weighing one to two grammes when purified, but it was noticeable that more acid was required in order to effect a good flocculent separation of the mucin. Even with a fourth extraction of the tissue a little mucin was obtained, showing as a decided turbidity when the alkaline fluid was made distinctly acid, but it was not until four or five days' standing that a distinct precipitate settled out even on the addition of stronger hydrochloric acid. The amount so obtained, however, was very small.

The composition of the main product obtained from the 1,700 grammes of tissue when dried at 110° C., until of constant weight, was as follows :

PREPARATION NO. 2.

I. 0.3194 gramme of substance gave 0.5659 gramme of CO_2 = 48.32 per cent. C, and 0.1815 gramme of H_2O = 6.31 per cent. H.

II. 0.4197 gramme of substance gave 0.7471 gramme of CO_2 = 48.54 per cent. C, and 0.2446 gramme of H_2O = 6.47 per cent. H.

III. 0.4051 gramme of substance gave 0.7189 gramme of CO_2 = 48.39 per cent. C, and 0.2353 gramme of H_2O = 6.45 per cent. H.

IV. 0.2519 gramme of substance gave, by the Kjeldahl method, 0.02965 gramme of nitrogen = 11.77 per cent. N.

V. 0.2578 gramme of substance gave, by the Kjeldahl method, 0.03026 gramme of nitrogen = 11.74 per cent. N.

VI. 0.2954 gramme of substance gave, by the Kjeldahl method, 0.03446 gramme of nitrogen = 11.67 per cent. N.

VII. 0.6610 gramme of substance gave, by fusion with $NaOH + KNO_3$, 0.1131 gramme of $BaSO_4$ = 2.35 per cent. S; after deducting sulphur of ash = 2.32 per cent. S.

VIII. 0.5248 gramme of substance gave, by fusion with $NaOH + KNO_3$, 0.0936 gramme of $BaSO_4$ = 2.45 per cent. S; after deducting sulphur of ash = 2.42 per cent. S.

IX. 0.6724 gramme of substance gave, by fusion with $NaOH + KNO_3$, 0.1140 gramme of $BaSO_4$ = 2.33 per cent. S; after deducting sulphur of ash = 2.30 per cent. S.

X. 0.3735 gramme of substance gave 0.0025 gramme of ash = 0.67 per cent. ash.

XI. Ash from 0.3735 gramme of substance gave 0.00082 gramme of $BaSO_4$ = 0.03 per cent. S.

PERCENTAGE COMPOSITION OF THE ASH-FREE SUBSTANCE.

										Average.
C.	48.64	48.87	48.72	.....	.....	.....	.....	.....	.....	48.74
H.	6.36	6.52	6.50	.....	.....	.....	.....	.....	....	6.46
N.	.....	.....	.....	11.85	11.82	11.74	.....	.....	.....	11.80
S.	.....	.....	.....	.....	.....	.....	2.32	2.42	2.30	2.35
O.	.....	.....	.....	.....	.....	.....	.....	.....	.....	30.65

A third specimen of mucin was prepared as follows: Fifteen hundred grammes of ox tendons were finely divided, the tissue extracted for twenty-four hours with three liters of ten per cent. salt solution, and then with water until the salt was wholly removed. The tissue was next extracted for sixty hours with three liters of half saturated lime water. From this solution the mucin could be only partially separated by the addition of 0.2 per cent. hydrochloric acid, quite a quantity of ten per cent. acid being required to effect a flocculent precipitation of the substance. This was purified by itself and not subjected to analysis. The tendons were again extracted with three liters of half saturated lime water for forty-eight hours, and from this solution the mucin was

separated as a flocculent precipitate by the addition of 0.2 per cent. hydrochloric acid. This precipitate was purified by washing with 0.2 per cent. hydrochloric acid, solution in 0.5 per cent. sodium carbonate, reprecipitation with 0.2 per cent. hydrochloric acid, etc., as described under the last preparation. The yield of dry product from this second extraction of the tissue with lime water amounted to 6.5 grammes. Dried at 110° C. until of constant weight, this preparation gave the following results on analysis:

PREPARATION NO. 3.

I. 0.3598 gramme of substance gave 0.6292 gramme of CO_2 = 47.69 per cent. C, and 0.2072 gramme of H_2O = 6.40 per cent. H.

II. 0.2939 gramme of substance gave 0.5150 gramme of CO_2 = 47.79 per cent. C, and 0.1725 gramme H_2O = 6.52 per cent. H.

III. 0.3154 gramme of substance gave 0.5536 gramme of CO_2 = 47.87 per cent. C.

IV. 0.1644 gramme of substance gave 0.0944 gramme of H_2O = 6.38 per cent. H.

V. 0.1965 gramme of substance gave, by the Kjeldahl method, 0.02255 gramme of nitrogen = 11.47 per cent. N.

VI. 0.2495 gramme of substance gave, by the Kjeldahl method, 0.02825 gramme of nitrogen = 11.32 per cent. N.

VII. 0.2574 gramme of substance gave, by the Kjeldahl method, 0.02930 gramme of nitrogen = 11.38 per cent. N.

VIII. 0.6046 gramme of substance gave, by fusion with $NaOH + KNO_3$, 0.1045 gramme of $BaSO_4$ = 2.38 per cent. S; after deducting sulphur of ash = 2.31 per cent. S.

IX. 0.5408 gramme of substance gave, by fusion with $NaOH + KNO_3$, 0.0931 gramme of $BaSO_4$ = 2.37 per cent. S; after deducting sulphur of ash = 2.30 per cent. S.

X. 0.3128 gramme of substance gave 0.0031 gramme of ash = 0.99 per cent. ash.

XI. Ash from 0.3128 gramme of substance gave 0.00152 gramme of $BaSO_4$ = 0.07 per cent. S.

PERCENTAGE COMPOSITION OF THE ASH-FREE SUBSTANCE.

										Average.
C.	48.17	48.26	48.34	.....	.....	.....	.....	.....	.....	48.26
H.	6.46	6.59	.....	6.44	.....	.....	.....	.....	.....	6.49
N.	.....	.....	.....	.....	11.59	11.43	11.50	.....	.....	11.51
S.	.....	.....	.....	.....	.....	.....	.....	2.31	2.30	2.31
O.	.....	.....	.....	.....	.....	.....	.....	.....	.....	31.43

A comparison of the composition of these three preparations of mucin with each other, and with the mucin analyzed by Loebisch and by Hammarsten, brings out certain points of interest which merit attention:

	Mucin from Tendons.				Snail Mucin. Hammarsten.	Submaxillary Mucin. Hammarsten.
	Preparation 1.	Preparation 2.	Preparation 3.	Loebisch's Average.		
C.....	49.29	48.74	48.26	48.30	50.32	48.84
H.	6.63	6.46	6.49	6.44	6.84	6.80
N.....	11.94	11.80	11.51	11.75	13.65	12.32
S	2.34	2.35	2.31	0.81	1.75	0.84
O	29.80	30.65	31.43	32.70	27.44	31.20

Loebisch analyzed three distinct preparations of mucin from ox tendons, in which the carbon, hydrogen, and sulphur showed practically no variation. The nitrogen, however, varied from 11.59 to 11.84 per cent. The average content of nitrogen in his three preparations was 11.75 per cent., identical with the average of our three preparations. It is to be noticed, however, that the carbon of our preparations shows decided variation, and it is also to be observed that a diminution in the percentage of carbon is attended in each case with a diminution in nitrogen. We may suppose that Preparation No. 3 is the purest of our products, and it is seen to agree most closely with the results obtained by Loebisch, except in the content of sulphur. The mucin from the submaxillary gland, as well as the snail mucin, are both characterized by a comparatively high content of nitrogen, while the latter product also shows a higher percentage of carbon.

Our results seemingly justify the assumption that white fibrous connective tissue contains more than one mucin, or else that the mucin obtainable from this tissue is prone to carry with it a certain amount of some other form of proteid matter which the ordinary methods of purification are not wholly adequate to remove. Our experience leads us to the belief that the surest way of obtaining a pure mucin from tendons, or at least one with a low content of carbon and nitrogen, is first to extract the finely divided tissue with ten per cent. salt solution, then after removal of the salt with water to extract the tissue with half saturated lime water in the proportion of two cubic centimeters for every gramme of moist tissue for about twenty-four hours at ordinary room temperature. This extract may be rejected, as it is very liable to yield a mucin with a higher content of nitrogen and carbon. By extracting the tissue a second time with lime water a mucin may be obtained with a lower content of carbon and nitrogen, as in our third preparation. It is

purely an assumption, however, to say that this body with its lower percentage of carbon and nitrogen is *pure* mucin. There is at the present time no standard of purity with regard to this body, and it is quite as probable that fibrous connective tissue contains two or more mucins as that there is only one mucin in the tissue, and that any deviation from the figures obtained by Loebisch or by us in Preparation No. 3 is due to the presence of a larger or smaller amount of proteid impurity.

Undoubtedly, preliminary extraction of the tissue with salt solution tends to remove a certain amount of proteid matter, especially globulins, which might otherwise render the product impure, and possibly this is in part the cause of the lower content of carbon and nitrogen in Preparation No. 2 as contrasted with Preparation No. 1. Still there is no certainty on this point, for it is to be remembered that precipitation of the mucin requires the addition of considerable hydrochloric acid beyond neutralization of the alkaline fluid, and this excess of acid would naturally exert a marked solvent action upon any albuminous matter present. That the first lime water extract is liable to yield a mucin with a higher content of both carbon and nitrogen the results fully indicate, and as a direct illustration of the difference in the content of nitrogen in mucin obtained from a first and second extract, we may instance the following experiment: Fifteen hundred grammes of tendons finely divided, as usual, were extracted with ten per cent. salt solution for two days, then washed with water and placed in three liters of half saturated lime water for forty-eight hours. This first extract was then strained off, and the tissue treated a second time with a like volume of half saturated lime water, thus giving a second extract. From the first extract, the mucin was precipitated by hydrochloric acid slightly above 0.2 per cent., the precipitate washed with 0.2 per cent. hydrochloric acid, then with water, and lastly dissolved in 0.5 per cent. sodium carbonate. From this filtered solution a portion of the mucin was precipitated by addition of 0.2 per cent. hydrochloric acid, while a second portion separated only on addition of a somewhat increased strength of acid. These two fractions were washed thoroughly with 0.2 per cent. acid, then with water, and finally boiled with alcohol-ether until quite free from soluble matter. The yield in the first fraction

was 1.4 gramme, and in the second fraction 1.0 gramme. From the second lime water extract the mucin was precipitated with 0.2 per cent. hydrochloric acid, after which it was purified by washing with 0.2 per cent. acid, solution in 0.5 per cent. sodium carbonate, reprecipitation with 0.2 per cent. acid, etc. The content of nitrogen in the three products, when dried at 110° C., was as follows, calculated on the ash-free substance:

First Extract.		Second Extract.
First Fraction.	Second Fraction.	
12.26 N.	11.91 N.	11.51 N.

It is thus seen that the first extraction with lime water furnishes a mucin with a considerably higher percentage of nitrogen than the second extract. It is equally noticeable that the mucin first precipitated — as in the first fraction of the first extract — has a higher percentage of nitrogen than the second fraction, thus indicating that the higher content of nitrogen and probably of carbon also belongs to some body more readily precipitated by acid than the mucin with 11.51 per cent. of nitrogen. In view of the great care exercised in all of these preparations, and the ready solubility of ordinary forms of albuminous matter in an excess of hydrochloric acid, especially after they have once been dissolved in an alkaline fluid, we are very much inclined to believe in the existence of several related mucins as components of ordinary white fibrillar connective tissue.

Such a view presents no great difficulty. Submaxillary mucin, for example, differs from tendon mucin by only 0.5 per cent. of carbon (48.84 per cent.) and about 0.5 per cent. of nitrogen (12.32 per cent.), although it shows some other points of difference, such as a tendency to undergo alteration by the action of lime water and by being soluble in 0.2 per cent. hydrochloric acid. Indeed, all of the various mucins described show minor points of difference, although agreeing in their general reactions, and it is easy to conceive of the presence of two or more closely related mucins, in tendons, with different elementary composition.

The most remarkable thing, however, connected with the mucins that we have separated from this form of fibrillar connective tissue is the amount of sulphur present in the purified products. In snail mucin, Hammarsten has shown the presence of 1.75 per cent. of

sulphur, but in the mucin from the submaxillary gland and in the mucin described by Loebisch as contained in tendons, the amount of sulphur has been placed at 0.84 to 0.81 per cent. In all three of our preparations, however, the sulphur present has amounted to at least 2.30 per cent., and, moreover, the agreement in the several products has been very close indeed. The greater portion of this sulphur is closely combined, a small amount only being in the form of the mercaptan group and responding to the reaction with potassium hydroxide and plumbic acetate. We present these figures with some doubt in our own minds, but, having obtained them as the result of most careful work, we see no possible explanation other than that this amount of sulphur is actually present in the mucin molecule. The determinations of sulphur were made after the usual method recommended by Hammarsten — viz., oxidizing the mucin with a mixture of ten grammes NaOH and two grammes KNO_3 in a silver crucible, etc. The sodium hydroxide employed was chemically pure, having been prepared from the metal, and, furthermore, several blank tests were made to prove the freedom of the various chemicals from sulphur. This percentage of sulphur is greater than has ever been accredited to a true mucin, although the mucin from the snail's membrane (mantle-mucin), which is somewhat related to keratin, has been found by Hammarsten to contain a fairly large amount of this element — viz., 1.79 per cent.

With regard to the *reactions* of the several products that we have studied, there is nothing special to be said. They all show the ordinary reactions of mucin as described by Loebisch, and we can simply substantiate what has long been published by him upon this point.

The most characteristic feature of mucin is the peculiar cleavage it undergoes when heated with dilute hydrochloric acid, by which a substance with reducing action upon alkaline copper solution results. Albumose and peptone are likewise formed by the action of the hot acid. We have tried several preliminary experiments in this direction, the results of which may be briefly stated: 3.25 grammes of mucin of preparation No. 2 were heated in a boiling water-bath with one hundred cubic centimeters of two per cent. hydrochloric acid for five hours. At the end of this

period the solution was of a deep-brown color, while suspended through the fluid was a large amount of gelatinous matter more or less brown in color. This was filtered off, washed with water, in which it was wholly insoluble, until the washings gave no proteid reaction. It was then tested with the following results: It was insoluble in dilute and stronger hydrochloric acid, but readily soluble in 0.5 per cent. sodium carbonate and in very dilute (0.5 per cent.) potassium hydroxide. From the solution in sodium carbonate, it was reprecipitated by neutralization, and was then readily soluble in a slight excess of 0.2 per cent. hydrochloric acid. It gave the ordinary color reactions characteristic of proteid matter. Warmed at 40° C. with an active gastric juice containing 0.2 per cent. hydrochloric acid, it was wholly unaffected even after twenty-four hours, but when warmed with an alkaline pancreatic juice it was readily dissolved, and almost completely converted into products soluble even on neutralization of the fluid, thus attesting its conversion into soluble albumoses and peptones. These reactions suggest that the substance in question is a form of antialbumid.

The original acid fluid containing the soluble products formed in the cleavage of the mucin was made neutral, by which a slight neutralization precipitate resulted, evidently syntonin from the reactions tried. The neutral fluid was then concentrated to a sirup, a strong caramel-like odor being developed during the process, and while still warm the residue was treated with a large excess of ninety-five per cent. alcohol, by which a thick gummy mass was formed, hard and brittle on cooling. While warm, the alcoholic fluid was quite clear and yellowish-red in color, but on cooling, a light-yellow precipitate, very small in quantity, formed, which was soluble in water, and gave a strong reducing action with Fehling's solution. It was too small in quantity, however, to study further. The gummy precipitate was washed by warming it repeatedly with fresh quantities of alcohol. It was readily soluble in water, gave more or less of a proteid reaction, and showed a fairly strong reducing action with Fehling's solution. Tested with phenylhydrazine hydrochloride, and sodium acetate, only an amorphous precipitate resulted from which a crystalline osazone could not be obtained. On boiling the gummy mass

with two per cent. hydrochloric acid, however, and then extracting the neutralized and evaporated fluid with alcohol, a very small amount of a crystalline osazone was obtained by application of the hydrazine test, apparently identical with that described further on.

The original alcoholic solution from the above gummy precipitate was evaporated to a small bulk on the water-bath, the residue taken up with fifteen cubic centimeters of water, forming a clear solution. This solution showed strong reducing action with alkaline copper solution, and evidently contained the greater portion of the reducing body formed from the cleavage of the mucin. To the main bulk of this solution was added one gramme of phenylhydrazine hydrochloride and 1.5 grammes of sodium acetate, after which the mixture was heated on the water-bath for an hour and a half, the volume of the fluid being kept at fifteen to twenty cubic centimeters. While hot the fluid was perfectly clear and reddish in color. After standing an hour in a cool place there was a marked separation of amorphous particles and oily globules, but no crystals could be detected under the microscope. After standing fifteen hours the amorphous particles were almost wholly transformed into fine crystals. These crystals were light yellow in color, and were mostly arranged in rosettes or balls of fine yellow needles, somewhat resembling lactosazone. The oily globules were unchanged. These crystals were purified by dissolving them in cold alcohol, followed by the addition of water, and heating the solution until the alcohol was practically all removed, when the crystals again separated out as the fluid cooled. The crystals were also insoluble in the hot *concentrated* fluid. In this way the crystals were gradually freed from the oily globules spoken of above and rendered fairly pure. Each time the crystals were filtered they were also washed with a little cold water. During the process of purification the crystals changed their appearance somewhat, tending to take on the branching form characteristic of dextrosazone. This crystalline osazone, when purified as much as possible, was readily soluble in warm water, in alcohol, ether, chloroform, and, to a certain extent, in benzol. The amount of the purified osazone was so small that the melting point alone could be determined. This was done as usual in a capillary tube. When the temperature reached 140° C. the

substance commenced to darken slowly, and at 160° C. it began to melt. Further recrystallization of the osazone did not alter this melting point. In melting point, therefore, this osazone, if pure, differs widely from dextrosazone or lactosazone. In general appearance and solubility, as well as in its melting point, it appears to resemble very closely the osazone obtained by Hammarsten from the cleavage product of the peculiar nucleoproteid described by him as present in the pancreas.* Whether this body is a pentaglucose, however, we can not definitely say. We had hoped, especially in view of the strong reducing action of the above alcoholic solution, to obtain a fairly large amount of an osazone, sufficient to determine its content of carbon and nitrogen, but the yield of purified product was very small indeed.

In order to verify the above results, a second portion of mucin was decomposed with dilute acid — 4.75 grammes of mucin with two hundred and fifty cubic centimeters of 2.0 per cent. hydrochloric acid — the mixture being heated directly over a lamp for about five hours. The flask was connected with an inverted Liebig's condenser to prevent concentration, and the mixture was kept in a state of gentle ebullition. In this case there was much less of the antialbumid-like body so prominent in the first decomposition, the amount being less than one fifth that found before. The neutralization precipitate, however, was considerably larger, and albumose and peptone were both present in abundance. The caramel-like body precipitated by alcohol was naturally more abundant than in the first case, but on analysis it was found to contain a large percentage of nitrogen, so that its fancied resemblance to caramel is purely superficial. By evaporation of the alcoholic extract containing the greater portion of the reducing body a residue was obtained as before, from which a crystalline osazone was formed agreeing in all its properties' with the body previously described. The purified osazone melted at 158° to 160° C. It is thus evident that the mucin or mucins present in ox tendon yield on cleavage with dilute hydrochloric acid a carbohydrate body which forms a well-defined and crystalline osazone, although at present we can not state definitely the exact nature of this carbohydrate substance.

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ANIMAL COLORING MATTERS.

By William J. Gies.

Many of the animal coloring matters are substances of considerable functional consequence. Some, for example, are of special service in respiration; others appear to be important factors in vision; a large number afford protective effects; several, also, are attractive in their influence. A majority, however, seem to be without any apparent physiological relations and not a few are purely excretory products.

I. Classification.

The multitude of animal pigments may be arranged conveniently in the following general groups:

1. Respiratory Pigments. — These coloring matters are very important functionally. Most of them are carriers of oxygen, with which they unite loosely, receiving it in the organs of respiration, conveying it to the body parts, and there giving it up to the tissues. The leading ones are compound ("chromo") proteids. Among them are haemoglobin, haemocyanin, haemerythrin and chlorocruorin.

2. Derivatives of Respiratory Pigments. — Some of the best-known animal coloring matters are derivatives of haemoglobin, and many of the colored substances in the lower animals are undoubtedly formed from their blood pigments. Prominent derivatives of haemoglobin are bilirubin (haematoidin), stercobilin (urobilin), urochrom and haematoporphyrin.

3. Lipochromes. — These substances, yellow or yellowish-red for the most part, are very numerous. They are found particularly in adipose tissue, yolk of egg, butter, and in the tissues and epidermal structures of the lower animals. In solubilities they are much like the fats, and they show absorption bands toward

the violet end of the spectrum. Little is known of their chemical composition. They appear to consist of only carbon, hydrogen and oxygen. Among them are serum lutein, tetronerythrin and the "chromophanes."

4. MELANINS. — These are brownish-black pigments occurring especially in epidermal structures. They consist of carbon, hydrogen, nitrogen and oxygen. Nearly all contain sulphur: a few, iron. It is thought by some that they are derivatives of haemoglobin; by others, modified lipochromes. They have been produced outside of the body from simple proteids by prolonged hydration ("melanoidins"), which fact suggests, of course, that they may be so derived within the system. Among the typical members of the group are fuscin, phymatorhusin and sepic acid.

5. CHROMOGENS. — These are the colorless, or less colored precursors of actual pigments occurring in nature. The leading ones are indoxyl compounds, which give rise to red and blue indigo; melanogen; uroroseinogen; the chromogen of the suprarenal medulla, related probably to the pigment of the skin in Addison's disease; and urobilinogen. The so-called "humous substances," obtained by destructive chemical methods, and such bodies as proteinochromogen (tryptophan), which merely form colored combinations with various reagents, are, of course, purposely excluded here.

6. MISCELLANEOUS PIGMENTS. — This residual group includes a very large number of protective, attractive and other coloring matters, characteristic especially of the lower animals, studied only spectroscopically for the most part. Among those whose chemical individuality is not disputed are turacin, carminic acid, punicin, chlorophyll and lepidotic acid.

II. DISTRIBUTION.

LOWER ANIMALS. — Coloring matters are widely distributed throughout the whole of the animal kingdom. In some animals they occur only in the body fluids, in others they are also diffused throughout various tissues. In many they occur in the form of granules in certain cells or cellular layers. "Coloring matters are often collected in special sacs which open and shut, producing the 'shot' or play of color of the chameleon, dolphin, cuttlefish

and other animals. In many low animals the color of the pigment is characteristic of genera, families or even higher groups, as among infusorians, etc." Many of the lowest types, such as infusoria, sponges and hydroids, contain *chlorophyll* (green) in granular form and some ciliated animalcules are colored by *stentorin* (blue). Chlorophyll is found in several mollusks, crustacea and insects, and also in the so-called livers of many invertebrates (*enterochlorophyll*). The latter organs also contain a ferruginous pigment, *ferrin* (brown) and *cholechrom* or *hepatochrom* (reddish yellow), a lipochrom; also *helicorubin* (orange red). *Haematoporphyrin* (purplish red), a derivative of *haemoglobin* (red), occurs in the integument of star fishes, slugs, the common earthworm and various sponges. A number of corals and hydroids, and some sea anemones, are colored by *actiniochrom* (red); also by *polyperythrin* (red), probably identical with haematoporphyrin. Some actiniae contain a coloring matter very similar to another derivative of haemoglobin, *haemochromogen* (red), and convertible into haematoporphyrin. Many echinoderms contain *pentacrinin* (red and purple) and the following pigments give special coloration to the lower species from which the terms are derived: *aplysiopurpurin* (purple), *bonellein* (green), *echinastrin* (red), *astroidin* (yellow), *rhizostomin* (violet), *ophiurin* (yellowish brown), *asterocyanin* (bluish violet) and *comatulin* (red). *Punicin* (purple) is derived from the colorless secretions of various mollusks on exposure to light, and *carminic acid* (red) is the pigment characteristic of the cochineal.

The shells of some mollusks, and also some corals, contain "lipochromoids" and "melanoids." The brownish-black ink of *Sepia officinalis*, used to color the sea water and cover the flight of the animal, contains a melanin, *sepic acid* (black). The green (*chlorophan*), yellow (*xanthophan*) and red (*rhodophan*) pigments, "chromophanes," of the oil droplets in the retinal cones of birds, reptiles and fishes, as well as the yellow substance in the yolk of egg (*ontochrin*), are lipochromes. The egg of the water spider is colored by the two lipochromes, *vitellorubin* (red) and *vitellolutein* (yellow). Some of the characteristic coloring matters in decapod crustacea are lipochromes. The red *crustaceorubin* is closely related to hepatochrom (cholechrom) in the livers of these animals.

The eggs of the river crab and the lobster contain the same bluish pigment as that in the carapace of the animals. This pigment, called *cyanocrystallin*, becomes red with acid and on boiling in water. Crustaceorubin appears to be derived from it. The shells of various birds' eggs are pigmented by haemoglobin derivatives, among which are *biliverdin* (green); *oöcyanin* (blue), closely related to biliverdin; *oörhodein* (reddish brown), probably identical with haematoporphyrin; *oöchlorin* (yellow) and *oöxanthin* (red).

In certain butterflies the white pigment of the wings consists of *uric acid;* the yellow pigment, of *lepidotic acid*, which yields uric acid on hydration. The red pigment of the body scales is closely related to lepidotic acid. The wing covers of beetles contain *coleopterin* (red). The showy colors in the plumage of birds are due in part to the influence on light which the feathers themselves exert, causing the so-called "interference colors"; in great part, however, to pigments. *Turacin* (red) is one of the best known of these. Among the many other feather pigments are *turacoverdin* (green), *zoönerythrin* (red), *zoörubin* (brown), *zoöfulvin* (yellow), *picofulvin* (yellow), *turacobrunin* (brown) and *psittacofulvin* (yellow). Nearly all of these, "lipochromoids" and "melanoids," seem to be very closely related to the numerous skin pigments in birds, and scale and flesh pigments in fishes, such as *tetronerythrin* (red) and *coriosulfurin* (yellow); and to *lacertofulvin* (yellow), *lipochrin* (yellowish green) and others, in the skin of reptiles and various amphibia. The red pigment, *diemyctylin*, of *Diemyctylus viridescens*, like lepidotic acid, yields uric acid on hydration. Many invertebrates contain "histohaematins," haemoglobin derivatives, chief of which is *myohaematin* (*myochrom*) of the red muscles; found in the muscles of insects and mollusks, also, whose haemolymph does not contain haemoglobin. The characteristic color of the muscles of the salmon and other related fishes seems to be due to a red lipochrom identical with tetronerythrin. The nerves, particularly the ganglia, of some worms are colored bright red by haemoglobin.

Haemoglobin is present in the circulating fluid of many species of the invertebrate subkingdoms. It has been found in several species of the starfish family; in no lower invertebrate forms, however, but in most species of all genera higher up the scale.

The corpuscles in the hydrolymph of sea urchins contain *echinochrom* (yellow), a "lipochromoid," with possibly respiratory function. The haemolymph of various invertebrates is colored yellowish to yellowish green by lipochromes; violet to purplish red by "floridins," of which *haemerythrin* (red) is the best known. Haemerythin, and also *chlorocruorin* (green), replace haemoglobin in the haemolymph of worms; *haemocyanin* (blue) in that of most mollusks, crustacea, and some members of the spider family. In the haemolymph of crustacea the lipochrom, tetronerythrin (crustaceorubin, zoönerythrin), is also frequently found along with the haemocyanin. The blood of the common house fly, and other like species, contains haemoglobin, but that of butterflies and many related insects is green, and contains chlorophyll derived from the food; although chlorophyll occurs in other parts as well. The blood of many insects turns brown to black when it is shed, to which process the term "melanosis" has been applied.

HIGHER ANIMALS.— The various tissues and fluids of the higher animals owe their color, very often, to mixtures of several pigments. Colored granules are frequently derived directly from external sources; into the lungs (pneumonokoniosis), such as coal dust (anthracosis), iron particles (siderosis), etc., whence they are sometimes distributed to the liver, lymphatic glands, kidneys and other organs. They result, also, from medicinal introduction, as reduced silver in the alimentary tract, skin, liver, kidneys, etc. (argyria). They enter through the skin, also (tattoo).

The following concise arrangement gives practically all the more important pigments found in man and mammalia generally, and will aid to reference to more extended accounts than can be given here. The terms in italics indicate the pigments occurring only under unusual or abnormal conditions:

ADIPOSE TISSUE — lipochrom.

BILE — bilirubin, biliverdin; also biliprasin and urobilin in some; *bilifuscin*, *cholohaematin* (from chromogen), *hydrobilirubin*, *haemoglobin*, *methaemoglobin*, *haematin*. BILIARY CALCULI — bilirubin, biliverdin, bilicyanin, bilifuscin, bilihumin (?), biliprasin, cholet-elin (hydrobilirubin ?). BLOOD — (*a*) CORPUSCLES: oxyhaemoglobin, haemoglobin; (*b*) PLASMA: serum lutein, bilirubin (in some); *haemoglobin and direct derivatives*, *haemoglobin compounds with*

poisonous substances, *hepatogenous pigments*, *melanin*. BLOOD CLOTS (OLD) — haematoidin (bilirubin), rubigin or haemosiderin (ferric hydroxide). BONE — lipochrom in ossein and yellow marrow; haemoglobin in red marrow; *haematogenous pigments* in ossein.

CONJUNCTIVA — *bile pigments*. CONNECTIVE TISSUES — lipochrom, melanin; *bile pigments*. CONTUSION — bile pigments, haematoidin. CORPUS LUTEUM — lutein, *haematoidin* (?) CYSTS — lipochrom: haemoglobin derivatives, including bile pigments.

EYE — (*a*) CHOROID AND IRIS, fuscin; (*b*) RETINA, (1) *Rods* — visual purple (rhodopsin), visual yellow (xanthopsin); (2) *Pigment layer* — fuscin, lipochrin.

FAECES — stercobilin (urobilin), indigo chromogens, urobilinogen, sulphide of iron; pigments from food, such as carrotin, chlorophyll, haematin; *haemoglobin* and *siderous haematogenous pigments*, *bile* and *drug pigments*. FRECKLES — haematogenous pigment.

GANGLION-CELLS — lipochrom. GASTRO-INTESTINAL MUCOSA — *haemoglobin* and its *direct derivatives* (haematochromatosis). GLANDS IN GENERAL — haemoglobin in capillaries, chromogens, *haematogenous pigments*.

HAIR — lipochrom, melanin.

INTESTINE — (*a*) CONCRETIONS: hepatogenous pigment; (*b*) CONTENTS: essentially same as faeces, including bile pigment and hydrobilirubin normally.

LEUCOCYTES (phagocytic cells) — *any pigment* found elsewhere in the body. LIVER — ferrin, cholechrom, *rubigin*, *non-siderous haematogenous* and also *bile pigments*. LUNGS — *Inhaled particles*, *haemosiderin*, *melanin* (?) LYMPHATIC (*a*) FLUIDS — serum lutein, *haematogenous* and *hepatogenous pigments*; (*b*) GLANDS: *haemoglobin derivatives*.

MECONIUM — bile pigments, *haemoglobin* and its *derivatives*. MENSTRUAL FLUID — haemoglobin and direct derivatives. MILK (cream, butter, cheese) — lipochrom; "blue milk," *triphenylrosanilin* (*B. cyanogenous*); "red milk," *pigment* by *M. prodigiosus*; "yellow milk," *pigment* by *B. synxanthum*. MOLE (naevus) — haematogenous pigment. MUSCLE — myochrom (diffused haemoglobin ?), myohaematin (haemochromogen ?).

PANCREAS — haematogenous pigment. PLACENTA — haemoglobin, haematoidin, haematochlorin (biliverdin ?). PUS — lipochrom, pyocyanin (*B. pyocyaneus*), pyoxanthose, bilirubin, indigo blue (?), haemoglobin and decomposition products.

SEBACEOUS SECRETIONS — lipochrom. SKIN — melanin, *bile pigments* (haemochromatosis), *histohaematins* (?). SPLEEN — haemoglobin, *rubigin*, *non-siderous haematogenous pigment*. SPUTUM — *blood*, *bile*, and *pus pigments*; also inhaled particles. STOMACH CONTENTS — food pigments; *blood and bile coloring matters*. SUPRARENALS — haemochromogen and chromogen yielding red pigment on exposure to light. SWEAT — *pyocyanin*, *indigo blue* (?), *bile pigments*; *haemoglobin and derivatives* ("red sweat"). Hippopotamus and kangaroo: reddish-brown pigment; dwarf antelope: blue pigment.

TISSUES GENERALLY — coloration effects due to blood in capillaries; *bile pigments*, *haemoglobin and haematogenous pigments*. TUMORS — phymatorhusin, sarcomelanin, lipochrom, haemoglobin and derivatives. Horse: hippomelanin.

URINE — (*a*) PIGMENTS: urochrom, urobilin, uroerythrin, haematoporphryrin (urospectrin), *skatoxyl red*, *melanin*, *indigo* (blue and red), *bile pigments*, *haemoglobin and direct derivatives*, *drug coloring matters*; (*b*) CHROMOGENS: indoxyl and skatoxyl compounds; precursors of haematoporphyrin and urorosein (urorhodin, urorubin, etc.); urobilinogen, *hydroxybenzene derivatives* ("*alkaptonuria*"), *melanogen*. URINARY CALCULI AND SEDIMENTS — uroerythrin, urochrom; *haematoidin*, *indigo blue*, *bile pigments*, *haemoglobin products*.

VOMIT — blood, bile, food and drug pigments.

III. CHEMICAL AND PHYSICAL QUALITIES.

The animal pigments have been the subject of many laborious researches, but, owing to the great difficulties they present to the investigator, our knowledge of the chemical characters of most of them is very slight and uncertain. The primary obstacle in the way of their proper chemical study is the strikingly minute amount in which they commonly occur, and, as nearly all of them have very great tinctorial power, their coloration effects, therefore, are usually out of all proportion to the actual quantity in which

they are present in any medium. Further, isolation of the pigments by chemical means is apt to induce radical changes in them, for many are very unstable and much confusion has resulted from failure to recognize this important fact. Nearly all of the animal coloring matters seem to have definite and characteristic effects on the spectrum, and may be differentiated, to a certain extent, by the number and position of their absorption bands. But even the extremely delicate indications of the spectroscope have undoubtedly led to error in some cases, since very wide spectroscopic differences may be brought about by very slight changes of molecular structure or physical condition, such as often result from ordinary chemical treatment. Consequently, there is good reason for believing that not a few of the coloring matters which have been dignified with special names are merely closely related artificial derivatives (oxides, reduction products, etc.) of several antecedent pigments or chromogens.

It would carry us far beyond the scope of this particular article to present detailed reference to each of the pigments already mentioned. All of the most important are given due notice in more extended accounts of blood, urine, faeces, bile, etc., in these volumes,* so that it will be sufficient here to describe, in conclusion, a few of the best known of those found in the lower animals.

HAEMOCYANIN (blue), CHLOROCRUORIN (green).— Each of these pigments is analogous to haemoglobin in chemical structure and in function, the first replacing it in the haemolymph of mollusks and related forms, the second in that of worms. Both, like haemoglobin, unite loosely with oxygen ; oxyhaemocyanin is blue, haemocyanin itself is colorless. Haemocyanin contains copper in place of iron and has no special influence on the spectrum. Chlorocruorin, on the other hand, yields haematin and shows characteristic absorption bands.

TURACIN is a red, feather pigment. It possesses a spectrum which is almost identical with that of oxyhaemoglobin. It contains seven per cent. of copper, besides carbon, hydrogen, nitrogen and oxygen. The quantity of turacin in the feathers of a single bird does not exceed two or three grains. It may be extracted from the feathers with 0.1 per cent. alkali and precipitated

* Reference Handbook of the Medical Sciences.

from its solution with dilute acid. It is insoluble in water, alcohol and ether.

CARMINIC ACID (CARMIN). — The female cochineal (*Coccus cacti*) contains from twenty-five to fifty per cent. of this coloring matter. The pigment is also found in the blossoms of certain plants. Its composition is shown by its formula: $C_{17}H_{18}O_{10}$. Some of its compounds produce effects on the spectrum analogous to those of oxyhaemoglobin. Carminic acid is a glucoside; when it is boiled with dilute acids, and thereby hydrated, it yields an optically inactive, non-fermentable sugar and also "carmin red" ($C_{11}H_{12}O_7$):

$$C_{17}H_{18}O_{10} + 2H_2O = C_{11}H_{12}O_7 + C_6H_{10}O_5.$$

Carminic acid may be extracted from the cochineal with warm water. The pigment is soluble in alcohol and dilute acids, and forms salts with alkalies and metallic compounds.

PUNICIN. — The colorless secretion of a glandular organ situated at the lower part of the mantle, between the gill and the rectum of various species of *Murex* and *Purpura*, assumes, on exposure to light, a bluish-green color at first, then red, and lastly a purple-violet. This coloring matter, "Tyrian purple," is the "purple of the ancients" and for centuries was the dye of greatest beauty and value. Punicin is the name of the pigment; the chromogen has not been isolated. Punicin is insoluble in water, alcohol and ether; soluble in boiling glacial acetic acid. It dissolves readily in boiling aniline, from which it separates, on cooling, in crystalline form.

CHLOROPHYLL. — This important plant pigment is found in *Hydra viridis*, *Spongilla fluviatilus*, in the elytra of cantharides beetles, in the blood of many insects, in the so-called livers of many invertebrates, etc. It is insoluble in water, but dissolves in alcohol and ether, and consists of carbon, hydrogen, nitrogen and oxygen, and possibly iron. Chlorophyll, treated with concentrated acid, yields phyllocyanin. The latter, on fusion with caustic soda, is transformed into phylloporphyrin ($C_{16}H_{18}N_2O$), a close relative of haematoporphyrin ($C_{16}H_{18}N_2O_3$), which may be produced from haemoglobin, on treatment with acids, and is isomeric with bilirubin ($C_{16}H_{18}N_2O_3$). Phylloporphyrin and haematoporphyrin

are probably oxides of one and the same radicle. This kinship corresponds to analogous physiological relations of the pigments from which each can be derived.

TETRONERYTHRIN (CRUSTACEORUBIN, ZOÖNERYTHRYN). — The red pigment in the warty integument around the eyes, and also in the feathers of various birds, and in the hypoderm and haemolymph of many invertebrates, is one of the most widely distributed of all the pigments. It is soluble in ether, alcohol and chloroform, and shows the absorption bands and gives the reactions of a typical lipochrom.

LEPIDOTIC ACID. — The yellow pigment in the wings and excrements of butterflies (*Pieridinae*). It may be extracted with hot water or dilute alkalies, and is precipitated from such extracts on acidification. Its solutions show a greenish fluorescence and, on warming with dilute nitric acid, it yields uric acid. Warmed with dilute sulphuric acid a purple product, lepidoporphyrin, is obtained, which shows two characteristic absorption bands. This substance may also be derived directly from uric acid. The close relation of lepidotic acid to xanthin and uric acid is shown by the figures for their percentage composition :

	C.	H.	N.	O.
Xanthin (dioxypurin)	39.4	2.6	36.8	21.1
Lepidotic acid	38.1	3.5	37.1	21.3
Uric acid (trioxypurin)	35.7	2.4	33.3	28.6

The above paper was written in the spring of 1900. Additional facts may be found in the following publications :

Griffiths. Ueber den Farbstoff von *Echinus esculentes*. Chemisches Central-Blatt, 1900, ii, p. 638.

Neumann. Das Pigment der braunen Lungeninduration. Jahresbericht über Thier-Chemie, 1900, xxx, p. 882.

Rosenfeld. Ueber das Pigment der Haemochromatose des Darmes. Ibid., p. 918.

Henze. Zur Kenntniss des Haemocyanins. Zeitschrift für physiologische Chemie, 1901, xxxiii, p. 370.

Alexander. Das Labyrinth Pigment des Menschen und der höheren Säugethiere, etc. Centralblatt für Physiologie, 1901, xv, p. 293.

V. Furth und Schneider. Ueber thierische Tyrosinasen und ihre Beziehungen zur Pigmentbildung. Beiträge zur chemischen Physiologie und Pathologie, 1901, i, p. 229.

Jones and Auer. On the oxidation of native pigments. American Journal of Physiology, 1901, v, p. 321.

Ducceschi. Ueber die Natur der Melanine und einiger verwandter Körper. Jahresbericht über Thier-Chemie, 1901, xxxi, p. 64.

Kuenen. Hämolyse und hämatogene Pigmentbildung. Ibid., p. 867.

Zeynek. Ueber den blauen Farbstoff aus den Flossen des *Crenilabrus pavo*. Zeitschrift für physiologische Chemie, 1901, xxxiv, p. 148; 1902, xxxvi, p. 568.

Dubois. Ueber den inneren Mechanismus der Purpurbildung. Chemisches Central-Blatt, 1902, i, p. 535. Also, Ueber die Bildung des Purpurs bei *Purpura lapillus*. Ibid., 1903, i, p. 473.

Häcker and Meyer. Ueber die blaue Farbe der Vogelfedern. Centralbatt für Physiologie, 1902, xvi, p. 153.

Lubarsch. Ueber fetthaltige Pigmente. Ibid., p. 754.

Oppenheim. Zur Frage der Pigmentbildung aus Tyrosin. Ibid., p. 755.

Zdarek und Zeynek. Zur Frage über den Eisengehalt des Sarkommelanins vom Menschen. Ibid., p. 757.

Zumbusch. Beiträge zur Charakterisirung des Sarkommelanins vom Menschen. Zeitschrift für physiologische Chemie, 1902, xxxvi, p. 511.

Levrat and Conte. Origin of the natural coloration of the silks of *Lepidoptera*. Journal of the Society of Chemical Industry, 1902, xxi, p. 1392.

Schulz. Die physiologische Farbstoffbildung beim höheren Tiere. Ergebnisse der Physiologie, erster Jahrgang. I. Abteilung, p. 505.

Sieber-Schumoff. M. v. Nencki's Untersuchungen über den Blutfarbstoff und dessen Beziehungen zum Blattfarbstoff. Biochemisches Centralblatt, 1903, i, p. 86.

Mörner. Kleinere Mittheilungen. III. Die sogenannten gefärbten Kalkkörper im Lederhaut der Holothurien. Ibid., p. 185.

Wychgel. Onderzoeingen over het pigment der huid, en de urine gedurende de zwangerschap. Ibid., p. 193.

Marchlewski. Studies on natural coloring matters. Ibid., p. 215.

Gamgee and Hill. Ueber die optische Aktivitat des Hämoglobins und des Globins. Beiträge zur chemischen Physiologie und Pathologie, 1903, iv, p. 1.

Spiegler. Ueber das Haarpigment. Ibid., p. 40.

MAY, 1903.

Reprinted from the Archives of Neurology and Psychopathology, 1899, ii, p. 557.

15

EMBRYOCHEMICAL STUDIES. I. SOME CHEMICAL CHANGES IN THE DEVELOPING EGG.

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I. Introduction.

In his remarkable book on general physiology, Max Verworn says: "Der Lebensvorgang beruht in dem Stoffwechsel der Eiweisskörper." I am not certain whether at the present state of science we are justified in making such positive statements that life is only a chemical process. However, it is evident to every biologist that the workings of all mechanisms in which life manifests itself to us, lead to constant wear of those mechanisms or organisms. Biologists have also observed long ago that the living organism possesses a peculiar ability of repairing its constant losses. In fact, there are but very few conditions in the organism when a substance cannot be classified among "the dead," and when the two processes, waste and repair, are not to be noticed. In most conditions of life we can well distinguish these two main functions, dying and growing. And the state of any living organism, its working capacity, its "quality," so to say, depends fully on the relation between these two functions, which Max Verworn calls "biotonus." He further very ingeniously presents the last in form of a fraction A/D. (A = processes of assimilation; D = processes of dissimilation.) Thus the different states of the biotonus might be represented as

$$\frac{A}{D} = 1\,; \quad \frac{A}{D} > 1\,; \quad \frac{A}{D} < 1.$$

The significance of this is self-evident. In one case the assimilation and dissimilation are in a state of equilibrium; in the other assimilation predominates; in the third, dissimilation takes the first place. It is further self-evident to any student of biology

that none of these processes is a single chemical reaction, that processes of formation, growth, as well as those of decomposition are very complicated; that before the body substance is transformed into final decomposition products, it undergoes many intermediate changes, and before food is assimilated and converted into a part of the body protoplasm, it undergoes numerous transformations. Thus, Verworn presents a general formula of the "biotonus," as

$$\frac{a + a_1 + a_2 \cdots a_m}{d + d_1 + d_2 \cdots d_m}.$$

This mathematical representation of the biotonus is true not only speculatively, but is also in accord with experimental evidence. It should be remarked that physiological chemistry (organic as well as inorganic) began its work, broadly speaking, with analytical experiments; it began by studying the path of transformation of that most complex substance protoplasm, into its final decomposition products, urea, CO_2, ammonia, etc. It first closely followed this path in the living organism, and finally succeeded in imitating the organism, and at the present day we may obtain nearly all the decomposition products met with in the organism, by mere chemical means. But if our knowledge of the process of dissimilation has become quite extensive, we must on the other hand own that the process of synthesis of living substance, even of proteids alone, is as dark to us to-day as it ever has been. And yet nature offers to us conditions when the growth of the organism is so much predominating over its wear that it seems there ought to be little difficulty in following the organism in its process of growing.

All the highest organisms develop from one single cell, and in many organisms their growth takes place outside of the body of the parent organism. In the animal kingdom the amphibia and birds, among others, belong to the last, and they offer good material for the study of the chemical changes in the growing tissue or organism.

It is singular that in the development of biology, the discoveries of botany nearly always preceded those of the animal biologist, and this has repeated itself again in the study of the relation of chemical changes in the growing or rather developing organism.

The work of E. Schulze and his school is remarkable in its results (and we refer the reader who is interested in the subject, to the original articles), but very little has been done in this direction by the animal physiologist.

The work we are publishing here is the beginning of a series of articles on the chemistry of the developing egg. We think that this general study ought to precede the special study of the development and growth of individual tissues, as muscular, nervous, and glandular tissues, and so on.

Of all the substances most peculiar to the living organisms are the different nitrogenous compounds that take part in formation of the proteid compounds and reappear on the decomposition of the latter. These compounds may be classified in a general way into two groups: First, those consisting only of C, H, O and N, and second, those in which some other elements, mainly S, P and Fe (each of them separately, or all together), join the former in the formation of their molecule.

The first group may be again divided into substances with a well-defined acid nature, as the monoamido acids, like leucin, and into those of a well-defined basic nature, which are very numerous and quite different in their composition.

The second group again may be divided into simple proteids, containing only C, N, H, O and S, and combined proteids as nucleo-compounds, mucin, etc. It is the molecule of the latter compounds that may contain besides C, H, O and N, also P and Fe.

The aim of this work was to study the distribution of N among the main groups just enumerated in different stages of the development of the egg, or, to be more precise, we attempted to estimate the quantity of N in the form of compounds not basic by nature, like amidoacid — those in the form of bases and finally those in the form of proteids. Further, an attempt was made to ascertain whether in the course of development a new formation of the combined proteids (only the nucleo-compounds were dealt with) was taking place or not. The amounts of ash and water were also estimated.

The material used was the egg of the codfish. It was examined in the following four stages: unfertilized; 24 hours after fertilization; 11 days and about 20 days after fertilization.

All the material was furnished to us by the courtesy of the U. S. Fish Commission, and we wish to express our indebtedness to Doctor Bumpus and Mr. Locke, who were kind enough to supply us with fish eggs. It was only through their kind assistance that this work could be carried out.

II. METHODS.

Total nitrogen was determined, after the material was dried to constant weight at 105° C., by Kjeldahl's method. The nitrogen in the form of monoamido acids and related compounds was estimated by the following method:

The dry substance was extracted for 24 hours with 0.2 per cent. HCl solution. The mixture was then treated with phosphotungstic acid, and after standing twenty-four hours the precipitate containing the insoluble part of the tissue and the phosphotungstic precipitate digested by Kjeldahl's method (K_2SO_4 and $CuSO_4$ used for digestion). For estimation of the proteid nitrogen, the substance was first extracted in a Kjeldahl digestive flask, for twenty-four hours with boiling alcohol, then washed with ether and alcohol, and treated with boiling water and a few drops of acetic acid for about ten hours and with cold water for about ten hours more, and then the N estimated by Kjeldahl's method. (All the extracts were tested for proteids. The results were negative.)

To study the changes in the quantity of nucleo-compounds and nucleo-bases, the eggs were extracted with cold and hot alcohol, then dried in air, pulverized, again extracted with hot alcohol, cold and hot ether; again dried, first in air, then at 105° C.

To estimate the nuclein bases, the substance was heated on a water-bath in a flask with a return condenser with 2 per cent. H_2SO_4 for about ten hours. The acid was partly neutralized by Ba (OH_2), the filtrate concentrated, the silver salts of the nuclein bases obtained and weighed as such.

Another part of the same material which was used for determination of the nuclein bases was digested with pepsin-hydrochloric acid for a week, and the digestive fluid changed every second day. The residue was then washed with water, until the latter gave a negative biuret reaction and contained no HCl. It was then washed with alcohol, ether, dried and weighed.

To ascertain whether the residue was really a nuclein or a substance rich in nucleins, the P was estimated; but only in one case, as in the other two the quantity was not sufficient for a satisfactory P estimation.

We present below all the results in tabular form.

III. RESULTS OF ANALYSIS.*

I. H_2O AND ASH DETERMINATIONS.

	Subst.	Dry. Subst.		Ash	
	In grms.	In grm.	Per Cent.	In grm.	Per Cent.
F–O	9.7612	0.5737	5.33	0.0580	10.09
F–I	8.2201	0.4760	5.20	0.6480	17.17
F–II	7.0600	0.5640	7.98	0.0990	17.55
F–III	8.0975	0.5315	6.31	0.1045	19.66

II. DISTRIBUTION OF NITROGEN.

	Subst.	Total N		
	In grm.	in grm.	Per Cent.	Per Cent.
F–O	0.5405	0.059568	11.01	10.90
	0.4030	0.043800	10.80	
F–I	0.3914	0.039858	10.16	9.96
	0.4299	0.042048	9.77	
F–II	0.2985	0.033288	11.15	11.22
	0.3225	0.036354	11.29	
F–III	0.3180	0.029346	9.52	9.52

III. N IN PHOSPHOTUNGSTIC PRECIPITATE = PROTEIDS + BASES.

	Grm. substance.	Grm.	Per Cent.	Per Cent.
F–O	0.3670	0.030660	8.32	8.50
	0.2956	0.026280	8.88	
F–I	0.1791	0.014016	7.82	7.83
	0.3296	0.025842	7.84	
F–II	0.2855	0.024528	8.52	8.67
	0.3366	0.029784	8.85	
F–III	0.2251	0.021462	9.53	9.53

IV. PROTEID NITROGEN.

F–O	0.1650	0.012264	7.43	72.9
	0.2940	0.020824	7.15	
F–I	0.5267	0.028470	5.40	5.33
	0.5504	0.028808	5.26	
F–II	0.5535	0.041610	7.52	7.27
	0.6540	0.045990	7.03	
F–III	0.2575	0.017520	6.84	6.84

* F–O = unfertilized; F–I = 24 hours after fertilization; F–II = 11 days after fertilization; F–III = 20 days after fertilization.

V. PROPORTIONS OF ACIDS, BASES AND PROTEIDS.

	F-O		F-I	
	Per Cent. of Dry Subst.	Per Cent. of Total N.	Per Cent. of Dry Subst.	Per Cent. of Total N.
N in Monoamido compounds.....	10.90—8.60 = 2.30	21.10	9.96—7.83= 2.13	21.37
N in form of bases.....	8.60—7.29 =1.31	12.07	7.83—5.33= 2.50	25.10
N in form of proteids	7.29	66.00	5.33	53.57
	F-II		F-III	
	Per Cent. of Dry Subst.	Per Cent. of Total N.	Per Cent. of Dry Subst.	Per Cent. of Total N.
N in Monoamido compounds ...	11.22—8.67= 2.55.	22.72	9.52—9.53 = —.01	0
N in form of ..... bases.....	8.67—7.27= 1.40	12.48	9.53—6.84 =2.69	28.25
N in form of proteids	7.27	64.79	6.84	71.84

VI. RESULTS OF DIGESTIVE EXPERIMENTS.

	Subst. in grms.	Residue in grm.	Per Cent.
F-I	2.0442	0.0428	2.08
F-II	1.6980	0.0570	3.35
F-III	1.7767	0.1297	7.24

P.—Determination in the residue of F-III: 0.137 grm. of the residue = MgP_2O_8 = 0.014 gr. P = 2.65 %.

VII. DETERMINATION OF THE NUCLEO-BASES.

	Subst. in grms.	Grm. bases.	Per Cent.
F-O	1.8611	0.0022	0.12
F-I	2.0227	0.0438	2.16
F-II	1.5190	0.0325	2.14
F-III	1.2132	0.0455	3.75

IV. GENERAL REMARKS.

I think it would be premature to draw any very broad conclusions from the little work completed at present. Such conclusions should be deferred until the data have increased considerably.

The results of this work, however, tend to indicate that in the developing egg the processes of synthesis are preceded by those of decomposition (consult Table V.). In the first stage after fertilization the proteids diminish in quantity; basic nitrogenous substances are formed at their expense. Later the basic substances decrease in quantity and proteids grow. Whether the molecules of those proteids are formed from the basic substances will be investigated in the future.

It is also seen that the character of the proteids is changed during the development of the egg; the combined proteids as we may term them, such as nucleoproteids, increase greatly in quantity. The importance of mineral salts for the formation of tissues can be illustrated by the increasing quantity of mineral substances in the egg in the course of its growth.

I take occasion to acknowledge my indebtedness to Professor Chittenden for all the kindness shown by him to me while I was engaged in this work in the laboratory of Physiological Chemistry at Columbia University.*

May 22, 1899.

BIBLIOGRAPHY.

A. Tichomiroff. Chemische Studien über die Entwicklung der Insecteneier. Zeitschr. f. physiol. Chemie, IX., 578.

A. Kossel. Weitere Beiträge zur Chemie des Zellkerns. Zeitschr. f. physiol. Chemie, X., 248.

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* See Preface, page 7.

B. PATHOLOGICAL AND TOXICOLOGICAL.

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THE INFLUENCE OF BORAX AND BORIC ACID UPON NUTRITION, WITH SPECIAL REFERENCE TO PROTEID METABOLISM.

By R. H. Chittenden and William J. Gies.

[From the Sheffield Laboratory of Physiological Chemistry, Yale University.]

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In view of the wide-spread use of borax and boric acid as food preservatives it is somewhat singular that our knowledge of the influence of these substances upon the nutritional processes of the body is so slight and uncertain. E. de Cyon,* M. Gruber,† and J. Forster ‡ have indeed studied the action of these agents upon proteid metabolism, but with results which are utterly lacking in harmony. Thus Cyon's work with borax seemingly indicates that proteid metabolism is diminished under its influence, *i. e.*, that borax tends to protect the consumption of proteid matter in the tissues. Gruber's experiments, on the other hand, indicate with equal positiveness that borax has no proteid sparing power, but really leads to an increase in the rate of proteid metabolism. To add to the uncertainty, the experiments with boric acid carried out under Forster's supervision tend to show that this

* Cyon. Sur l'action physiologique du borax. Comptes rendus, 1878, tome 87, p. 845.

† Gruber. Ueber den Einfluss des Borax auf die Eiweisszersetzung im Organsmus. Zeitschr. f. Biol., 1880, Band 16, p. 198.

‡ Forster. Ueber die Verwendbarkeit der Borsäure zur Conservirung von Nahrungsmitteln. Nach Versuchen von Dr. G. H. Schlencker aus Surakarta. Archiv. f. Hygiene, 1884, Band 2, p. 75.

agent is wholly without influence upon proteid metabolism. Obviously, conclusions which are so much at variance cannot be accepted without careful consideration.

Cyon's experiments were conducted simultaneously on three full-grown dogs which were fed upon a diet almost exclusively proteid. His observations were practically limited to determining changes in body-weight during short periods, with an estimation of the nitrogen of the urine. He found that during the period when borax was included in the food, the animals gained noticeably in body-weight and that less nitrogen was contained in the excreta than in the ingesta. From these very crude observations the conclusion was drawn that borax, even to the extent of 12 grams per day, may be ingested with the food, especially when the latter is essentially proteid in nature, without provoking the slightest disturbance in general nutrition. Further, Cyon appeared to see in his results evidence that borax, if substituted for common salt in food, will facilitate the assimilation of the latter and bring about a great increase in the weight of the animal. Such deductions, however, were wholly unwarranted from the data at hand, for not only were the periods of observation exceedingly short, but, as pointed out by both Gruber * and C. Voit,† the animals at the beginning were much emaciated and received throughout the experiment such excessive quantities of meat that increase of body-weight would have inevitably followed without the presence of borax. Consequently, all that can be inferred legitimately from Cyon's experiments is that assimilation and general metabolism were not seriously affected by borax in the quantities given.

In Gruber's work more scientific methods were pursued, but it may well be questioned whether the conditions under which the experiments were conducted were adapted for bringing out clearly the full action of borax upon proteid metabolism. The two dogs employed were fed simply upon meat and water, and were presumably in a condition of nitrogenous equilibrium. In the first experiment, when the animal received daily 1,500 grams of meat and 200 c.c. of water, the daily excretion of urea in the urine

* Gruber. Loc. cit.

† Voit. Hermann's Handbuch der Physiologie, Band 6, Theil I. p. 165

varied from 75.82 grams to 110.30 grams during the six days prior to the administration of borax. Then 20 grams of borax were introduced with the food, an amount so large that vomiting was at once produced, leading to a loss of about 5 grams of the borax and about 100 grams of the meat, with most of the water. On this day, however, 108.20 grams of urea were excreted in the urine, although the food consumed was 100 grams less than the usual quantity. On the two following days, without borax and with the full complement of food, the excretion of urea amounted to 109.00 and 107.60 grams respectively. From these results Gruber concludes that the borax increased the excretion of urea 4–6 per cent. In the second experiment, with a dog of 34 kilos body-weight, fed on a daily ration of 1,100 grams of meat and 200 c.c. of water, the daily excretion of urea varied from 70.86 grams to 80.60 grams for the four days of the normal period, while the administration of 10 grams of borax was accompanied by an excretion of 82.14 grams of urea, and, on the second day following, the introduction of 20 grams of borax was accompanied by an excretion of 85.25 grams of urea. Further, on this latter day the volume of urine rose to 1,310 c.c., while the largest daily excretion prior to this day was 1,040 c.c. Gruber, therefore, concludes that borax does not spare proteid as Cyon asserts, but, just as in the case of common salt, sodium sulphate, and other neutral salts, it causes an increase in the elimination of water from the body and induces therewith an increased proteid catabolism. It is not to be inferred from this statement that there is simply an increased washing out of urea from the tissues, for, as Voit * has pointed out, the amounts of urea excreted on the days following the ingestion of borax simply fall back to the neighborhood of the average for the normal period, and do not drop below that average. Gruber also concludes that borax has no unfavorable influence upon the assimilation of food, since the quantity of feces, their content of solid matter and of nitrogen are within the limits of the normal elimination during periods when meat alone is fed. Further, no harmful influence, even after the ingestion of the largest dose — 20 grams — was to be observed, and the appetite of the animal was found to be undiminished on the days fol-

* Voit. Loc. cit., 165.

lowing that upon which borax was given. The objection we would make to accepting Gruber's conclusions in their entirety is that they are based solely upon the results following the administration of two large doses of borax, 10 and 20 grams, whereas, to our minds, longer periods with a dosage of borax continued for several days in succession would seemingly render the conditions much more favorable for an accurate judgment as to the character of the influence exerted by the substance on tissue changes. Further, since urea alone was determined in the urine, possible minor changes connected with the presence of the salt would naturally be overlooked. Lastly, we are inclined to the view that it is extremely hazardous to draw such sweeping conclusions from one or two short experiments of this nature, especially where, as in the animal body, individual characteristics not infrequently give rise to exceptional results quite foreign to those ordinarily obtainable.

In Forster's work with boric acid, Dr. Schlencker experimented on himself, using a mixed diet and taking boric acid in daily doses of 1–3 grams. Each experiment consisted of three periods, of three days each, the boric acid being taken in the middle period. The conclusions arrived at were that proteid metabolism is not influenced, the excretion of urea in the boric-acid period being midway between that of the fore and after periods. It was noticed, however, that the quantity of ethereal sulphuric acid in the urine was considerably lessened in the boric-acid period and in the period following, thus implying an inhibitory influence upon the putrefactive processes of the intestine. Further, it was observed that the amount of the feces, together with the contained nitrogen, was greatly increased under the influence of boric acid, from which it was inferred that this agent interferes with the assimilation of the food and perhaps, at the same time, gives rise to an increased secretion of mucus with a possible increase in the discharge of epithelial cells from the intestinal mucosa. This latter, however, is purely conjectural. Increased secretion of bile is also said to result from the action of boric acid. On the pulse and temperature no action was observed.

It is thus quite evident that the influence of borax and boric acid on nutrition, and especially their influence on proteid metabolism, is by no means wholly settled. The preceding statements

clearly emphasize the uncertainty of our present information on the more essential features of the question before us, and we have therefore deemed it desirable to carry out, as thoroughly as possible, a series of experiments upon the action of both borax and boric acid on proteid metabolism and related phases of nutrition.

Conduct of the Experiments. — The experiments were conducted wholly upon full-grown dogs ranging in weight from 8 to 12 kilos. The animal was confined in a suitable cage partially lined with galvanized iron and with the floor so arranged that both fluid and solid excreta could be collected in their entirety, while the upper portions of the cage were so constructed as to permit unrestricted circulation of air. In view of the length of the experiments — ranging from twenty-seven to fifty-six days each, with periods of eight to ten days' duration — it seemed inadvisable as well as unnecessary to empty the bladder each day with a catheter. Such diurnal variations as might possibly occur from incomplete emptying of the bladder at the end of the twenty-four hours would obviously be neutralized in periods of the above length, and consequently the urine was collected as naturally excreted, thus avoiding any possible disturbance of the normal condition of the bladder, etc. At the end of each twenty-four hours, the urine collected was combined, and its volume, specific gravity, etc., determined, after which the bottom of the cage, was rinsed with a little distilled water and these washings added to the main fluid. The latter was then made up to some convenient volume in preparation for the daily analysis.

The feces whenever passed were collected in a weighed dish, the mass thoroughly desiccated over a water-bath, and the dry weight ascertained. The dried material was then pulverized and the nitrogen-content as well as the ether-soluble matter determined in sample portions. The nitrogen determinations were always made in duplicate by the Kjeldahl method and rarely varied more than 0.05 per cent. Whenever, as sometimes occurred, hair accumulated in the cage it was likewise collected and the nitrogen determined. The ether-soluble matter was determined by extraction of the dried feces in a Soxhlet apparatus.

The animals were fed during the experiments on a mixed diet composed of fresh lean beef, cracker dust, lard and water. The

meat was prepared as follows: fresh lean beef, freed as far as possible from all adherent fat and connective tissue, was run through a hashing machine, after which it was enclosed in a bag of thin cloth, placed under a heavy press, and kept there under increasing pressure for several hours, the bloody fluid which drained off being thrown away. By this method there results a mass of tissue free from surplus moisture, and which, when enclosed in a bottle, will keep perfectly fresh on ice for seven to ten days without separation of fluid. Several advantages accrue from this method. Thus, we have a perfectly homogeneous mixture which can be drawn from for at least a week with surety that its nitrogen-content is constant. There is therefore no necessity for a daily determination of nitrogen in this portion of the diet, for each sample can be analyzed when prepared and the data accepted as long as the meat keeps fresh. Further, meat prepared in this manner at different times, if subjected to essentially the same pressure, varies only slightly in its content of nitrogen. We have invariably analyzed each lot when prepared to avoid any possibility of error, but, as the following results show, the differences in composition are very slight and necessitate very little alteration in the proportion of meat when changing from one lot to another. The following results are a few of the many obtained:

	Weight of Meat.	Absolute Content of Nitrogen.	Percentage of Nitrogen.
1.	0.8703 gram.	0.03041 gram.	3.49
	0.7710 "	.02682 "	3.48
	0.7631 "	.02628 "	3.44
2.	0.7673 "	0.02716 "	3.54
	0.9228 "	.03238 "	3.51
	1.0591 "	.03723 "	3.52
3.	0.8478 "	0.03015 "	3.56
	1.0014 "	.03591 "	3.59
	0.8876 "	.03152 "	3.55
4.	1.0082 "	0.03642 "	3.61
	1.0445 "	.03783 "	3.62
	1.0803 "	.03961 "	3.67
5.	1.1977 "	0.04265 "	3.56
	0.8142 "	.02902 "	3.56
	0.9793 "	.03463 "	3.54

The carbohydrate element in the diet, as already stated, was supplied by commercial cracker dust. This was purchased in

large quantity and preserved in well-stoppered bottles. It contained on an average 1.46 per cent. of nitrogen. The lard employed was entirely free from any recognizable amount of nitrogen.

The daily diet was divided into two equal portions, one half being fed at 8 A. M. and the other half at 6 P. M. When borax or boric acid was given, the daily dose was likewise divided and given either with the food or directly after. The body-weight of the animal was taken each morning just before feeding. Each day's urine included the fluid passed from 8 A. M. of one day to 8 A. M. of the next day.

Methods of Analysis. — Nitrogen was determined wholly by the Kjeldahl method, viz., in the daily analyses of the urine, feces and food material. All analyses were made in duplicate, and the figures given are based upon the averages of closely agreeing results. In analysis of the urine 5 c.c. were used for each determination, oxidation being carried out in a long-necked Kjeldahl flask with 10 c.c. of sulphuric acid and a crystal of cupric sulphate, thus doing away with the necessity of adding sodium sulphide in the distillation. The ammonia formed was distilled into quarter-normal hydrochloric acid, the latter being titrated with quarter-normal ammonia, using congo red as an indicator.

Sulphur and phosphorus were determined in the customary manner by evaporating a given volume of the urine — 25 c.c. for each determination — in a roomy silver crucible with 10 grams of pure sodium hydroxide (made from the metal) and 2 grams of potassium nitrate, igniting the residue until oxidation was complete and treating the fused mass with water. For sulphur, the mixture was acidified with hydrochloric acid, evaporated to dryness, the residue moistened with a few drops of hydrochloric acid and dissolved in hot water. The filtered solution was then precipitated in the usual manner with barium chloride, the resultant barium sulphate filtered, ignited and weighed, thus giving data for calculation of the total sulphur. For phosphorus, the aqueous extract of the oxidized urine was acidified with nitric acid, evaporated to dryness, the residue moistened with nitric acid and dissolved in warm water. From this solution the phosphoric acid was precipitated in the usual manner with molybdic solution and eventually transformed into ammonio-magnesium phosphate. From the weight

of magnesium pyrophosphate obtained, the total phosphorus of the urine was calculated.

Uric acid was determined by the well-known Salkowski-Ludwig silver method, using 100–200 c.c. of urine.

Phosphoric acid was determined by Mercier's * modification of Neubauer's method, *i. e.*, by titration of 50 c.c. of urine with a standard solution of uranium nitrate and tincture of cochineal as an indicator.

Total sulphuric acid was estimated by diluting 25 c.c. of urine with 3–4 volumes of water, adding 5 c.c. of dilute hydrochloric acid, heating to boiling and precipitating hot with barium chloride. The barium sulphate so obtained, after standing twenty-four hours in a warm place, was washed with hot water until free from chlorides and lastly with hot alcohol, ignited and weighed.

Combined sulphuric acid was determined by Baumann's method, using 100 c.c. of urine.†

Chlorine was determined in 10 c.c. of urine by Neubauer and Salkowski's modification of Mohr's method.‡ Other methods occasionally made use of are referred to in their appropriate places.

First Experiment. With Borax. — The animal made use of in this experiment was a short-haired mongrel bitch weighing about 12 kilos. She was brought into a condition approximating to nitrogenous equilibrium only after a preliminary period of nearly three weeks, during which time superfluous fat was lost and she became wholly accustomed to her surroundings. The daily food, at the time the experiment actually commenced, consisted of 250 grams of the prepared meat, 70 grams of cracker dust, 40 grams of lard and 500 c.c. of water. It contained 9.814 grams of nitrogen. This diet, with the above content of nitrogen, was adhered to throughout the entire experiment of twenty-seven days, the only variation being the slight changes in the amount of nitrogen, to be seen in the tables, incidental to the use of different lots of meat and in the employment of gelatin capsules during the borax period. These gelatin capsules, in which the borax was administered, contained 14.95 per cent. of nitrogen, the four cap-

* See Neubauer und Vogel's Analyse des Harns, neunte Auflage, p. 450.

† Ibid., p. 447.

‡ Ibid., p. 437.

sules used each day during the borax period containing 0.12 gram of nitrogen. This amount was naturally included in the nitrogen of the food.

The experiment extended through twenty-seven days and was divided into three periods of nine days each: a fore or normal period during which no borax was given, a borax period during which 45 grams of borax (5 grams a day) were administered, and an after period when normal conditions again prevailed. During the borax period of nine days the quantity of borax given per day amounted to nearly 0.6 per cent. of the total food and drink ingested, while of the solid food it formed 1.3 per cent. This dosage of borax, considering the size of the animal, was fairly large, and with this particular dog considerable difficulty was experienced in inducing the animal to take it. At first the borax was simply mixed with the food, but its presence was quickly detected and the food refused, although it was eventually coaxed down, but with some difficulty. After this first day the borax was given in capsules, as already stated, and no further difficulty of this sort was experienced. Three times during the borax period, however, the animal was nauseated and vomited a portion of the food, thus showing that this quantity of borax was sufficient to disturb the physiological equilibrium of the animal. The vomited matter was eventually eaten, however, later in the day, so that this occurrence did not disturb the validity of the experiment. It will be remembered that in Gruber's experiment with a much larger dog (39 kilos) 20 grams of borax likewise caused vomiting. In his experiment, however, the entire dose of borax was taken at one time, while in our case, 2.5 grams were given in the morning and a like quantity at night. Hence, taking into account the weight of the dog, it might perhaps be argued that 0.25 gram of borax to 1 kilo of body-weight will produce vomiting. This, however, is very questionable, for in the above exeriment the dog did not vomit until the afternoon of December 5, when she had already taken 12.5 grams of borax. In other words, the animal was without doubt suffering in part from the cumulative action of the salt. Thus, there was a slight attack of vomiting again on the fifth day (December 7) and a final attack on the eighth day (December 10). During the after period of

nine days the animal was perfectly normal, and at the close of the period, to again test the action of the borax, 5 grams were given at one time shortly after the morning meal. Forty-five minutes afterwards the animal vomited, and this occurred three times during the forenoon. We are inclined to lay particular emphasis upon this action of the borax because it tends to show that in this first experiment the dosage of borax throughout the nine days' period was as large as it well could be for this particular animal without vitiating the experiment, and that the conditions were therefore well adapted for bringing out distinctly any possible influence the borax might have upon the metabolic phenomena of the body.

We would also call attention to the obvious advantage — in spite of the greater labor involved — of continuing experiments of this character over comparatively long periods of time. To be sure, in some cases where the substance being tested has a marked physiological action, a single dose may show at once the character of the influence exerted, but too often erroneous conclusions are arrived at through negligence of this precaution. Where, however, the substance under examination is given for five to ten days consecutively, with careful examination of the excreta, the chances of detecting minor influences are greatly increased, and at the same time the danger of being led astray by a single exceptional result — or by other possible errors — is greatly diminished.

The table on page 335 contains the analytical results obtained throughout the experiment.*

Referring now to the table containing the results of the first experiment, it is to be noted that in the fore period of nine days the total nitrogen ingested amounted to 88.326 grams, while in the urine excreted during this period there were contained 87.185 grams of nitrogen, and in the feces 2.122 grams, making a total of 89.307 grams of nitrogen; hence the nitrogen balance for the period of nine days is — 0.981 gram. The body-weight remained

* The arrangement of daily records and summaries of the metabolism experiments has been somewhat altered in reprinting. The data have been put in more condensed form than in the original print. Nothing has been omitted. Daily averages have been added to the summaries.

TABLE I. FIRST EXPERIMENT.

Date. 1896.	Body. Weight.	Food. Nitrogen	Borax.	Urine. Vol.	Sp. gr.	Reaction.	Nitrogen.	Uric Acid.	Phosphorus.	Sulphur.	Total SO_3.	Comb'd SO_3.	Feces. Dry Weight.	Nitrogen.
	kilos.	grams.	gm.	c. c.		litmus	grams.							
I. *Fore Period. Nine Days.*														
Nov.														
24	10.9	9.814		505	1018	Acid.	7.945	0.038	0.468	0.491	0.962	0.058	....	....
25	10.9	9.814		716	1018	"	11.361	.049	.646	.720	1.388	.075	.....	.....
26	10.9	9.814		773	1017	"	11.367	.061	.688	.671	1.343	.077	.....	.. ..
27	11.0	9.814		786	1016	"	12.476	.049	.763	.737	1.521	.084	.....	.. ....
28	11.0	9.814		650	1017	"	10.069	.047	.585	.586	1.214	.064	.....	.....
29	11.0	9.814		415	1017	"	6.102	.040	.325	.381	0.765	.032	.....	.....
30	10.8	9.814		770	1019	"	12.302	.066	.760	.758	1.554	.084	.....	.....
Dec.														
1	10.9	9.814		575	1017	"	8.995	.040	.505	.570	1.148	.062	.....	.....
2	11.0	9.814		439	1018	"	6.568	.038	.410	.405	0.804	.055	38.15	2.122
II. *Borax Period. Nine Days.*														
3	10.9	9.903	5	796	1021	Acid.	13 344	0.054	0.821	0.789	1.631	0.097	.....	.....
4	11.1	9.933	5	368	1022	Alkaline	5.909	.032	.321	.371	0.705	.039	.....	.....
5	11.2	9.933	5	485	1025	"	9.183	.039	.535	.527	1.103	.057	.....	.....
6	11.1	9.933	5	520	1027	"	10.043	.042	.568	.592	1.197	.060	.....	.....
7	11.1	10.016	5	686	1024	"	12.823	.050	.818	.754	1.526	.068	35.91	2.292
8	11.2	10.100	5	422	1024	"	7.412	.051	.444	.426	0.825	.042	.....	.....
9	11.2	10.100	5	604	1023	"	10.742	.049	.615	.596	1.228	.069	.....	.....
10	11.3	10.100	5	498	1026	"	9.846	.031	.521	.600	1.174	.060	.....	.....
11	11.3	10.100	5	602	1020	"	8.825	.063	.456	.554	1.040	.063	24.68	1.627
III. *After Period. Nine Days.*														
12	11.5	9.981		488	1019	Acid.	8.727	0.042	0.441	0.596	1.024	0.055	.....	.....
13	11.3	9.981		670	1018	"	10.632	.053	.589	.716	1.247	.073	.....	.....
14	11.4	9.981		691	1017	"	10 047	.039	.621	.742	1.265	.083	. .....	.....
15	11.5	9.981		551	1016	"	7.804	.032	.482	.601	0.978	.049	.....	.. ..
16	11.4	9.981		681	1018	"	10.549	.051	.694	.736	1.345	.073	33.25	1.995
17	11.5	9.981		595	1019	"	10.121	.036	.662	.662	1.213	.062	.....	.....
18	11.5	9.981		572	1018	"	9.232	.036	.587	.613	1.119	.069	.....	.. ..
19	11.5	9.981		630	1017	"	9.587	.056	.574	.610	1.083	.068	.....	.....
20	11.5	10.036		549	1019	"	9.678	.044	.574	.616	1.180	.069	25.45	1.629

GENERAL SUMMARY.

Periods.	Total Nitrogen. Ingested.	Excreted.	Balance.	Urine. Vol.	Nitrogen.	Uric Acid.	Phosphorus.	Sulphur.	Total SO_3.	Comb'd SO_3.	Feces. Dry Weight.	Nitrogen.
	grams.			c.c.	grams.							
Period Totals.												
I.	88.326	89.307	—0.981	5629	87.185	0.428	5.150	5.319	10.699	0.591	38.15	2.122
II.	90.118	92 046	—1.928	4981	88.127	.411	5.099	5.209	10.429	.555	60.59	3.919
III.	89.884	90.001	—0.117	5427	86.377	.389	5.224	5.892	10.454	.601	58.70	3.624
Daily Averages.												
I.	9.814	9.923	—0.109	625	9.687	0.048	0.572	0.591	1.189	0.066	4.24	0.236
II.	10.013	10.227	— .214	553	9.792	.046	.567	.579	1.159	.062	6.73	.435
III.	9.987	10.000	— .013	603	9.597	.043	.580	.655	1.162	.067	6.52	.403

practically constant. The slight excess of nitrogen excreted over the amount ingested in this period is due possibly to lack of complete involution of the mammary glands;* the deficiency, however, is too slight, considering the length of the period, to need much consideration. For comparison, the results of the three periods, showing the relative excretion of nitrogen, may be arranged in tabular form:

	Fore Period.		Borax Period.		After Period.	
Nitrogen of Food		88.326		90.118		89.884
Nitrogen of Urine	87.185	89.307	88.127	92.046	86.377	90.001
Nitrogen of Feces	2.122		3.919		3.624	
Nitrogen Balance		—0.981		—1.928		—0.117
Ratio of Urine Nitrogen to Food Nitrogen		98.6 per cent.		97.7 per cent.		96.0 per cent.

It is thus evident that in this experiment, in spite of the large doses of borax and the length of the period, proteid metabolism is not modified in any noticeable degree. The amount of nitrogen eliminated through the urine in proportion to the nitrogen of the food, during the borax period, differs from that of the fore period only to a slight extent, and this difference is due apparently to a diminished assimilation of the proteid food. The change in the nitrogen balance of the borax period is plainly caused by a slight increase in the amount of fecal nitrogen, and not to increased metabolism, thus indicating that the borax has a tendency to diminish somewhat the absorption of proteid food, or possibly leads to an increased secretion of mucus. When, however, the nitrogen of the feces of the borax period is compared with both that of the fore and after periods the increase is seen to be so slight that it is perhaps unwise to attach much importance to it. Certainly the borax, though given in doses sufficiently large to keep the animal on the verge of nausea, did not in this experiment interfere greatly with the digestion of any of the food-stuffs, since the feces of the borax period are not much greater in amount than those of the after period, though somewhat larger in quantity than those of the fore period.

The weight of the animal during the twenty-seven days' period showed a tendency to rise somewhat, *i. e.*, from 10.9 kilos to 11.5

* Marcuse. Ueber den Nährwerth des Caseins. Pflüger's Archiv. f. d. ges. Physiol., 1896, Band 64, p. 223.

kilos. This, however, is not to be attributed to a laying on of fat nor to a retention of nitrogenous matter by the body, but is the result simply of a diminished excretion of water due to the presence of the borax. The results in this connection are in direct opposition to those obtained by Gruber with single doses of borax. There is here no suggestion whatever of an increased excretion of water, but on the contrary, a very marked decrease. Thus, by reference to Table I., it will be observed that during the fore period the total volume of urine amounted to 5629 c.c. and the body weight remained practically constant, *i. e.*, 10.9–11.0 kilos. During the borax period, however, the volume of urine fell to 4981 c.c. and the body weight gradually rose to 11.3 kilos, while in the after period the volume of urine rose to 5427 c.c. with a constant body weight of 11.5 kilos. It is thus quite clear that borax may decidedly check the output of water through the kidneys, and lead, as in this case, to its retention within the body.

Very noticeable also, in this experiment, was the sudden change in the specific gravity of the urine, as also in the reaction of the fluid, when borax was given. Thus, in the fore period the specific gravity of the urine stood at 1017–1018, but at the opening of the borax period it rose at once to 1022–1027, dropping back, however, as the borax was discontinued. Similarly, the reaction of the normal urine was acid to litmus, but on exhibition of borax, the reaction quickly changed to alkaline. The marked rise in the specific gravity of the urine during the borax period is not due solely to diminished elimination of water nor to increase in the proportion of metabolic products, but mainly to the borax itself, which is rapidly eliminated through the urine. We have not made any special trial to ascertain how soon the borax appears in the urine after its administration, but we have observed that the urine collected on the first day of the borax period gives, after acidulation with hydrochloric acid, a strong reaction with turmeric paper for boric acid. Further, that the elimination of borax in the urine is very rapid is manifest from the fact that, at the end of the borax period, the animal having received 45 grams of the salt, no trace of a reaction could be obtained with turmeric paper on the *second* day of the after period. In other words, elimination of the borax was practically complete twenty-four to thirty-six hours after the last

dose had been taken. These observations accord with Johnson's statements * that borax and boric acid begin to be eliminated through the urine a short time after their administration.

While it is clear from a study of the nitrogen excretion that proteid metabolism, under the conditions of this experiment, is not materially affected by borax, the other analytical results must not be overlooked. Thus, in the borax period the excretion of phosphorus, sulphur, total sulphuric acid and combined sulphuric acid is slightly below that of the fore and after periods. The differences, however, are so small that it is perhaps unwise to draw any positive conclusions from them, other than to admit their negative character. It can certainly be asserted with perfect safety that the borax has failed to exert any marked influence upon the excretion of either sulphur or phosphorus. In this connection it will be remembered that Forster † found, on feeding boric acid to man, a marked increase in the output of phosphoric acid. Borax, however, certainly fails to produce any such results, its presence in the body (of the dog) tending on the other hand to reduce the output of phosphorus. Further, it is evident that the slight diminution in the excretion of combined sulphuric acid is not sufficient to indicate any inhibitory influence upon intestinal putrefaction. Lastly, the figures obtained in connection with uric acid are such as to indicate a purely negative action.

Second Experiment. With Boric Acid.—The animal experimented on was a short-haired mongrel bitch weighing 8 kilos. Nitrogenous equilibrium was quickly established on a daily diet composed of 160 grams of the prepared meat, 40 grams of cracker dust, 30 grams of lard and 400 c.c. of water. This diet contained 6.144 grams of nitrogen and was practically adhered to throughout the experiment. The latter was of thirty days' duration, *i. e.*, three periods of ten days each. During the middle, or boric acid period, 1–2 grams of boric acid were given daily mixed with the food, the animal taking it without the slightest reluctance and without any apparent effect upon the appetite. No sign of nausea

* Johnson. Ueber die Ausscheidung von Borsäure und Borax aus dem menschlichen Organismus. Jahresbericht ü. Thierchemie, 1885, p. 235. See also Vigier: Note préliminaire sur l'action physiologique du borate de soude. Comptes rend. Soc. de Biol. Paris, 1883, p. 44.

† Forster. Archiv. f. Hygiene, 1884, Band 2, p. 75.

or vomiting was seen. With 2 grams of boric acid per day the mixture of food and drink contained 0.31 per cent., while the dry food contained 0.86 per cent. of boric acid. The total amount of boric acid given during the ten days was 14.5 grams.

During the fore period of ten days the animal received a total of 61.440 grams of nitrogen. The nitrogen excreted through the urine for this period amounted to 58.119 grams, while the feces contained 3.203 grams, thus making a total of 61.322 grams of nitrogen excreted, with a nitrogen balance of + 0.118 gram. Plainly the animal was in a condition of nitrogenous equilibrium. The table on page 341 contains the various data obtained.

The relative excretion of nitrogen for the three periods may be seen in the following summary:

	Fore Period.	Boric Acid Period.	After Period.
Nitrogen of Food..	61.440	62.032	61.943
Nitrogen of Urine.....	58.119 } 61.322	59.600 } 63.538	58.979 } 62.923
Nitrogen of Feces.....	3.203 }	3.938 }	3.944 }
Nitrogen Balance	+ 0.118	— 1.506	— 0.980
Ratio of Urine Nitrogen to Food Nitrogen.....	94.5 per cent.	96.7 per cent.	95.2 per cent.

From these figures it would appear that there is a slight tendency toward stimulation of proteid metabolism. When it is remembered, however, that the nitrogen balance for the boric acid period, —1.506, is the result of ten days' consecutive feeding with boric acid, it is manifest that the stimulating action is very slight, and our results may perhaps be considered as practically in accord with those reported by Forster, who found that in man on a mixed diet, boric acid in moderate doses (1–3 grams) was without influence on proteid decomposition as measured by the excretion of urea. Upon the assimilation of the proteid food there is no evidence of any action, *i. e.*, the nitrogen content of the feces during the boric acid period is essentially the same as that of the fore and after periods. Further, the total weight of feces for each of the three periods is so nearly the same, it is quite evident that assimilation has not been materially interfered with. In this respect our results fail to agree with those reported by Forster, who found that small doses of boric acid (1 gram in two days) given to a man on a mixed diet, and on a milk and egg diet, increased the excretion of feces; this increase being due, according to Forster,

not to any decrease in the assimilation of fat nor to increase in the volume of the secretions, but to a decreased assimilation of the proteid food under the influence of the boric acid. This difference in our results may of course depend upon the difference in the character of the animal species. In our experiment, the weight of the animal remained perfectly constant throughout the entire period of thirty days.

Unlike borax, boric acid fails to produce any change in the volume of the urine. Thus, in the fore period of ten days the total volume excreted amounted to 4,647 c.c., while in the boric acid period of the same length the total volume was 4,665 c.c., and in the after period 4,644 c.c. Further, there is no marked difference, to be measured by litmus paper, in the reaction of the fluid, although, as Table II shows, alkaline reaction is more common in the normal periods than in the boric acid period. In the latter period, however, the specific gravity of the urine, as might be expected, shows a higher average than in the two normal periods. This is due, as in the case of borax, to the rapid elimination of the boric acid through the urine. The latter shows the presence of the acid by the turmeric test on the first day of the boric acid period, while on the second day of the after period all trace of a reaction disappears, thus showing that the acid is rapidly eliminated from the body and is practically completely removed twenty-four to thirty-six hours after the last dose.

Upon the elimination of uric acid, boric acid appears to have a slight inhibitory effect, at least under the conditions of this experiment, but upon the excretion of total and combined sulphuric acid, chlorine and phosphoric acid, no tangible effect is produced. Certainly, the results in connection with combined sulphuric acid do not indicate any retarding effect upon the putrefactive processes in the intestine. In this connection it will be remembered that in Forster's experiments on man doses of boric acid, corresponding to those used by us, apparently gave rise to a marked retardation in the amount of ethereal sulphate excreted. As a result, Forster arrived at the conclusion that boric acid materially reduces intestinal putrefaction. Our results, however, show no action of this kind in the dog, and we are inclined to the view that both borax and boric acid are too rapidly eliminated from the system to be

TABLE II. SECOND EXPERIMENT.

Date. 1897.	Body. Weight. kilos.	Food. Nitrogen. grms.	Boric Acid. gm.	Urine. Vol. c.c.	Sp gr.	Reaction. litmus.	Nitrogen. grms.	Uric Acid. grms.	Total SO_3. grms.	Comb'd SO_3. grms.	Chlorine. grms.	Total P_2O_5. grms.	Feces. Dry Weight grms.	Feces. Nitrogen grms.
I. *Fore Period. Ten Days.*														
Feb.														
24	7.9	6.144		500	1015	Acid.	6.642	0.037	0.682	0.023	0.354	0.950	.....	.....
25	7.9	6.144		486	1012	"	5.051	.055	.533	.021	.404	0.726	.....	.....
26	7.9	6.144		460	1014	"	5.741	.048	.638	.029	.340	0.789	6.96	0.450
27	8.0	6.144		410	1015	"	4.956	.049	.560	.024	.379	0.665	.....	.....
28	7.9	6.144		581	1014	"	7.605	.096	.830	.036	.573	1.053	.....	.....
Mar.														
1	8.0	6.144		325	1014	"	4.067	.033	.477	.020	.210	0.506	.....	.....
2	7.9	6.144		525	1016	Alkaline.	7.613	.052	.807	.040	.404	1.008	11.90	0.780
3	7.9	6.144		440	1014	"	5.425	.057	.581	.022	.407	0.722	10.50	0.657
4	8.0	6.144		370	1014	"	4.119	.026	.464	.016	.291	0.540	.....	.....
5	7.9	6.144		550	1015	Acid.	6.900	.051	.749	.026	.462	1.004	17.30	1.316
II. *Boric Acid Period. Ten Days.*														
6	7.9	6.144	1	470	1016	Acid.	5.915	0.040	0.678	0.025	0.347	0.911	.....	.....
7	7.9	6.144	1	505	1016	"	6.390	.031	.712	.032	.451	0.946	.....	.....
8	8.0	6.183	1	380	1014	"	4.479	.028	.511	.020	.259	0.550	10.20	0.710
9	7.9	6.223	1	525	1017	"	7.280	.041	.767	.034	.356	1.073	.....	.....
10	8.0	6.223	1.5	400	1016	Alkaline.	4.166	.026	.481	.017	.333	0.586	9.75	0.660
11	7.9	6.223	1.5	530	1017	Acid.	7.460	.062	.803	.034	.555	1.017	.....	.....
12	7.9	6.223	1.5	460	1017	"	6.000	.040	.664	.031	.538	0.849	16.30	1.317
13	7.9	6.223	2	470	1017	"	6.035	.041	.682	.031	.565	0.841	.....	.....
14	7.9	6.223	2	480	1017	"	6.032	.035	.648	.027	.488	0.828	11.60	0.882
15	7.9	6.223	2	445	1017	"	5.843	.042	.683	.027	.425	0.899	5.45	0.369
III. *After Period. Ten Days.*														
16	7.9	6.223		432	1016	Acid.	6.100	0.057	0.670	0.026	0.346	0.813	5.45	0.369
17	8.0	6.223		360	1014	Alkaline.	4.318	.028	.526	.020	.226	0.526	7.71	.476
18	7.9	6.223		560	1016	"	7.630	.096	.874	.048	.604	1.106	.....	.....
19	7.9	6.223		485	1015	Acid.	6.284	.052	.717	.033	.448	0.890	8.82	.655
20	8.0	6.223		425	1013	Alkaline.	4.412	.040	.514	.023	.366	0.674	.....	.....
21	7.9	6.223		560	1017	Acid.	7.947	.069	.937	.053	.742	1.205	12.25	.814
22	7.9	6.041		490	1015	Alkaline.	5.922	.044	.678	.033	.528	0.831	.....	.....
23	7.9	6.188		450	1015	"	4.940	.036	.575	.022	.416	0.739	10.47	.798
24	7.9	6.188		480	1016	"	6.827	.037	.749	.038	.296	0.979	.....	.....
25	7.9	6.188		402	1015	"	4.599	.049	.571	.020	.260	0.686	10.90	.832

GENERAL SUMMARY.

Periods.	Total Nitrogen. Ingested. grms.	Total Nitrogen. Excreted. grms.	Balance. grms.	Urine. Vol c.c.	Nitrogen. grms.	Uric Acid. grms.	Total SO_3. grms.	Combined SO_3. grms.	Chlorine. grms.	Total P_2O_5. grms.	Feces. Dry Weight grms.	Feces. Nitrogen grms.
Period Totals.												
I.	61.440	61.322	+0.118	4647	58.119	0.504	6.321	0.257	3.824	7.963	46.66	3.203
II.	62.032	63.538	—1.506	4665	59.600	.386	6.629	.278	4.317	8.500	53.30	3.938
III.	61.943	62.923	—0.980	4644	58.979	.508	6.811	.316	4.232	8.449	55.60	3.944
Daily Averages.												
I.	6.144	6.132	+0.012	465	5.812	0.050	0.632	0.026	0.382	0.796	4.67	0.320
II.	6.203	6.354	— .151	467	5.960	.039	.663	.028	.432	.850	5.33	.394
III.	6.194	6.292	— .098	464	5.898	.051	.681	.032	.423	.845	5.56	.394

very effective in the intestine. As already stated, the elimination of borax and boric acid through the urine commences almost immediately after their ingestion, and it is very questionable, therefore, whether, with moderate doses of these substances, enough would remain unabsorbed at the lower end of the small intestine to exert much influence upon the growth and development of microörganisms. Certainly, the feces do not ordinarily contain any appreciable amount of borax or boric acid after these substances have been administered in moderate quantities, although obviously the length of time the feces are forming will have some influence upon their content of soluble matter. In only one instance, to be detailed later, where a very large dose of borax was given, could any decided reaction for boric acid be obtained in the feces. Johnson * states that in the case of the human organism borax and boric acid show great irregularity in their appearance in the feces, and that he was able to detect them in the latter only in six cases out of fourteen, although daily doses of 0.9–3.0 grams of boric acid were given.

Lastly, it is to be noted that in our experiment with boric acid there is no such increase in the excretion of phosphoric acid through the urine as was observed by Forster ; our results, indeed, fail to show any distinct influence exerted by boric acid upon the metabolism of phosphorized matter.

Third Experiment. With Borax and Boric Acid. — This experiment was divided into seven periods of eight days each, thus making a total of fifty-six consecutive days during which the variations in the composition of the urine and feces were followed as before, under the influence of both borax and boric acid. The object in extending the experiment through this lengthy period was to ascertain whether prolonged treatment with borax and boric acid might not eventually result in such a disturbance of physiological equilibrium that more positive data would be obtained. With this end in view, a mongrel bitch of ten kilos body-weight was brought into nitrogenous equilibrium, after which the urine and feces were analyzed for eight consecutive days, *i. e.*, the fore period. Borax was then given with the food for eight

* Johnson. Ueber die Ausscheidung von Borsäure und Borax aus dem menschlichen Organismus. Jahresbericht ü. Thierchemie, 1885, p. 235.

days, making the first borax period. This was followed by another period of eight days during which neither borax nor boric acid were administered, after which came a third period of eight days when boric acid was fed. This, in turn, was succeeded by a normal period of equal length, followed by eight days of borax treatment — the second borax period — concluding with a final after period of eight days, *i. e.*, a total of fifty-six days. By thus keeping the same animal under continuous observation for this length of time it might reasonably be expected that any cumulative action — assuming it to exist — would be clearly manifest. Further, considerably larger daily doses of borax and boric acid were administered than in the preceding experiments.

The daily diet made use of throughout the entire experiment consisted of 160 grams of the prepared meat, 40 grams of cracker dust, 30 grams of lard and 430 c.c. of water. Its exact content of nitrogen is shown in the table of the fore period. (Table III.) The total amount of nitrogen ingested during the fore period was 52.163 grams. The amount excreted during the same period was 51.734 grams, thus showing a nitrogen balance for the eight normal days of + 0.429 grams. The dog used in this experiment, although short-haired, lost considerable hair daily. This was therefore collected and at the end of each period its content of nitrogen was determined and the amount added to the nitrogen of the urine and feces, as seen in the accompanying table. It is interesting to note in this connection that the loss of hair in periods of eight days' duration may be considerable; so large, indeed, that an appreciable loss of nitrogen may result. Thus, in the seven periods of this experiment the total amount of hair shed was 61.98 grams, *i. e.*, 8–10 grams for each period, the total nitrogen thrown off in this manner amounting to 7.856 grams. These figures show that the hair shed contained 12.6 per cent. of nitrogen. Obviously, in careful experiments, this source of loss cannot be overlooked.

In the first borax period of eight days the daily dose of borax ranged from 2 to 5 grams, the total amount administered being 32.5 grams. In the following boric acid period the daily dose ranged from 1 to 3 grams, a total of 17 grams of boric acid being given. On commencing the second borax period the daily

TABLE III. (FIRST PART). THIRD EXPERIMENT.

Date.	Body.	Food.	Drug.	Urine.								Feces.	
1897. April.	Weight.	Nitrogen.		Vol.	Sp. gr.	Reaction.	Nitrogen.	Uric Acid.	Total SO_3.	Comb. SO_3	Total P_2O_5.	Dry Weight.	Nitrogen.
	kilos.	grms.		c.c.		litmus.	grms.						
I. *Fore Period. Eight Days.*													
21	10.0	6.593		490	1015	Acid.	6.160	0.040	0.525	0.042	0.981	.....	.....
22	10.0	6.770		470	1013	"	5.050	.032	.437	.036	0.697	.....	.....
23	10.0	6.770		540	1016	"	7.139	.042	.641	.071	1.117	.....	.....
24	10.1	6.406		440	1014	"	5.231	.028	.489	.059	0.779	.....	.....
25	10.0	6.406		640	1015	"	7.685	.060	.746	.093	1.329	14.33	0.846
26	10.0	6.406		465	1012	"	4.643	.031	.420	.039	0.638	.....	.....
27	10.0	6.406		525	1014	"	5.641	.047	.544	.061	0.862	.....	.....
28	9.9	6.406		626	1015	"	7.544	.035	.713	.094	1.335	10.36	0.571
II. *First Borax Period. Eight Days.*													
			Borax										
29	10.0	6.406	2	400	1015	Alk.	4.025	0.039	0.401	0.049	0.597	2.96	0.163
30	10.1	6.406	3	400	1022	"	6.738	.043	.677	.091	1.142	.....	.....
May													
1	10.0	6.406	4	591	1018	"	6.542	.042	.704	.089	1.107	.....	.....
2	10.0	6.406	4	470	1021	"	7.028	.042	.797	.126	1.089	.....	.....
3	9.9	6.406	4.5	520	1017	"	5.916	.031	.565	.072	0.781	20.10	0.990
4	10.1	6.285	5	380	1017	"	4.041	.024	.372	.040	0.409	.....	.....
5	10.1	6.285	5	460	1022	"	6.531	.040	.597	.082	0.977	.....	.....
6	10.0	6.285	5	540	1022	"	7.503	.032	.792	.113	1.272	20.69	1.023
III. *First After Period. Eight Days.*													
7	10.1	6.285		410	1015	Acid.	5.687	0.036	0.575	0.054	0.810	.....	.....
8	10.2	6.285		430	1012	"	4.330	.034	.449	.040	0.458	.....	.....
9	10.1	6.285		590	1016	"	7.671	.044	.623	.117	1.187	.....	.....
10	10.2	6.428		390	1014	"	4.717	.014	.563	.065	0.599	.....	.....
11	10.0	6.428		597	1015	"	7.425	.036	.872	.106	1.423	19.55	0.745
12	10.0	6.428		530	1013	"	5.952	.029	.586	.060	1.066	.....	.....
13	10.1	6.428		525	1014	"	5.894	.029	.620	.065	1.017	11.90	.627
14	10.1	6.428		490	1013	"	5.754	.026	.568	.055	0.959	8.21	.473
IV. *Boric Acid Period. Eight Days.*													
			Boric Acid.										
15	10.1	6.428	1	525	1015	Acid.	5.677	0.039	0.558	0.068	1.003	2.73	0.157
16	10.1	6.396	1	441	1015	"	5.424	.035	.627	.066	0.785	.....	.....
17	10.2	6.396	1.5	401	1014	"	4.247	.053	.454	.038	0.502	.....	.....
18	10.2	6.396	2	490	1015	"	5.909	.018	.637	.076	0.927	9.90	.529
19	10.1	6.396	2.5	555	1016	"	6.934	.031	.734	.100	1.184	.....	.....
20	10.2	6.396	3	465	1016	"	6.131	.041	.606	.080	0.806	9.68	.557
21	10.2	6.396	3	400	1014	"	4.588	.034	.457	.042	0.467	.....	.....
22	10.3	6.396	3	500	1018	"	7.029	.059	.689	.099	1.080	11.86	.579
V. *Second After Period. Eight Days.*													
23	10.3	6.396		402	1015	Acid.	5.424	0.051	0.597	0.075	0.671	3.95	0.192
24	10.3	6.396		445	1010	Alk.	3.957	.028	.394	.031	0.289	.....	.....
25	10.1	6.410		620	1014	Acid.	7.224	.066	.787	.077	1.115	.....	.....
26	10.2	6.410		521	1013	"	5.730	.051	.541	.045	0.911	9.76	.542
27	10.1	6.410		550	1014	"	5.614	.039	.601	.050	0.892	.....	.....
28	10.1	6.410		470	1016	"	6.518	.033	.722	.066	1.103	.....	.....
29	10.2	6.410		455	1013	Alk.	4.994	.041	.549	.043	0.692	.....	.....
30	10.3	6.410		480	1017	Acid.	6.977	.045	.769	.065	1.113	15.04	.731

TABLE III (SECOND PART). THIRD EXPERIMENT.

Date. 1897. May.	Body. Weight. kilos.	Food. Nitrogen grms.	Drug.	Urine. Vol. c.c.	Sp. gr.	Reaction. litmus.	Nitrogen. grms.	Uric Acid. grms.	Total SO_3. grms.	Comb. SO_3. grms.	Total P_2O_5. grms.	Feces. Dry Weight. grms.	Feces. Nitrogen. grms.
VI. *Second Borax Period. Eight Days.*													
31	10.1	6.410	Borax 10	530	1027	Alk.	7.711	0.053	0.836	0.070	1.474	.....	.....
June 1	10.1	6.410	10	420	1029	"	6.384	.037	.671	.052	1.102	.....	.....
2	10.3	6.410	5	360	1026	"	6.627	.029	.761	.099	0.998	19.10	0.874
3	10.4	6.410	6	342	1020	"	4.574	.029	.495	.038	0.336	.....	.....
4	10.3	6.392	7	540	1022	"	8.025	.042	.828	.113	1.112	.....	.....
5	10.2	6.374	10	450	1025	"	5.634	.031	.610	.077	0.680	19.40	1.019
6	10.2	6.374	8	502	1023	"	6.495	.040	.629	.070	1.005	.....	.....
7	10.3	6.374	8	513	1023	"	6.913	.034	.683	.076	0.809	17.55	0.844
VII. *Third After Period. Eight Days.*													
8	10.3	6.374		411	1016	Acid.	6.213	0.042	0.567	0.033	0.644	.....	.....
9	10.3	6.374		525	1013	"	5.834	.029	.558	.043	0.686	.....	.....
10	10.4	6.374		422	1011	"	4.284	.042	.447	.047	0.399	.....	.....
11	10.4	6.374		500	1014	"	6.149	.037	.638	.058	0.872	.....	.....
12	10.4	6.374		525	1016	"	7.560	.051	.781	.075	1.344	22.80	0.895
13	10.3	6.409		503	1013	Alk.	5.158	.043	.518	.030	0.644	.....	.....
14	10.2	6.445		652	1015	Acid.	7.917	.040	.856	.082	1 445	.....	.....
15	10.2	6.445		512	1012	"	5.663	.044	.631	.065	0.793	20.06	1.194

GENERAL SUMMARY.

Period.	Total Nitrogen. Ingested. grms.	Excreted. grms.	Balance. grms.	Urine. Vol. c. c.	Nitrogen. grms.	Uric Acid. grms.	Total SO_3. grms.	Comb. SO_3. grms.	Total P_2O_5. grms.	Feces. Dry Wght. grms.	Feces. Nitrogen. grms.	Hair. Nitrogen. grms.
Period Totals.												
I. Normal....	52.163	51.734	+0.429	4196	49.093	0.315	4.515	0.495	7.738	24.69	1.417	1.224
II. Borax (1).	50.885	51.686	—0.801	3761	48.324	.293	4.905	.662	7.374	43.75	2.176	1.186
III. After.....	50.995	50.334	+0.661	3962	47.430	.248	4.856	.562	7.519	39.66	1.845	1.059
IV. Boric Acid	51.200	49.026	+2.174	3777	45.939	.310	4.762	.569	6.754	34.17	1.822	1.265
V. After.....	51.252	49.130	+2.122	3943	46.438	.354	4.960	.452	6.786	28.75	1.465	1.227
VI. Borax (2).	**51.154**	**56.032**	**—4.878**	**3657**	**52.363**	**.295**	**5.513**	**.595**	**7.516**	**56.05**	**2.737**	**0.932**
VII. After.....	51.169	51.830	—0.661	4050	48.778	.328	4.996	.433	6.827	42.86	2.089	0.963
Daily Averages.												
I. Normal....	6.520	6.467	+0.053	525	6.137	0.039	0.564	0.062	0.967	3.09	0.177	0.153
II. Borax (1).	6.361	6.461	—0.100	470	6.041	.037	.613	.083	.922	5.47	.272	.148
III. After.....	6.374	6.292	+0.082	495	5.929	.031	.607	.070	.940	4.96	.231	.132
IV. Boric Acid	6.400	6.128	+0.272	472	5.742	.039	.595	.071	.844	4.27	.228	.158
V. After.....	6.406	6.141	+0.265	493	5.805	.044	.620	.056	.848	3.59	.183	.153
VI. Borax (2).	**6.394**	**7.004**	**—0.610**	**457**	**6.545**	**.037**	**.689**	**.074**	**.939**	**7.01**	**.342**	**.116**
VII. After	6.396	6.479	—0.083	506	6.097	.041	.624	.054	.853	5.36	.261	.120

dose of borax was placed at 10 grams. This was continued for two days, but on the third day after taking the morning dose of 5 grams the animal's appetite began to fail so that it became necessary to coax her considerably in order to have the day's ration consumed. On this day, therefore, only 5 grams were given, but on the following day the appetite was nearly normal and 6 grams of borax were given. The dose was then raised to 10 and 8 grams daily, as shown in Table III. a total of 64 grams of borax being given in this period of eight days. Throughout the entire experiment of fifty-six days the animal remained perfectly well, kept a fairly constant body-weight, and showed no symptoms of nausea or vomiting during the administration of either borax or boric acid. The only noticeable effect was a seeming loss of appetite on one day, as mentioned above. At the termination of the final after period, a single dose of 5 grams of boric acid was given. This resulted in vomiting 4–5 hours afterward.

The relative excretion of nitrogen for the seven periods is shown in the following summary :

	1. Fore Period.		2. First Borax Period.		3. First After Period.	
Nitrogen of Food.....		52.163		50.885		50.995
Nitrogen of Urine.....	49.093		48.324		47.430	
Nitrogen of Feces.....	1.417	51.734	2.176	51.686	1.845	50.334
Nitrogen of Hair..... ..	1.224		1.186		1.059	
Nitrogen Balance ...		+ 0.429		— 0.801		+ 0.661
Ratio of Urine and Hair Nitrogen to Food Nitrogen.....	96.4 per cent.		97.2 per cent.		95.0 per cent.	

	4. Boric Acid Period.		5. Second After Period.		6. Second Borax Period.	
Nitrogen of Food.....		51.200		51.252		51.154
Nitrogen of Urine.....	45.939		46.438		52.363	
Nitrogen of Feces.....	1.822	49.026	1.465	49.130	2.737	56.032
Nitrogen of Hair	1.265		1.227		0.932	
Nitrogen Balance ...		+ 2.174		+ 2.122		— 4.878
Ratio of Urine and Hair Nitrogen to Food Nitrogen.....	92.2 per cent.		93.0 per cent.		104.1 per cent.	

7.
Third After Period.

Nitrogen of Food.....		51.169
Nitrogen of Urine.....	48.778	
Nitrogen of Feces.....	2.089	51.830
Nitrogen of Hair.....	0.963	
Nitrogen Balance		— 0.661
Ratio of Urine and Hair Nitrogen to Food Nitrogen.....		97.2 per cent.

In the first borax period of eight days with a total consumption of 32.5 grams of borax, *i. e.*, an average of 4 grams per day, there is practically no change in the rate of proteid metabolism. There is, however, a slight rise in the amount of fecal nitrogen similar to that noticed in the first experiment with borax, by which the nitrogen balance is somewhat changed, but there is plainly no effect produced on proteid metabolism. In the second borax period, on the other hand, there is evidence for the first time of a distinct and unquestionable influence upon proteid metabolism. In this period of eight days 64 grams of borax were administered, and under its influence the excretion of nitrogen through the urine was greatly increased. As in the other experiments, the proportion of nitrogen in the feces was likewise increased, implying decreased assimilation of proteid food, but the nitrogen balance of — 4.878 is mainly due to direct stimulation of proteid metabolism. When, however, it is considered that to accomplish this result a daily dose of 8 grams of borax was required, and for eight consecutive days, with a dog weighing only 10 kilos, it is very plain that proteid metabolism is not readily affected by borax.

In the boric acid period of eight days, with a total dosage of 17 grams of the acid, there is some evidence of the diminished proteid metabolism. The excretion of nitrogen through the urine is certainly diminished ; there appears to be a sparing of proteid, but it is to be noticed that in the period following, the nitrogen balance remains unaltered, which fact casts some doubt upon the assumption that the result is due solely to the acid. It is of course possible that the action of the boric acid may be continued into the after period, but this we should hardly expect in view of the rapid elimination of boric acid from the system. Further,

after the second borax period, where the nitrogen balance is so noticeably disturbed, there is a quick return to the normal, the nitrogen balance for the final period dropping back to — 0.661 gram. Consequently, while the analytical data show a retention of nitrogen during the boric acid period, thus indicating diminished proteid metabolism, we feel some hesitation in attributing the result wholly to the boric acid, particularly as the earlier experiment with boric acid gave essentially negative results.

Especially noticeable in this experiment, as in the earlier experiment with borax, is the action of the latter agent in reducing the volume of the urine. (See Table III, General Summary.) In both borax periods the total volume of urine excreted is distinctly reduced, and the same holds true in this experiment with the boric acid. It is quite probable that the somewhat larger daily dose of boric acid made use of in the present experiment is responsible for this result, although it is possible of course that the personality of the animal may have had some influence. In the previous experiment with boric acid, where the maximum daily dose was 2 grams, the volume of the urine was unaltered. In view of these facts it is perhaps proper to consider the larger dosage of boric acid used in the present experiment as responsible for the apparent action upon proteid metabolism likewise.

Also noticeable in this experiment is the influence of the larger doses of borax upon the excretion of total and combined sulphuric acid. Both of these are distinctly increased in amount during the last borax period, in harmony with the increase in proteid metabolism, and there is a suggestion of the same influence in the first borax period. Moreover, in the last borax period the excretion of phosphoric acid is noticeably increased, while the elimination of uric acid is slightly diminished. It is thus plainly evident, as already stated, that while moderate doses of borax, even long-continued, are without influence upon the nutritional processes of the body, large doses may distinctly increase the rate of proteid metabolism, giving rise not only to an increased excretion of nitrogen, but also of sulphuric acid and phosphoric acid.

In all of these experiments with borax there is constant evidence of an increase in the weight of the feces during the borax periods. This increase in weight is due in part to an increased

output of nitrogenous matter through this channel, but whether the latter is caused by diminished digestion and absorption of the proteid food or to a stimulation of the mucus or other secretions from the gastro-intestinal tract is not so clear. It has been plainly shown, however, in another connection * that while borax in moderate quantities has no inhibitory action whatever on either gastric or pancreatic digestion of proteids, larger proportions do retard the proteolytic action of both digestive fluids. Further, retardation of proteolysis with borax is much more pronounced than with boric acid; hence it seems quite probable that the increased bulk of feces and the higher content of nitrogen therein during the borax periods is due mainly to slight retardation in the assimilation of proteid food.

Large amounts of borax likewise interfere with the assimilation of fatty foods; a statement which does not appear to be true of boric acid. In the accompanying table (Table IV) are given the results of our analyses of the dry feces, from a study of which it is plain that under the influence of large doses of borax — first and second borax periods of experiment third — both the total and percentage amounts of ether-soluble matter in the feces are greatly increased. Boric acid, on the other hand, produces no such effect. In the first experiment, with borax, the evidence of decreased fat absorption is less pronounced, although both the dosage of borax and the amount of fat fed were greater than in the first borax period of experiment third. Quite possibly this apparent difference in action may be due to the personality of the animal. However this may be, it is plain that large doses of borax are prone to increase somewhat the bulk of the feces, in part by diminishing slightly the assimilation of both proteid and fatty food, and in part, we think, through a tendency to increase the secretion of mucus. Thus, we observed in the last experiment, during the period when the largest doses of borax were given, that the feces were more slimy than in the normal periods, and appeared to contain more mucus than ordinarily. Further, it is to be noted that under the influence of large doses of borax there is a tendency toward diarrhœa; not very marked to be sure, but sufficient to render the discharge of feces somewhat watery.

* Chittenden. Influence of Borax and Boric Acid on Digestion. Dietetic and Hygienic Gazette, 1893, vol. 9, p. 25.

In spite of these evidences of minor action in the intestinal tract with large doses of borax, there is no evidence whatever of any influence exerted upon intestinal putrefaction, either by borax

TABLE IV. CONTENT OF FAT AND OTHER ETHER-SOLUBLE MATTER IN THE FECES.

EXPERIMENT I.

Date. 1896.		Feces. Dry Weight. Grams.	Ether-soluble Matter. Per cent.	Ether-soluble Matter. Grams.	Period.
Dec.	2	38.15	35.03	13.362	Fore
	7	35.91	33.60	12.067	
	11	24.68	25.23	6.227	
		60.59	30.02	18.294	Borax
	16	33.25	36.51	12.140	
	20	25.45	24.36	6.198	
		58.70	31.24	18.338	After

EXPERIMENT II.

Date. 1897.		Feces. Dry Weight. Grams.	Ether-soluble Matter. Per cent.	Ether-soluble Matter. Grams.	Period.
Feb.	26	6.96	23.70	1.649	
Mar.	2	11.90	17.88	2.128	
	3	10.50	16.95	1.770	
	5	17.30	20.82	3.602	
		46.66	19.61	9.149	Fore
	8	10.20	18.87	1.924	
	10	9.75	17.67	1.723	
	12	16.30	20.31	3.311	
	14	11.60	20.60	2.390	
	15	5.45	20.54	1.119	
		53.20	19.67	10.467	Boric Acid
	16	5.45	20.54	1.119	
	17	7.71	26.63	2.053	
	19	8.82	20.28	1.789	
	21	12.25	20.72	2.538	
	23	10.47	20.01	2.095	
	25	10.90	19.31	2.105	
		55.60	21.04	11.699	After

EXPERIMENT III.

Date. 1897.		Feces. Dry Weight. Grams.	Ether-soluble Matter. Per cent.	Ether-soluble Matter. Grams.	Period.
Apr.	25	14.33	28.91	4.134	
	28	10.36	29.09	3.029	
		24.69	29.01	7.163	Normal
	29	2.96	29.09	0.840	
May	3	20.10	36.35	7.306	
	6	20.69	37.06	7.671	
		43.75	36.15	15.817	Borax
	11	19.55	36.18	7.091	
	13	11.90	23.50	2.797	
	14	8.21	25.89	2.117	
		39.66	30.27	12.005	After
	15	2.73	25.89	0.705	
	18	9.90	33.19	3.286	
	20	9.68	25.76	2.499	
	22	11.86	24.13	2.858	
		34.17	27.36	9.348	Boric Acid
	23	3.95	24.13	0.953	
	26	9.76	24.20	2.372	
	30	15.04	29.54	4.443	
		28.75	27.02	7.768	After
June	2	19.10	45.01	8.596	
	5	19.40	39.06	7.579	
	7	17.55	33.94	5.940	
		56.05	39.46	22.115	Borax
	12	22.80	39.27	8.954	
	15	20.06	29.99	6.028	
		42.86	34.96	14.982	After

or boric acid. Even with the largest doses of borax the combined sulphuric acid of the urine is raised rather than lowered, and careful examination of the urine daily with Jaffe's indoxyl test ailed to reveal any indications pointing to an inhibitory influence

exerted by either borax or boric acid upon the production of indican. If, however, one studies carefully the output of combined sulphuric acid as shown in the various tables it will be noticed that the highest figures are generally obtained on the day (or the day preceding that) on which the dog defecates; while after defecation the combined sulphuric acid of the urine falls at once. In other words, the natural obstruction of the intestine favors, as is well known, the absorption of putrefactive products, and thus leads to an increase of combined sulphuric acid in the urine. When, on the other hand, defecation occurs, the combined sulphuric acid of the urine is at once diminished in amount. Upon these natural fluctuations of combined sulphuric acid even the largest doses of borax and boric acid are without effect, not because these agents are without influence upon microörganisms, but because they are too rapidly and completely absorbed from the intestine to exert much influence upon intestinal putrefaction. In only one instance were we able to detect any boric acid in the feces, viz., on June 5, at a time when the largest doses of borax were being given; and at the close of this period the boric acid reaction could be obtained with the urine only on the first day of the after period, so rapidly was the borax passed out of the body.

Lastly, attention may be called to the constant presence, in appreciable amounts, of uric acid in the urine of all animals experimented with, in opposition to the older statements of Liebig * and others that kynurenic acid may entirely replace uric acid in the urine of the dog. Our results, so far as they extend, are thus wholly in accord with the recent observations of Solomin.† We have, however, made no attempt to determine the amounts of kynurenic acid present.

General Conclusions. — Moderate doses of borax up to 5 grams per day, even when continued for some time, are without influence upon proteid metabolism. Neither do they exert any specific influence upon the general nutritional changes of the body. Under no circumstances, so far as we have been able to ascertain, does borax tend to increase body-weight or to protect the proteid matter of the tissues.

* Liebig. Annalen d. Chem. u. Pharm. Band 86, p. 125.

† Solomin. Zur Kenntniss der Kynurensäure. Zeitschr. f. physiol. Chem., 1897, Band 23, p. 497.

Large doses of borax, 5–10 grams daily, have a direct stimulating effect upon proteid metabolism, as claimed by Gruber; such doses, especially if continued, lead to an increased excretion of nitrogen through the urine, also of sulphuric acid and phosphoric acid.

Boric acid, on the other hand, in doses up to 3 grams per day, is practically without influence upon proteid metabolism and upon the general nutrition of the body.

Borax, when taken in large doses, tends to retard somewhat the assimilation of proteid and fatty foods, increasing noticeably the weight of the feces and their content of nitrogen and fat. With very large doses there is a tendency toward diarrhœa and an increased excretion of mucus. Boric acid, on the contrary, in doses up to 3 grams per day, is wholly without influence in these directions.

Borax causes a decrease in the volume of the urine, changes the reaction of the fluid to alkaline, and raises the specific gravity, owing to the rapid elimination of the borax through this channel. Under no circumstances have we observed any diuretic action with either borax or boric acid. The latter agent has little effect on the volume of the urine.

Both borax and boric acid are quickly eliminated from the body through the urine, twenty-four to thirty-six hours being generally sufficient for their complete removal. Rarely are they found in the feces.

Neither borax nor boric acid have any influence upon the putrefactive processes of the intestine as measured by the amount of combined sulphuric acid in the urine, or by Jaffe's indoxyl test. Exceedingly large doses of borax are inactive in this direction, not because the salt is without action upon microörganisms, but because of its rapid absorption from the intestinal tract.

Borax and boric acid, when given in quantities equal to 1.5–2.0 per cent. of the daily food are liable to produce nausea and vomiting.

Owing to the rapid elimination of both borax and boric acid, no marked cumulative action can result from their daily ingestion in moderate quantities.

At no time in these experiments was there any indication of abnormality in the urine; albumin and sugar were never present.

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ON THE INFLUENCE OF THE CONTENTS OF THE LARGE INTESTINE UPON STRYCHNIN.

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[A preliminary communication of an experimental study from the Rockefeller Institute for Medical Research. Carried out under the direction of Dr. S. J. Meltzer, New York.]

In the study of Meltzer and Salant * on the effect of subminimum doses of strychnin upon nephrectomized rabbits the remarkable fact was found that notwithstanding the removal of the chief eliminating organs, the kidneys, a good deal more than the fatal dose can be gradually injected into these animals with hardly any cumulative effect. As a possible explanation of this phenomenon it occurred to us that after the removal of the kidneys perhaps vicarious elimination into the gastrointestinal canal becomes developed. The appearance of urea in the alimentary tract in advanced cases of nephritis seems to favor such a suggestion.

To test experimentally this hypothesis, I set out to examine for strychnin the contents of the gastrointestinal canal of nephrectomized rabbits, which gradually received a considerable dose of strychnin. Of the methods for the separation of strychnin I first employed those of Otto Stas and of Dragendorff, but later I followed out scrupulously the method described by Haines.†

In four experiments in which the nephrectomized rabbits received gradually doses of strychnin amounting respectively to 2, 3.5, 6 and 8 mgrs. the entire contents of the gastrointestinal canal including the feces were carefully searched, but no strychnin could be detected. To test the efficiency of the method 1 mgr. of strychnin was added respectively to blood, crushed brains, liver, etc.; here strychnin was easily detected.

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* Meltzer and Salant. Journal of Experimental Medicine, 1902, Vol. iv.

† Haines. See Allen McLane Hamilton's System of Legal Medicine, 1894, Vol. i, pp. 451 to 459.

However, before deciding that in our nephrectomized rabbits the strychnin was not eliminated into the alimentary canal, 1 mgr. of strychnin was added to the gastrointestinal contents of these animals, and, to our surprise and disappointment, the strychnin could not be detected. To exclude the bare possibility that in nephrectomized animals some unknown substance is deposited in the gastrointestinal canal which prevents the detection of strychnin, the contents of the gastrointestinal canals of normal rabbits were taken for further experimentation.

In three experiments 1 mgr. of strychnin was added to the entire gastrointestinal contents of one animal; 2 mgrs. was added to the entire gastrointestinal contents of the second animal, and 1 mgr. was added to half of the contents of a third rabbit; in none of these experiments could strychnin be found, carefully as it was searched for. Since there was no difficulty in detecting strychnin in any other mixture of organic substance, the failure to detect it here could not be ascribed to imperfect methods or faulty technic.

But now this outcome appeared to have an important practical bearing. In numerous medicolegal cases it was claimed that strychnin was found in the stomach of poisoned human beings, and this finding had probably assisted more than once in deciding the fate of an accused. The question was now, How did the medicolegal experts succeed in separating and detecting strychnin in the contents of the gastrointestinal canal? Is it different with the contents of the gastrointestinal canal in human beings? Here another idea occurred to us. In most of these cases it was the contents of the stomach alone in which strychnin was found. We therefore started to examine separately the contents of some of the sections of the alimentary tract of the normal rabbit, with the following remarkable results:

One mgr. of strychnin added to the contents of the stomach alone: strychnin easily detected; 1 mgr. of strychnin added to the contents of the small intestine: strychnin detected; 1 mgr. of strychnin added to the contents of cecum and colon: no reaction of strychnin. The same uniform results were obtained in repeated experiments. It is, then, the contents of colon and cecum alone in which strychnin cannot be detected.

This failure to recover strychnin could be interpreted to mean simply that the methods followed for its detection in organic substances are not adapted for its separation from the contents of the cecum and colon of rabbits. However, we have seen that strychnin could not be detected in the contents of the entire gastrointestinal canal, which could only mean that the presence of the contents of the large intestine prevents the detection of strychnin, even when dissolved in another medium. This fact was now established again directly by the following experiments :

One mgr. of strychnin was added to two thirds of the contents of the stomach, with which one third of the contents of the large intestine was mixed : strychnin could not be detected. One mgr. of strychnin added to the contents of the small intestine, to which some of the contents of the large intestine were admixed : here, likewise, no strychnin was found. The presence of some of the contents of the large intestine then prevents the detection of strychnin in any other part of the contents of the alimentary canal.

This series of experiments leads up apparently to the very remarkable conclusion that the contents of the large intestine of normal rabbits contains something which, to say the least, interferes with the detection of strychnin (even 2 mgs.) by the methods at our disposal.

By experiments which are now in progress we will soon be able to state whether and how far the contents of the large intestine interfere also with the physiologic effect of strychnin.

In conclusion, I take the opportunity of acknowledging my indebtedness to Dr. W. J. Gies, professor of physiological chemistry at the College of Physicians and Surgeons, Columbia University, for his generosity in according to me all the privileges of his laboratory, in which the chemical work of this research was carried out.

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A FURTHER STUDY OF THE INFLUENCE OF THE CONTENTS OF THE LARGE INTESTINE UPON STRYCHNIN.

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In a previous communication to this journal[1] "On the Influence of the Contents of the Large Intestine Upon Strychnin," it was reported that small quantities of strychnin (2 mgs.) added to the contents of the cecum and colon of normal rabbits could not be detected when examined according to the method recommended by Haines.[2]

In a number of control experiments, however, with 1 mg. of strychnin mixed respectively with gastric contents, liver, crushed brains or urine, carried out with the same method, strychnin was easily found. The conclusion was therefore drawn that the contents of cecum and colon of normal rabbits contain something which interferes with the detection of strychnin by the methods at our disposal. Beside the method referred to, other methods, such as those of Draggendorff and Otto Stas, were employed at first, but were abandoned in favor of the Haines' method. I have recently made use also of Blyth's method,[3] with similar results.

A study of the physiologic effect of a mixture of strychnin and contents of cecum and colon of normal rabbits was next undertaken. A quantity containing a maximum of $\frac{1}{20}$ mg. of strychnin nitrate and injected into a frog of about 30 gms. induced a typical tetanus shortly after. This certainly proves that strychnin is not destroyed by the contents of the large intestine of normal rabbits. The failure to detect strychnin could be due, therefore, either to its destruction by heat used in the process of separation or to loss during the numerous manipulations involved in the various methods I employed. The first suggestion was put to an experimental test. A mixture of strychnin and con-

tents of large intestines of normal rabbits was heated on the water-bath for four to five hours at a temperature of 75° C. to 80° C., and injected into frogs. The results obtained, although not constant, have shown that this temperature does not destroy strychnin in the presence of the contents of the cecum and colon of normal rabbits. I therefore set out now to simplify the method of obtaining strychnin. This was accomplished as follows:

After adding strychnin to the contents of the cecum and colon of a normal rabbit the mixture was acidified with acetic acid. To this 95% alcohol was added and both were digested on the water-bath for several hours at 75° C. to 80° C. Strong alcohol was now added again, and the whole filtered, the residue washed with alcohol several times. A second extract was made by treating the residue with alcohol and filtering as before. The two filtrates were united and evaporated to about 2 ounces at the same temperature as before. This was now shaken up with chloroform in the separatory funnel and the chloroform drawn off. Chloroform was then added again and the solution made alkaline with KOH. After shaking vigorously the chloroform was drawn off. A second chloroform extract, to insure complete removal of the strychnin, was made, the two extracts united and evaporated. The residue was dissolved with acetic and water and filtered. The filtrate was made alkaline and shaken up with chloroform, which on evaporation gave a typical strychnin reaction with potassium bichromate and concentrated sulfuric acid. I found, however, that when the original mixture is digested at room temperature for 24 hours and the filtrate evaporated at 30° C. to 40° C. a much purer chloroform extract is obtained. I never failed to detect strychnin, even 2 mgs., in the contents of cecum and colon by the method as outlined when the operations were carried on at a low temperature. It is, therefore, the method that was at fault in the failure in the early experiments to detect strychnin in the contents of the cecum and colon of normal rabbits. Why should a simplified method give different results? This may be explained as follows: If a careful study be made of the various methods I have employed, such as the Otto Stas, Draggendorff, Haines, and Blyth, it may be seen that in all of them the number of manipulations is quite large. The solution is filtered many times and shaken up in the separatory funnel a number of times successively with several reagents before the alkaloid is ready for the final

test. As only small quantities of strychnin were experimented with, the loss of even a small portion during any of these processes would be sufficient to prevent its detection. But the same method was successfully employed for the separation of even smaller quantities of strychnin (1 mg.) from gastric contents, liver, brain, urine, etc. This may be explained by the fact that the organic impurities are not so numerous, and filtration much better; hence fewer manipulations with less loss of substance. While the acid solution of the large intestine had to be shaken up many times with amyl alcohol, benzin, etc., one extraction with amyl alcohol was all that was necessary for the purification of gastric content, etc. As amyl alcohol takes up water, it is not at all improbable that some of the strychnin was lost in this way. This would also explain why in many cases of strychnin poisoning the alkaloid has not been found. The large number of manipulations involved in the methods generally employed probably interfered with the detection of strychnin.

BIBLIOGRAPHY.

[1] *American Medicine*, August 18, 1902.
[2] Allen McLane Hamilton's System of Legal Medicine, Vol. i, pp. 451–59, 1895.
[3] Poisons: Their Effects and Detection, third edition, p. 331.

LYMPH FORMATION

ASHER AND GIES.

Untersuchungen über die Eigenschaften und die Entstehung der Lymphe.

Dritte Mittheilung

von

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IV. Ueber den Einfluss von Protoplasma-Giften auf die Lymphbildung.

Die Anwendung von Giften zur Erforschung der Bedingungen, unter welchen die Lymphe entsteht, ist schon mehrfach erfolgt, so z. B. durch Merunowicz, durch Camus und Gley, durch Spiro und durch Tschirwinsky. Die hierbei zu Grunde liegende Idee wechselte, je nach den theoretischen Vorstellungen, welche die betreffenden Beobachter über die Bildung der Lymphe hatten. Die Gifte wurden angewandt entweder weil sie den Blutdruck beeinflussten oder neuerdings, weil sie gewisse Secretionen förderten oder hemmten. Auf diesem Wege sollte also entweder die mechanische Lymphtheorie, die Abhängigkeit der Lymphbildung vom Blutdrucke, oder die Abhängigkeit vom secretorischen Vermögen der Capillarendothelien, Heidenhain's secretorische Lymphtheorie, geprüft werden. In der voraufgegangenen Mittheilung[1]) war zum ersten Male der Versuch gemacht worden,

1) L. Asher, Untersuchungen über die Eigenschaften und die Entstehung der Lymphe. 2. Mittheilung. Zeitschr. f. Biol. 1898, N. F. Bd. 19 S. 261.

Gifte zu benutzen, um die »physiologische« und die »physikalische« Componente bei den Erscheinungen der experimentell erzeugten Lymphbildung zu trennen. In dieser Mittheilung gedenken wir, den dort entwickelten Plan weiter auszuführen.

Unter »physiologischer« Componente verstehen wir denjenigen Theil der Lymphbildung, welcher bedingt ist durch die specifische Lebensthätigkeit der Zellen desjenigen Gewebes oder Organes, aus welchem die Lymphe jeweilig stammt. Unter normalen Verhältnissen ist es die Thätigkeit der lebenden Zelle, sind es die Bedürfnisse des lebendigen Protoplasmas, welche die Menge und die chemische Zusammensetzung der gebildeten Lymphe bestimmen. Diese Anschauung, welche als die cellular-physiologische Theorie der Lymphbildung bezeichnet werden kann, steht im Einklange mit den Thatsachen und mit weitverbreiteten biologischen Principien. In Bezug auf die Principien bedarf es nur des Hinweises, dass für die innere Athmung und für den Stoffwechsel der Nahrungsmittel die hier vorgetragene Anschauung fast gleichlautend ziemlich unbestritten gilt. Dass merkwürdiger Weise für den unmittelbarsten Vermittler der Stoffwechselvorgänge das cellulare Princip bisher geringere Bedeutung besessen hat, ist offenkundig und rührt daher, dass eine grosse Reihe von experimentellen Erzeugungsarten von Lymphbildung eine Erklärung zuliessen, welche mit verhältnissmässig einfachen mechanischen Vorstellungen auskam. Der Mechanismus der Zellen selbst blieb hierbei ganz aus dem Spiele. Thatsache aber ist es, wie Barbèra und der Eine von uns in der ersten Mittheilung[1]), sowie der Eine von uns in der zweiten zu beweisen versuchte, dass sowohl bei der normalen wie auch bei der künstlich erzeugten Lymphbildung nichts constanter Hand in Hand damit auftritt, als Thätigkeit der Organe, weshalb die Lymphe als ein Produkt der Arbeit der Organe bezeichnet wurde. Es mag betont werden, dass an dieser Aussage nichts Hypothetisches ist; die Hypothese beginnt erst bei der Erklärung des Zusammenhanges zwischen Organarbeit und Lymphbildung. Auch bei den Vertretern mechanischer Anschauungen beginnt der von uns aufgestellte Satz:

1) Diese Zeitschrift 1897, Bd. 36, N. F. Bd. 18 S. 154.

»Die Lymphe ist ein Produkt der Arbeit der Organe« Anerkennung zu finden. So hat W. Róth[1]) sich hierzu bekannt. Er verknüpft mit unserer Lehre freilich eine Reihe von physikalischen Vorstellungen, denen gegenüber mit aller Bestimmtheit daran erinnert werden muss, dass der Beweis für das factische Vorkommen im Organismus der von ihnen (zum Theil im Anschlusse an Korányi) angenommenen »rein physikalischen« Vorgänge noch zu erbringen ist. Wie wenig wir die Berechtigung des Versuches leugnen, den Mechanismus der Lymphbildung durch bekannte physikalisch-chemische Vorgänge dem Verständnisse näher zu rücken, geht wohl daraus zur Genüge hervor, dass wir selbst eine Vorstellung entwickelten, welche auf den möglichen Antheil der Transsudation und der Osmose hinwies. Aber wir betonten den hypothetischen Charakter dieser Vorstellung, indem wir erklärten: »Bei diesem Stande der Dinge kann die Vorstellung, welche wir über die Bildung der Ernährungsflüssigkeit haben, nur den Werth einer mehr oder weniger beglaubigten Hypothese besitzen«. Die wichtigsten Gründe, warum diese Einschränkung geboten ist, sind die folgenden: 1. Alle Versuche werden nicht an der Ernährungsflüssigkeit, sondern an der abfliessenden Lymphe angestellt. (Dieser Unterschied wurde schon in unserer ersten Mittheilung S. 228 ausführlich erörtert, und wir kommen daher hier nicht darauf zurück.) 2. Die Vorgänge in den Gewebsspalten lassen sich nicht ohne Weiteres aus den Erfahrungen ableiten, welche durch osmotische Experimente an Membranen gewonnen werden; denn jene Vorgänge werden durch das lebendige Protoplasma beeinflusst, dessen physikalisch-chemische Eigenschaften recht wenig bekannt sind. 3. Da die einzelnen Organe in Bezug auf ihren Chemismus specifisch verschieden sind, dürften deren Zellen in entsprechend verschiedener Weise an der Lymphbildung mitwirken; die bisherigen mechanischen Hypothesen berücksichtigen diese Unterschiede nicht, sondern sprechen nur von überall gleichen Kräften. 4. Die angenommenen und

1) W. Róth, Ueber die Permeabilität der Capillarwand und deren Bedeutung für den Austausch zwischen Blut und Gewebsflüssigkeit. Archiv f. Anat. u. Physiol. Phys. Abth. 1899, S. 416.

theoretisch durchaus möglichen osmotischen Kräfte lassen sich gar nicht selten bei reinen physiologischen Versuchen — z. B. bei den schönen Resorptionsversuchen Cohnheim's, den wichtigen, von den Mechanisten noch nicht hinreichend gewürdigten Erfahrungen Hamburger's an der Halslymphe des Pferdes oder den interessanten Versuchen von Cushny und Wallace über Abführmittel — direkt ausschliessen. Da wo scheinbar osmotische Kräfte zur Erklärung glatt ausreichen, wie bei den Versuchen Róth's an der Peritonealhöhle, handelt es sich oft um die Untersuchung eines Vorgangs, welcher gar nicht zu den normalen Functionen des betreffenden Körpertheils gehört. Man könnte also sagen, dass in Bezug auf einen solchen Vorgang die betreffenden Zellen ohne Leben seien.

Im Gegensatze zu dem hypothetischen Charakter der bisher vorgetragenen mechanischen Anschauungsweisen lässt sich die Arbeit der Organe als Faktor bei der Lymphbildung thatsächlich nachweisen. So stehen beispielsweise in Bezug auf die theoretisch äusserst wichtigen Lymphagoga als einzige gesicherte experimentelle Erkenntnisse da: erstens Heidenhain's Entdeckung eben ihrer eigenartigen lymphagogen Wirkung, zweitens unser Nachweis, dass dieselben die Leberthätigkeit intensiv steigern, weshalb wir vorschlagen, dieselben als Lebergifte zu bezeichnen. (In der vierten augenblicklich im Drucke befindlichen Mittheilung wird diese Frage im Anschlusse an die Untersuchung über die physiologische Arbeit der Leber eingehend behandelt werden.) Lehren, wie die Starling'sche von der Veränderung der Permeabilität der Capillarwand, oder Korányi's von dem durch Eiweisszerfall erhöhten osmotischen Druck, oder unsere eigene von der Aenderung der osmotischen Beziehungen zwischen Blut und Lymphe durch Dissimilationsprodukte der Zellen, sind entweder überhaupt nicht experimentell nachgewiesen oder gewinnen erst dadurch einen festen Ausgangspunkt der Prüfung, dass der physiologische Factor der Arbeit der Organe gesichert ist.

Während in der Arbeit der Organe wesentlich die »physiologische Componente« bei der Lymphbildung beruht, besteht daneben eine »physikalische Componente«, welche von der

specifischen Zellmechanik unabhängig ist. Die »physikalische Componente« tritt am besten bei gewissen künstlichen Steigerungen der Lymphbildung zu Tage und die Annahme liegt nahe, dass eben durch die Künstlichkeit der Versuchseingriffe diese Erscheinung begünstigt wird. Unter der »physikalischen Componente« bei der Lymphbildung verstehen wir alles das, was sich einwandsfrei und ausschliesslich auf die physikalischen Factoren der Filtration, der Diffusion und Osmose zurückführen lässt. Die Anerkennung einer Erscheinung als rein physikalisch verursacht, ist vor Allem abhängig von der Erfüllung der Bedingung, dass die specifische Zellthätigkeit nachweisbar bei der Mitwirkung ausgeschlossen ist — wir halten dies methodisch für eine unerlässliche Voraussetzung. Der Gang unserer Erkenntnisse in der Biologie ist zumeist der gewesen, dass eine Zeit lang die beobachtbaren Erscheinungen sich auf verhältnissmässig einfache, mechanische Weise erklären liessen, bei weiterer Analyse aber immer wieder, wie Heidenhain eindringlich betont hat, die »Vorgänge der lebenden Zelle« als mitwirkend erkannt wurden. Das ist auch der augenblickliche Stand der Lymphfrage.

Gemäss den entwickelten Anschauungen haben wir in dieser Mittheilung Giftwirkungen versucht, um die physiologische von der physikalischen Componente zu trennen. Die Anwendung von Giften ist insofern ein Nothbehelf, als die Giftwirkung meist sehr vielgestaltig, und die Art und Weise, wie sie die lebendige Zelle beeinflusst, ziemlich dunkel ist. Immerhin gibt es einige wenige Gifte mit gewissen so hervorstechenden Merkmalen, dass sie methodisch brauchbar erscheinen. Als solche boten sich für unsere Zwecke in erster Linie das Chinin und das Arsen dar.

Das Chinin gilt als ein ganz allgemeines Protoplasma-Gift; es sollte daher dazu dienen, zu prüfen, wie sich die Lymphbildung verhalten würde, wenn bekannte, lymphvermehrende Eingriffe statt hatten, während die specifischen Zellen gleichzeitig dem Einflusse eines allgemeinen Protoplasmagiftes unterworfen wären. Andererseits darf das Arsen auf Grund der Untersuchungen

von Böhm[1]) und besonders auch von Magnus[2]) als ein typisches Gefässgift bezeichnet werden; es sollte daher dazu dienen, zu untersuchen, welche Bedeutung einer bekanntermaassen vorhandenen, nicht etwa bloss hypothetisch angenommenen, erhöhten Permeabilität der Gefässwände für die Lymphbildung beizumessen sei. Dass die etwas schematische Trennung der beiden Gifte als Protoplasma- und Gefässgift eine streng durchführbare sei, liegt uns fern zu behaupten: es kommt nur darauf an, dass im Symptomenbild der Vergiftung quantitativ die Unterschiede der beiden Wirkungsarten so hervorstechende seien, dass etwaige Abweichungen von bekannten Vorgängen bei der Lymphbildung ohne Weiteres auf Protoplasma- oder Gefässvergiftung bezogen werden können.

Methodisches.

Die Präparation des Brustlymphganges geschah in der Art und Weise, wie sie in den früheren Mittheilungen geschildert wurde. Mit der Form der Canüle haben wir wiederum gewechselt, ein Ereigniss, was wohl manchem Untersucher des Lymphstromes als Nothwendigkeit sich aufgedrängt hat. Wir bedienten uns dieses Mal der Heidenhain'schen Form der Lymphcanüle, mit Weglassung der zweiten Biegung. Wir haben dieselbe nicht durch Nähte befestigt, sondern die Canüle wurde während der ganzen Beobachtungszeit von uns mit der Hand gehalten. Obwohl dies, namentlich während langdauernder Versuche, etwas unbequem ist, verlohnt es sich doch, der kleinen Mühe sich zu unterziehen; denn das Halten mit der Hand erwies sich desshalb so vortheilhaft, weil man den kleinsten Verlagerungen der Canüle, welche sich auch bei tiefer Narkose nicht vermeiden lassen, mit der nachgiebigen Hand sofort Rechnung tragen kann; hingegen ist man bei dem schweren Gewichte der Canüle durch das blosse Annähen an die Haut oder die Muskeln nicht vor unliebsamen Zerrungen oder Compressionen des Lymphganges geschützt. Die Bestimmung des Trockengehaltes der Lymphe geschah auf be-

1) Böhm u. Unterberger, Beiträge zur Kenntniss d. physiol. Wirkung der arsenigen Säure. Archiv f. exp. Pathol. u. Pharmak. 1874, Bd. 2 S. 89.

2) Magnus, Ueber die Entstehung der Hautödeme bei experimenteller hydrämischer Plethora. Archiv f. exp. Pathol. u. Pharm. 1899, Bd. 42 S. 250.

kannte Weise; es wurde, wenn möglich, jede aufgefangene Lymphportion auf ihre Concentration geprüft, weil, wie schon früher ausgeführt wurde, dem Trockengehalte der Lymphe in zahlreichen Fällen ein grösserer Werth zur Beurtheilung der Ereignisse im Quellgebiete der Lymphe zukommt als der blossen Ausflussmenge. Zur Zuckerbestimmung wurden Blut und Lymphe nach Drechsel's Methode vorbehandelt. Zunächst wurde eine abgemessene Portion in die zehnfache Menge 95proc. Alkohols langsam zugelassen; nach 24 Stunden wurde mit der Saugpumpe vom Niederschlage abfiltrirt und der gut ausgewaschene Niederschlag nochmals im Mörser mit Alkohol verrieben und filtrirt. Die vereinigten Filtrate wurden abgedampft und der Rückstand mit etwa 200 ccm heissen Wassers aufgenommen; hierzu wurden etwa 2 g reinen Paraffins und 6—7 Tropfen Phosphorsäure zugesetzt. Bei starkem Kochen ballen sich Verunreinigungen und Fett zusammen und nach dem Erkalten kann die klare Flüssigkeit von der festen Paraffindecke abfiltrirt werden. Der Paraffinkuchen wird noch drei Mal unter Zusatz von einem Tropfen Phosphorsäure mit Wasser aufgekocht. Die vereinigten sauren Flüssigkeiten wurden mit Na_2CO_3 neutralisirt und auf ein passendes Volum eingeengt. Der Traubenzucker wurde nach Kühne's Methode mit ammoniakalischer Kupfersulfatlösung bestimmt.[1]) Wir fanden es vortheilhaft, die auf Zucker zu prüfende Lösung ganz gleichmässig und allmählich zufliessen zu lassen, bis der Moment kam, wo die blaue Färbung entschieden abzublassen beginnt, dann nichts mehr zuzugeben und zwei Minuten lebhaft weiter zu kochen; das völlige Verschwinden der blauen Farbe nach zwei Minuten Kochen giebt die scharfe Endreaction. Bei den ersten Titrationen lässt man leicht zu viel Lösung zufliessen, man erhält aber bald constante Minimalwerthe.

Lymphbildung unter der Einwirkung von Chinin.

Alle Eingriffe, welche künstlich eine Beschleunigung des Lymphstroms, eine vermehrte und qualitativ veränderte Lymphbildung hervorrufen, sind unserer Auffassung nach auf das

1) O. Cohnheim, Ueber die Dünndarmresorption. Zeitschr. f. Biol. Bd. 36 N. F. Bd. 18 S. 134.

Innigste verknüpft mit veränderten Thätigkeitszuständen der Gewebe. Von solchen bekannten und sowohl von anderer Seite als auch von uns mehrfach discutirten Eingriffen unterzogen wir zunächst die Lymphbildung nach Injection von Traubenzucker einer Untersuchung auf ihr Verhalten unter der neuen Versuchsbedingung, dass gleichzeitig der Organismus einer starken Chininvergiftung ausgesetzt war. Wir wissen, dass die Injection von krystalloïden Substanzen zu einer regen Thätigkeit der verschiedensten drüsigen Organe Veranlassung gibt; leider liegen noch keine Untersuchungen über etwaige Differenzen je nach der angewandten Substanz vor, aber nach Allem, was wir über den Stoffwechsel wissen, müssen sich unzweifelhaft die Verhältnisse anders gestalten, je nachdem beispielsweise Zucker, Kochsalz oder Harnstoff injicirt wird. Es ist von allen Seiten zugestanden worden, dass gerade die Erscheinungen nach Injection von krystalloïden Substanzen zum guten Theile sich erklären lassen ohne Zuhilfenahme specifischer Zellthätigkeit. Da der Eingriff als solcher, vor allem in der bisher beliebten Methode, weit abweicht von physiologischen Zuständen, ist es nicht verwunderlich, dass die Antheilnahme der physiologischen Zellthätigkeit nicht ohne Weiteres entschleiert werden kann. In der letzten Mittheilung hat der Eine von uns eine Erscheinung beschrieben, welche als »physiologische Componente« bezeichnet wurde: es war das die Thatsache, dass nach Injection verhältnissmässig kleiner Mengen von krystalloïden Substanzen eine vermehrte Stoffabfuhr aus den Geweben durch die Lymphe stattfand. Wir haben zunächst geprüft, ob diese »physiologische Componente« irgendwie durch Chininvergiftung beeinflusst würde.

(Siehe Tabelle auf S. 188.)

Der Versuch ergab, dass eine wesentliche Veränderung in den Erscheinungen, trotz einer hohen Chinindosis, nicht zu erkennen war. Es trat sowohl nach intravenöser Injection einer nicht allzu grossen Menge Traubenzuckers eine merkliche, wenn auch nicht sehr grosse Lymphbeschleunigung ein, als auch erfolgte die charakteristische Vermehrung des Procentgehaltes der Lymphe an festen Substanzen. Diese vermehrte Stoffabfuhr durch

Tabelle I.

Vers. 1. Hund 7—8 kg. 24 Std. ohne Nahrung; Morphium-Aethernarkose.

Zeit	Lymphmenge in ccm	Lymphmenge pro Min. in ccm	Procentgehalt an festen Substanzen	Bemerkungen
10 h 8′ — 10 h 44′	4,3	0,12	5,27	
10 » 44′ — 11 » 20′	6,6	0,18	6,02	10 h 45′ — 50′. 30 ccm Kochsalzlösung enthaltend 10 g Traubenzucker + 0,5 g Chinin mur. in die Vena femoralis; keine anfängliche Verlangsamung; tropft sehr gut ab; Gerinnung viel weniger als früher.
11 » 21′ — 11 » 57′	6,0	0,17	5,53	
12 » 5′ — 12 » 41′	5,4	0,15	6,00	12 h 5′. 0,5 g Chinin mur. in die Vena femoralis.
12 » 45′ — 1 » 21′	3,0	0,08	6,41	
1 » 27′ — 1 » 37′	1,0	0,10		1 h 27′. 10 g Traubenzuck. in 30 ccm Kochsalzlösung in die Vena femoralis; keine anfängliche Verlangsamung.
1 » 37′ — 1 » 47′	1,2	0,12	6,31	
1 » 47′ — 1 » 57′	2,8	0,28		
1 » 57′ — 2 » 3′	2,1	0,35		
2 » 3′ — 2 » 13′	2,2	0,22		
2 » 13′ — 2 » 23′	2,3	0,23	6,20	
2 » 23′ — 2 » 33′	2,1	0,21		
2 » 33′ — 2 » 39′	0,5	0,08		

die Lymphe wurde auch nicht verringert, als in einer späteren Periode des Versuches durch eine abermalige Zuckerinjection eine erneute Lymphbeschleunigung hervorgerufen wurde. Aus dieser letzteren Thatsache geht hervor, dass die Concentrirung der Lymphe in späteren Stadien solcher Versuche nicht etwa darauf beruhe, dass der Lymphe nicht mehr genügende Wassermengen zur Verfügung stehen. Nachdem wir so erkannt hatten, dass dem Chinin nicht das Vermögen innewohne, die Vorgänge im Lymphsystem nach Injection von kleinen Mengen von Traubenzucker erkennbar zu beeinflussen, schritten wir zur Untersuchung der Lymphbildung unter dem gleichzeitigen Einflusse einer intravenösen Injection von grossen Mengen Traubenzuckers und einer starken Chininvergiftung. Es kam hierbei darauf an, folgende Momente zu berücksichtigen: die Vermehrung der Lymphmenge, die Verhältnisse der Concentration der Lymphe an festen Substanzen, die Zuckerausscheidung aus dem Blute und das Verhalten der Zuckerconcentration in der Lymphe. Die beiden letzten

Punkte beanspruchen desshalb besonderes Interesse, weil Heidenhain bekanntlich an ihnen einige auffallende Thatsachen entdeckte, in denen er Merkmale eines activen, secretorischen Eingreifens der Capillarendothelien sah.

Diese Annahme ist mit gewichtigen Gründen von Cohnstein und Starling bekämpft worden, und auch wir konnten uns, wenn auch aus ganz anderen Gründen wie die genannten Forscher, vorläufig der secretorischen Hypothese nicht anschliessen. Die Ergebnisse der besprochenen Versuche sind in Tabelle II niedergelegt.

(Siehe Tabelle auf S. 190.)

Diese Versuche lehren zunächst, dass trotz der Chininvergiftung nach Zuckerinjection eine erhebliche Beschleunigung des Lymphausflusses eintritt; vielleicht ist dieselbe nicht ganz so gross wie sie ohne Chinin gewesen wäre, wenigstens, wenn man als Maassstab die von Heidenhain in seiner grossen Arbeit mitgetheilten Zahlen wählt. Dort finden sich unter zwölf Versuchen Beschleunigungsquotienten, welche vom 4,8fachen bis zum 37,5fachen gehen. Doch wollen wir auf diesen Unterschied kein grosses Gewicht legen; zunächst kommen viele individuelle Schwankungen der Reaction auf Traubenzuckerinjection vor, wie sich am besten daraus ergibt, dass zwischen der pro 1 kg Körpergewicht injicirten Zuckermenge und dem Beschleunigungsquotienten gar keine Proportionalität nachweisbar ist; ferner haben wir bei einer so schweren Chininvergiftung, dass bald nach der Zuckerinjection der Tod eintrat, eine ganz ungemeine Beschleunigung des Lymphflusses sich entwickeln sehen. Auf dieses wichtige Experiment kommen wir weiter unten in einem anderen Zusammenhange zurück. Auch die Art und Weise, wie sich der Procentgehalt der Lymphe an festen Substanzen, namentlich aber wie sich die Ausscheidung des Zuckers aus dem Blute und die Anhäufung desselben in der Lymphe gestaltet, weicht nicht von den Befunden an unvergifteten Thieren ab. Ganz wie bei den letztgenannten verlässt der Zucker ausserordentlich rasch die Blutbahn und tritt in die Lymphe über, wo er sich so anhäuft, dass lange Zeit die Zuckerconcentration höher ist, nicht allein

Tabelle II.

Versuch 2. Hund 20 kg. Morphium-Aethernarkose.

Zeit	Lymphmenge in ccm	Lymphmenge pro 10 Min. in ccm	Zuckergehalt der Lymphe in Proc.	Procentgehalt an festen Substanz.	Bemerkungen
9h 20′— 9h 34′	11,0	7,8		4,90	
9 » 45′— 9 » 58′	24,0	16,2	0,451		9 h 45′—48′. 40 g Traubenzucker + 0,5 g Chinin mur. in 80 ccm Kochsalzlösung in die V. femoralis; keine anfängl. Verlangsamung.
9 » 58′— 10 » 12′	29,0	20,7	0,464	4,91	10 h 2′. 0,5 g Chinin mur. in die V. femoralis.
10 » 12′— 11 » 00′	32,0	6,7		3,38	10 h 22′. 28 ccm Blut aus der Art. femoralis, enthaltend 0,357% Zucker.
11 » 00′— 11 » 20′	9,8	4,9	0,364		
11 » 20′— 11 » 40′	12,0	6,0			11 h 25′. 54 ccm Blut aus der Art. femoralis; enthaltend 0,208% Zucker.
11 » 40′— 11 » 59′	10,1	5,3		5,54	
11 » 59′— 12 » 17′	10,2	5,6		5,49	12 h 17′. 50 ccm Blut aus der Art. femoralis; enthaltend 0,128% Zucker.

Versuch 3. Hund 20 kg. Morphium-Aethernarkose.

11h 4′— 11h 14′	5,3	5,3		6,30	
11 » 15′— 11 » 25′	8,2	8,2	0,843		11 h 15′—17′. 40 g Zucker + 1 g Chin. mur. in 80 ccm Kochsalzlösung in die V. femoralis; keine anfängliche Verlangsamung.
11 » 25′— 11 » 31′	9,6	16,0		5,46	11 h 28′. 35 ccm Blut aus der Art. femoralis, enthaltend 0,538% Zucker.
11 » 31′— 11 » 41′	10,0	10,0	0,870		
11 » 41′— 11 » 51′	9,0	9,0		3,86	11 h 45′. 33 ccm Blut aus der Art. femoralis, enthaltend 0,288% Zucker.
11 » 51′— 12 » 13′	12,2	5,6	0,748		12 h. 30 ccm Blut aus d. Art. femor., enthaltend 0,247% Zucker.
12 » 13′— 12 » 50′	10,0	2,7	0,376		
12 » 50′— 1 » 10′	5,6	2,3		5,27	
1 » 10′— 1 » 25′	7,0	4,7	0,518		1 h 10′—12′. 20 g Traubenzucker i. 100 ccm Kochsalzlösung in die V. femoralis.
1 » 25′— 1 » 40′	5,0	3,3	0,780		

als die gleichzeitige, sondern sogar als diejenige, die dreiviertel Stunden früher im Blute nachweisbar war. Aus dem dritten Experimente geht sehr deutlich hervor, dass selbst zwei Stunden nach der Einführung des Giftes auf eine erneute, gar nicht sehr grosse

Zuckerinjection hin Lymphbeschleunigung und Zuckeraustritt aus dem Blute in die Lymphe in charakteristischer Weise sich geltend macht. Das Gesammtergebniss unserer Versuche über combinirte Wirkung von Chinin- und Zuckerinjection auf die Vorgänge am Lymphstrome würde sich also dahin aussprechen lassen, dass Chinin dieselben nicht deutlich erkennbar zu beeinflussen vermag. Wenn die Voraussetzung richtig wäre, dass Chinin als allgemeines Protoplasmagift die specifischen Zellfunctionen tief schädigen müsse, so müsste man zu dem Schlusse gelangen, dass weder die Bildung einer vermehrten und anfänglich weniger, später mehr concentrirten Lymphe, noch die ungeheuer rasche Ausscheidung des Zuckers aus dem Blute, noch schliesslich das gänzlich unparallele Verhalten der Zuckerconcentration im Blute und in der Lymphe irgend etwas mit aktiver Zellthätigkeit zu schaffen haben. Man wird denjenigen, welche die geschilderten Vorgänge in bekannter, ausschliesslich mechanischer Weise zu erklären gewillt sind, zugeben müssen, dass die soweit mitgetheilten Versuchsergebnisse einen zwingenden Grund nicht enthalten, diesen Standpunkt zu verlassen, im Gegentheil eher eine Bestätigung desselben zu geben scheinen.

Eine nähere Discussion über die Wirkungen des Chinins auf den Organismus lehrt, dass die Verhältnisse nicht gar so einfach liegen. Leider ist manches, was über die Chininwirkungen als bekannt vorliegt, nicht eindeutig oder nicht hinreichend experimentell beglaubigt. Zunächst geben alle Beobachter an, dass toxische Dosen den Blutdruck erheblich mindern; nach der mechanischen Lymphtheorie soll die Lymphvermehrung nach Injection von Krystalloïden auf Capillardruckerhöhung beruhen: hier liegt also schon eine Schwierigkeit vor. Ferner scheint aus einer grossen Reihe von Beobachtungen hervorzugehen,[1]) dass toxische Dosen auf die Blutgefässe stark erweiternd wirken; unter diesen Umständen wird die Annahme nahe gelegt, dass die Capillarzellen selbst in ihrer Function leiden könnten. Da nun

1) Die Literatur hierüber findet sich in vorzüglicher Weise zusammengestellt in Wood, Therapeutics: its principles and practice. 9. Ed. Philadelphia 1894.

die Zuckerausscheidung aus dem Blute trotz Chininvergiftung ungestört verläuft, würden unsere Versuche eine weitere Stütze für die Ableugnung secretorischer Functionen der Capillarendothelien darbieten. Am wichtigsten erscheint uns aber, dass sowohl die Untersuchungen von Strassburg wie auch die von Chittenden ergaben, dass selbst grosse Dosen Chinins keine merkliche Störung der Kohlensäurebildung verursachten. (Etwas abweichend davon sind die Angaben von Boeck und Bauer.) Daraus geht hervor, dass durchaus nicht alle Stoffwechselvorgänge unter der Giftwirkung des Chinins zu leiden haben; unzweifelhaft hat aber die intravenöse Injection von Traubenzucker mit jenen Processen, welche zur CO_2-Bildung führen, enge Beziehungen. Diese Erwägungen führen zu dem naheliegenden Schlusse, dass möglicher Weise die Erscheinungen am Lymphstrome nach Injection von Krystalloïden nur desshalb nicht durch Chininvergiftung merklich geändert werden, weil das Chinin denjenigen physiologischen Processen gegenüber, welche durch intravenöse Zuckerinjectionen angeregt werden, machtlos ist. Wir müssen daher die Frage nach der physiologischen Componente bei der Lymphbildung in Folge von intravenöser Zuckerinjection als eine durch Chininversuche ungelöste bezeichnen.

Unsere nächste Aufgabe war, die Wirkung eines der Heidenhain'schen Lymphagoga unter gleichzeitiger Anwendung der Chininvergiftung zu prüfen. Wir hatten in unseren früheren Mittheilungen den Nachweis zu erbringen gesucht, dass die Vermehrung und gewaltige Veränderung in der Lymphbildung durch dieselben eine Theilerscheinung der intensiven Leberthätigkeit sei, welche durch jene Mittel ausgelöst würde. Da wir auf dieser Erkenntniss fussten, erschien die Anwendung des Chinins im Hinblick auf die ziemlich sichergestellte Thatsache (namentlich durch die Untersuchungen von Prior), dass durch Chinin die Harnstoffbildung sehr bedeutend herabgedrückt wird, geradezu geboten. Denn die letztere Thatsache weist ja auf eine tiefe Schädigung desjenigen Organes hin, dessen Thätigkeitsgrad besonders maassgebend für die Art und den Umfang der Lymph-

bildung ist, wie wir wiederholt nachgewiesen haben. Wir wandten für unsere Versuche als Lymphagogum (oder Lebergift) Extract von Blutegelköpfen an. Blutegelkopfextract hat vor manchen anderen Mitteln gleicher Wirkungsart den grossen Vortheil voraus, dass es dem Herzen und den Gefässen gegenüber in denjenigen Dosen, die zur Anregung der Lymphbildung erforderlich sind, unschädlich ist. Beim Pepton liegen die Verhältnisse viel verwickelter, da dasselbe nicht allein das Herz, sondern auch, wie aus den Untersuchungen von Thompson[1]) hervorgeht, sehr ausgeprägte Wirkungen auf die Gefässe besitzt. Worauf es aber wesentlich ankommt, das ist Pepton und Blutegelkopfextract gemeinsam: denn das Letztere regt in gleicher Weise, wie Barbèra und der Eine von uns und auch Gley für Pepton nachwiesen, nach Gley's in der Festschrift der Société de biologie (1900) niedergelegten Beobachtungen stark die Leberthätigkeit an. Wir wandten für unsere Versuche ein Blutegelinfus an, gestützt auf die Erfahrungen von Eguet[2]), der in Sahli's Klinik nachgewiesen hat, dass dieses Präparat am wirksamsten und von der grössten Constanz war. Ausser dem jeder Zeit frisch bereiteten Infus bedienten wir uns noch eines von Haussmann (St. Gallen) hergestellten Glycerinextractes, von dessen Wirksamkeit auf die Hemmung der Blutgerinnung wir uns durch einen eigenen Versuch überzeugten. Ein Cubikcentimeter dieses Extractes entspricht zwei Blutegelköpfen. Aus den Ergebnissen von Versuch 4 ist mit ziemlicher Deutlichkeit zu erkennen, dass die charakteristische Wirksamkeit des Blutegelinfuses auf die Lymphbildung durch die Chininvergiftung ganz wesentlich modificirt wird.

(Siehe Tabelle auf S. 194.)

Es wird zwar, wie Tabelle III lehrt, die Lymphmenge nach der Injection von Blutegelinfus recht erheblich gesteigert, aber das, was so charakteristisch für die Wirkung eines solchen

1) W. H. Thompson, The physiological effects of »peptone« when injected into the circulation. Journ. of. Physiol. 1899, Vol. 24 p. 874.

2) Eguet, Ueber den Einfluss des Blutegelinfuses auf die Thrombenbildung. Inaug.-Dissert. Bern 1894.

Tabelle III.

Versuch 4. Hund 10 kg 12 cg Morphium, später Aether.

Zeit	Lymphmenge in ccm	Lymphmenge pro Min. in ccm	Procentgehalt an festen Substanzen	Bemerkungen
9 h 48′ — 9 h 58′	2,5	0,25	7,34	
9 » 58′ — 10 » 11′	3,0	0,23	6,96	9 h 58′ — 10 h 8′. 0,6 g Chinin mur. in 80 ccm Kochsalzlösung in die V. femoralis.
10 » 11′ — 10 » 21′	1,8	0,18	6,93	
10 » 21′ — 10 » 33′	7,3	0,61	6,99	10 h 22′. 30 ccm Blutegelinfus in die V. femoral. (10 Blutegelköpfe in 50 ccm Salzlösung infundirt.) 10 h 27′ Ausfluss beschleunigt.
10 » 33′ — 10 » 42′	10,0	1,11	6,64	Lymphe gerinnt viel weniger. 10 h 37′—40′ 6 ccm Infus in d. V. fem.
10 » 42′ — 10 » 53′	6,6	0,60	6,34	10 h 47′—52′ der Rest des Infuses in die Vene.
10 » 53′ — 11 » 5′	11,0	0,92	5,41	10 h 54′—11 h 5′. 360 ccm 0,85 proc. Kochsalzlösung in die V. femor. 10 h 59′ deutl. Beschleunigung; vorher Verlangsamung.
11 » 5′ — 11 » 14′	19,0	2,11	4,00	
11 » 14′ — 11 » 23′	10,0	1,11	4,32	
11 » 23′ — 11 » 32′	5,8	0,64	4,71	

Mittels ist: die bedeutende Steigerung des Procentgehaltes der Lymphe, bleibt vollständig aus. Auf Grund aller bisherigen Beobachtungen wäre bei einem so ungemein hohen Beschleunigungsquotienten der Lymphe wie 4,5 im Gegentheil eine entsprechende grosse Vermehrung der festen Substanzen in derselben zu erwarten gewesen. In dem vorliegenden Versuche nimmt die Concentration unausgesetzt ab. Gerade dieser Contrast zwischen Menge und Concentration erscheint besonders werthvoll, weil er darauf hinweist, dass zwar dem Infus als solchem Wirksamkeit innewohnt, aber dessen Wirksamkeit durch das Eingreifen eines anderen Momentes in die durch dasselbe sonst ausgelösten Vorgänge gestört worden ist. Dieses andere Moment ist die Chininvergiftung. Die Chininvergiftung hat die Auslösung einer Leberthätigkeit von solcher Intensität durch das Blutegelinfus verhindert, dass dadurch nicht allein ein vermehrter Flüssigkeitsübertritt, sondern auch eine gesteigerte Stoffzufuhr in die Lymphe veranlasst würde. In der Thatsache, dass Chinin die charakteristische Wirkung der Lymphogoga erster Klasse

(Lebergifte) unterdrückt, liegt ein neuer Beweis dafür vor, dass der Erfolg derselben geknüpft ist an das Stattfinden einer erhöhten Leberthätigkeit. Wir haben im vorliegenden Versuche durch Injection einer grossen Menge von Kochsalzlösung zum Schlusse untersucht, ob die Permeabilitätsverhältnisse der Gefässwände irgendwie gelitten hätten: das aus dem Grunde, weil man geneigt gewesen ist, die Wirkung der Lymphagoga auf blosse passive Veränderung der Permeabilität der Gefässwände zurückzuführen. Der prompte Erfolg der Kochsalzinjection erwies, dass die Permeabilität der Gefässwände von der Norm nicht abwich; es ist somit der Einwand nicht zulässig, dass die Chininvergiftung durch Störung der Permeabilität der Gefässwände hinderlich gewesen sei. Andererseits ist die Schädigung der specifischen Leberfunctionen durch Chinin experimentell bewiesen; erstens durch den schon erwähnten, von Prior[1]) gelieferten Nachweis, dass gerade derjenige Stoffwechsel, an welchem die Leber einen so hervorragenden Antheil nimmt, unter Chininzufuhr stark darniederliegt, zweitens durch den neuerdings von Cavazzani[2]) erbrachten Beweis, dass Chinin die Glykogen bildende Function der Leber hemmt. Wir theilen in der folgenden Tabelle noch zwei weitere Versuche mit, wo nach der Chininvergiftung Blutegelinfus ohne jede Wirkung auf den Lymphstrom war.

(Siehe Tabelle IV auf S. 196.)

Im 5. Versuch, in welchem offenbar durch das Chinin ein hoher Grad der Prostration erzielt war, hatte Blutegelinfus überhaupt keinen nachweisbaren Einfluss auf die Lymphbildung. In Versuch 6 benützten wir als Injectionsweg für die anzuwendenden Mittel die V. lienalis; über die Methodik wird in der vierten Mittheilung berichtet werden. Auf diese Weise wurde sowohl das Chinin wie auch das Blutegelinfus direct der Leber zugeleitet und konnte so möglichst verdünnt in demjenigen Organe ihre Wirkungen entfalten, welches bei dem vorliegenden Probleme

1) Prior, Ueber den Einfluss des Chinins auf den Stoffwechsel des gesunden Organismus. Pflüger's Archiv 1886, Bd. 34 S. 237.

2) Cavazzani, Influence de la quinine sur la glycogenèse et sur la thermogenèse du foie. Arch. ital. de Biol. 1899, T. 32 p. 350.

Tabelle IV.

Versuch 5. Hund 6,25 kg. 6 cg Morphium, hernach Aether.

Zeit	Lymphmenge in ccm	Lymphmenge pro Min. in ccm	Procentgehalt an festen Substanzen	Bemerkungen
10 h 30′ — 10 h 53′	7,2	0,31	9,27	Lymphe von Anfang an blutig; 10 h 33′—53′ 0,5 g Chin. mur. in 40 ccm Kochsalzlösung in d. V. femoral.; viel Gerinnung in der Lymphe.
10 » 53′ — 11 » 8′	2,4	0,16	9,30	
11 » 8′ — 11 » 43′	1,2		9,43	11 h 9′—28′. 20 ccm Blutegelinfus (25 ccm = 7 Blutegelköpfe); fortwährende Gerinnung; 11 h 43′—55′ neue Canüle in den Brustlymphgang eingebunden.
11 » 55′ — 12 » 7′	6,2	0,52	8,40	11 h 58′ 5 ccm Blutegelinfus.
12 » 7′ — 12 » 18′	3,0	0,27	8,53	12 h 19′—22′ 8 ccm Glycerinblutegelextract in 30 ccm Kochsalzlösung in die V. fem.
12 » 18′ — 12 » 40′	6,2	0,28	8,65	
12 » 40′ — 12 » 53′	4,2	0,40	8,49	12 h 49′—53′ 8 ccm Glycerinblutegelextract in 30 ccm Kochsalzlösung in die V. fem
12 » 53′ — 1 » 15′	6,5	0,33	8,46	1 h 1′ 2 ccm Glycerinblutegelextract in 10 ccm Kochsalzlösung in die V. fem.
1 » 15′ — 1 » 30′	7,0	0,47	7,95	Während des ganzen Versuches tiefe Prostration des Thieres.

Versuch 6. Hund 12 kg. Morphium; dann Curare.

3 h 47′ — 4 h 2′	3,3	0,22	4,65	3 h 52′—4 h 8′ 1 g Chinin mur. in die Vena lienalis.
4 » 2′ — 4 » 12′	3,6	0,36	5,20	
4 » 12′ — 4 » 27′	2,8	0,11	5,13	4 h 12′—20′ Blutegelinfus aus 12 Blutegelköpfen in die V. lienalis. 4 h 15′ Speichelfluss; einige Beweg.
4 » 27′ — 4 » 43′	4,4	0,29	5,38	4 h 30′—39′ 6 ccm Glycerinblutegelextract in die V. fem
4 » 43′ — 5 » 2′	5,1	0,27	5,69	4 h 47′ Speichel fliesst a. d. Munde.
5 » 2′ — 5 » 12′	5,0	0,50	5,69	Curarewirkung vertieft sich während des Versuches.

überwiegend in Frage kam. Das Curare, welches wir anwandten, um vollkommene Bewegungslosigkeit zu erhalten, hat seinen bekannten Einfluss auf den Lymphstrom ausgeübt. Aus den Untersuchungen Paschutin's[1]) ist bekannt, dass nach dem Eintritte der Curarevergiftung die Geschwindigkeit der Absonderung wächst, sowie der Gehalt an festen Substanzen, namentlich

1) Paschutin, Ueber die Absonderung der Lymphe im Arme des Hundes. Ludwig's Arbeiten 1873, S. 197.

an Eiweiss, erheblich zunimmt. Hand in Hand mit der sich vertiefenden Curarevergiftung geht eine Concentrirung der Lymphe einher; der Hauptsprung erfolgt von der ersten zur zweiten Lymphportion, also vor jeder Beeinflussung durch Blutegelinfus. Das Infus selbst hat keine sich wesentlich bemerkbar machende Wirkung auf den Lymphstrom gehabt, und wir glauben nach Allem, was ausgeführt worden ist, dem Zusammenhange der Dinge am meisten durch die Annahme gerecht zu werden, dass auch hier die Chininvergiftung durch Hemmung der Thätigkeit der Leber eine Begleiterscheinung dieser Thätigkeit, nämlich die vermehrte und veränderte Bildung der Lymphe, unterdrückt habe.

Es erhebt sich die Frage, lehren die mitgetheilten Versuche etwas über die Betheiligung der Gefässwandzellen an der Lymphbildung? Leider sehen wir uns, wie bisher stets in dieser Frage, vor der Nothwendigkeit des Verzichtes auf unbedingt einwandsfreie oder überzeugende Auskunft. Chinin stört die Erscheinungen nach Zuckerinjection nicht, wohl aber diejenigen nach Injection von Blutegelinfus. Die Anhänger von Heidenhain's Anschauungen, denen zu Folge in beiden sich die active Thätigkeit der Capillarendothelien offenbart, müssen hierdurch in einige Verlegenheit gerathen, sich zu entscheiden, aus welchem Grunde sie für den einen Fall eine Gefässwandschädigung annehmen wollen, für den anderen aber nicht. Wenn man hingegen annehmen will, dass mit jeder Organthätigkeit normaler Weise ein besonderes Verhalten der Gefässwände auf das Innigste verbunden ist — eine Möglichkeit, auf welche wir wiederholt schon hinwiesen, — würde man schliessen können, dass in den zuletzt betrachteten Fällen das Chinin mit den Processen in den specifischen Leberzellen zugleich auch die dazugehörigen in den Gefässwandzellen betroffen habe. Aus biologischen Gründen wollen wir diese Auffassung nicht vollständig ablehnen, betonen aber, dass andererseits unsere Versuche Denjenigen nicht Lüge strafen, welcher eine active Betheiligung der Gefässwände leugnet.

Lymphbildung unter der Einwirkung von Arsen.

Die angestellten Betrachtungen über die etwaige Rolle der Gefässwände bei der Lymphbildung leiten zu den Versuchen mit einem typischen Gefässgifte über. Magnus hat in seiner oben citirtenArbeit die von Schmiedeberg aufgestellte Ansicht, dass Arsenik in eigenartiger Weise die Wandungen der Capillaren vergiftet, so dass ausser der Erweiterung eine tiefgreifende Störung des Stoffaustausches zwischen ihnen und den Geweben besteht, experimentell gut gestützt, indem er direct die Steigerung der Durchlässigkeit der Capillaren der Haut nachwies. Dass aber auch namentlich die Capillaren des Darmes betroffen werden, geht aus den Untersuchungen von Böhm und Unterberger, sowie von Pistorius (nähere Literaturangaben finden sich in Magnus' oben citirter Arbeit) hervor. Bei der Bedeutung, welche von vielen neueren Forschern der blossen Aenderung der Permeabilität der Gefässwände zugemessen wird, ist es sehr werthvoll, ein Mittel zu besitzen, welches nachweisbar diese Aenderung verursacht; es ist nun zu erwarten, dass durch das Experiment sich erkennen lässt, welche Beziehungen zwischen vermehrter Permeabilität der Capillaren und Lymphbildung bestehen. Auch für Heidenhain's Vorstellungen von der secretorischen Function der Capillarendothelien bietet sich in dem Arsenik, kraft seiner geschilderten Eigenschaften, ein willkommener Prüfstein dar.

Wir benutzten zur Injection in die Vena femoralis Lösung eines Präparates reinen arseniksauren Natriums in Kochsalzlösung; 1 ccm derselben entsprach 0,01g Natrium arsenicosum. In Tab. V (S. 199) sind Versuchsdaten niedergelegt, welche über mehrere der hier interessirenden Punkte Aufschluss geben. Arsenik vermehrt, wie mit aller Deutlichkeit aus dem Versuche hervorgeht, den Ausfluss der Lymphe aus dem Brustgang. Auf der Höhe der Arsenikbeschleunigung beträgt der Beschleunigungsquotient nicht weniger als 3,5. Hiermit ist der Nachweis geliefert, dass Arsenik ein lymphtreibendes Gift ist. Da sich keine mechanischen Verhältnisse, welche etwa nur die Austreibung einer durchaus nicht vermehrt gebildeten Lymphe begünstigen würden, ausgebildet haben, muss es sich um die vermehrte Bildung von

Tabelle V.

Versuch 7. Hund 17 kg. Morphiumnarkose.

Zeit	Lymphmenge in ccm	Lymphmenge pro Min. in ccm	Procentgehalt an festen Substanz.	Zucker in Procent	Bemerkungen
10 h 00′ — 10 h 10′	2,0	0,20	7,02		
10 » 10′ — 10 » 23′	3,2	0,25	6,98		10 h 11′—12′ 10 ccm Arsenlösung und um 10 h 12½′ bis 16½′ 20 ccm Arsenlösung in die V. fem. = 0,03 g Natr. arsenicosum.
10 » 23′ — 10 » 33′	2,8	0,28	7,08		10 h 36′—38′ 10 ccm Arsenlösung = 0,01 g Natr. ars.
10 » 33′ — 10 » 53′	6,2	0,31	7,17		
10 » 53′ — 11 » 10′	5,6	0,33	7,18		11 h 7½′—8½′ 10 ccm Arsenlösung = 0,01 g Natr. ars.
11 » 10′ — 11 » 20′	2,7	0,27	7,28		
11 » 20′ — 11 » 30′	3,4	0,34			11 h 20′—21½′ 10 ccm Arsenlösung, 11 h 25½′—26½′ 10 ccm Arsenlös. = 0,02 g Natr. ars.
11 » 30′ — 11 » 40′	4,2	0,42	7,31		11 h 30′—38½′ 30 ccm Arsenlösung = 0,03 g Natr. ars.
11 » 40′ — 11 » 50′	6,4	0,64			
11 » 50′ — 12 » 5′	10,4	0,70	7,38		
12 » 5′ — 12 » 12′	10,5	1,50	6,97		12 h 5′—8′ 30 g Traubenzucker + 0,01 g Natr. ars. in die V. fem.
12 » 12′ — 12 » 17′	17,0	3,48		1,411	
12 » 17′ — 12 » 22′	11,0	2,20			
12 » 22′ — 12 » 27′	7,0	1,40	5,73		Herzschlag nicht wie gewöhnlich bei Zuckerinjection verstärkt.
12 » 27′ — 12 » 32′	5,0	1,00		1,095	
12 » 32′ — 12 » 37′	4,0	0,80			
12 » 37′ — 12 » 57′	9,8	0,49		0,959	

Lymphe handeln. Was die Aenderung der mechanischen Verhältnisse durch das Gift anbetrifft, so liegen sie alle eher nach der Richtung der Hemmung für das Wegschaffen der Lymphe. Unzweifelhaft liegt der Blutdruck tief darnieder und sind eine Reihe motorischer Elemente, welche gleichfalls den Lymphausfluss fördern könnten, in einem lähmungsartigen Zustande. Unser Versuch liefert, wenn man von der durch Magnus gesicherten Erkenntniss der erhöhten Durchlässigkeit der Capillarwände ausgeht, einen neuen Nachweis dieser Thatsache für das grosse Gebiet der Eingeweidelymphe. Als weitere Stützen für die Ansicht, dass die vermehrte Lymphbildung durch Arsenikvergiftung auf

der erhöhten Permeabilität der Gefässwände beruhen müsse, können die bekannten, sehr heftigen Vergiftungserscheinungen an der Schleimhaut des Verdauungskanals angeführt werden, welche von jeher auf eine vermehrte Exsudation aus den Gefässen bezogen wurden. Da die Veränderung der Durchlässigkeit der Gefässe vornehmlich die Eingeweidegefässe betrifft, steht der Durchtritt einer wesentlich concentrirteren Flüssigkeit als sonst zu erwarten; das ist in der That der Fall.

An und für sich würde im Verlaufe eines länger dauernden Versuches die Concentration der Lymphe unausgesetzt sich mindern; in dem vorliegenden Versuche nimmt die Concentration von 7,02% bis zu 7,38% zu. Diese Zunahme ist nicht erheblich, aber immerhin mit Rücksicht auf die eben genannte, nicht zu vernachlässigende Thatsache eine ins Gewicht fallende. Ueberblicken wir die Voraussetzungen und die Erfolge des Versuches bis hierher, so haben wir fast alle Momente beisammen, welche bei der Einwirkung der Heidenhain'schen Lymphagoga (der Lebergifte) auf den Lymphstrom zur Beobachtung gelangen und welche von Seiten Starling's und seiner Anhänger zur Erklärung derselben angeführt werden. Nach Injection von Krebsmuskelextract, Blutegelextract, Pepton etc. wird die Lymphbildung vermehrt, die Lymphe concentrirter; beim Pepton ist zudem noch eine Beeinflussung der Gefässweite und der sog. »Vasomobilität« constatirt worden, welche die grösste Aehnlichkeit mit der Arsenikwirkung auf die Gefässe besitzt. Und doch besteht ein frappanter Unterschied, welcher auch in dem nächstfolgenden Versuche zur Geltung kommt.

(Siehe Tabelle S. 201.)

Auch dieser Versuch zeigt wiederum die Vermehrung des Lymphstromes und die Erhöhung der Concentration. Eine weitere Aehnlichkeit mit den Erfolgen der Injection von Lebergiften besteht ferner noch in den Concentrationsverhältnissen des Blutes; wie bei der letztgenannten steigert sich auch während der Arsenikvergiftung der Gehalt des Gesammtblutes an festen Bestandtheilen, woraus abermals folgt, dass Arsenik einen vermehrten Austritt von Plasma aus den Blutgefässen veranlasst.

Tabelle VI.

Versuch 8. Hund 12,5 kg. 16 cg Morphium; sehr tiefe Narkose.

Zeit	Lymphmenge in ccm	Lymphmenge pro Min. in ccm	Procentgehalt an festen Substanzen	Bemerkungen
9 h 40′ — 10 h 11′	5,4	0,25	6,40	10 h 4′ 2,1558 g Blut aus der Art. fem. mit 17,02% fester Substanz.
10 » 11′ — 10 » 33′	8,5	0,39		10 h 11′ — 18′ 0,03 g Natr. ars. in die V. fem.
10 » 33′ — 10 » 55′	9,2	0,42	6,59	0,03 g Natr. ars. in die V. fem.
10 » 55′ — 11 » 11′	8,7	0,44	6,05	10 h 59′ — 11 h 4′ 0,03 g Natr. ars. in die V. fem.
11 » 11′ — 11 » 39′	7,2	0,36	6,48	11 h 22′ 2,2795 g Blut aus der Art. fem. mit 18,29% fester Substanz.
11 » 39′ — 12 » 1′	10,3	0,47	6,54	
12 » 1′ — 12 » 15′	7,3	0,52	7,03	12 h 15′ Tod des Hundes; nach dem Tode Lymphfluss sehr langsam; fast ganz stockend v. 12 h 55′ an.
12 » 15′ — 1 » 15′	7,8	0,13		

Aber nicht minder tritt der Unterschied der Arsenikwirkung auf den Lymphstrom gegenüber derjenigen der Heidenhain'schen Substanzen zu Tage. Wie seltsam contrastiren beim Arsenik auf der einen Seite die tiefgreifenden Schädigungen der Gefäss- und Darmschleimhautzellen und die eventuellen profusen Exsudationen, auf der anderen Seite die verhältnissmässig geringfügige Beschleunigung und die sich in engen Grenzen haltende Concentrirung der Lymphe mit der gewaltigen Vermehrung der Lymphmenge und deren sehr starker Anreicherung an festen Substanzen durch die unvergleichlich unschuldigeren Lebergifte. Was den Contrast noch verschärft, ist, dass das Arsenik überall im Körper als ein Capillargift sich erweist, ein Lymphagogum aber nur auf dem beschränkten Gebiete der Leber und des Darmes (was übrigens bis jetzt nur für das Pepton erwiesen ist). Hierzu kommt ferner noch die Thatsache, dass Arsenik eine Steigerung des Zerfalls der Gewebszellen und so bedeutsame Stoffwechselveränderungen wie Fetttransporte nach besonderen Stellen des Körpers veranlasst; den Anschauungen zu Folge, welche wir bei früherer Gelegenheit entwickelt haben, müssen solche Vorgänge zur Bildung einer stoffreicheren Lymphe beitragen. Dieses Moment muss also mit der Erhöhung der Permeabilität der Gefässwände concurriren, wenn es sich um die

ursächliche Erklärung der Lymphbildung unter dem Einflusse von Arsenik handelt.

Wir glauben, durch die Darlegung der Unterschiede zwischen den Wirkungen des Arseniks einerseits, wie sie aus den zwei besprochenen und einem dritten sofort mitzutheilenden Versuche sich ergeben haben, andererseits denjenigen der Lymphagoga 1. Classe, neue Belege dafür erbracht zu haben, dass die Hypothese, nach welcher die Wirkung der letztgenannten Substanzen ausschliesslich auf Rechnung erhöhter Permeabilität der Unterleibsgefässe zu setzen sei, unhaltbar ist. Die Ueberlegenheit der Lebergifte als lympherzeugende Mittel gegenüber dem deletären Protoplasma- resp. Gefässgifte Arsen beruht auf dem Hinzutreten eines physiologischen Momentes, dem von uns nachgewiesenen gesteigerten Thätigkeitszustande der grössten Unterleibsdrüse.

Ein actives Eingreifen der Capillarendothelien in Heidenhain's Sinne würde gleichfalls die Ueberlegenheit der Lymphagoga vor dem Arsen erklären. Die Beobachtungen, welche wir im weiteren Verlaufe des 7. Versuches (Tabelle V) gesammelt haben, gibt uns auf neue Veranlassung, vorläufig von dem activen Eingreifen der Capillarendothelien wegen Mangels an bestimmten Beweisen für dasselbe abzusehen. Denn als auf der Höhe der Arsenikvergiftung eine intravenöse Traubenzuckerinjection gemacht wurde, traten die gewohnten Folgen am Lymphstrome auf. Zunächst einmal die sehr starke Beschleunigung des Lymphflusses. Das Gelingen dieser ausserordentlichen Beschleunigung — der Beschleunigungsquotient erreichte den hohen Werth **17,4** — beseitigt den etwaigen Einwand, dass die Schwere der Arsenikvergiftung verhindert habe, dass die Folgen der erhöhten Permeabilität der Gefässwände sich geltend machten. Worauf es aber im Augenblicke noch mehr ankommt, ist die Thatsache, dass die Zuckerausscheidung aus dem Blute mit so grosser Geschwindigkeit vor sich geht, dass schon in dem Zeitraume 4—14 Minuten nach der vollendeten Zuckerinjection die Zuckerconcentration der Lymphe den sehr hohen Werth 1,411 % erreicht hat. Man wird schwerlich annehmen können, dass ein so heftiges Capillargift wie das Arsen die Zuckerausscheidung ungestört belassen

hätte, wenn diese wirklich, wie Heidenhain andeutete, auf einer secretorischen Leistung der Capillarendothelien beruhte. Wir haben somit das interessante bisherige Ergebniss, dass sowohl Chinin wie auch Arsen auf die Entfernung des Zuckers aus dem Blute ohne Einfluss ist und erblicken darin experimentelle Stützen für die Annahme, dass den Capillarendothelien nicht das Vermögen zukommt, Zucker aus den Gefässen auszuscheiden. Es mag freilich noch einmal daran erinnert werden, dass den Chininversuchen, für sich allein betrachtet, keine erhebliche Beweiskraft aus früher erörterten Gründen beigemessen werden kann.

Die Permeabilitätsverhältnisse bei der Arsenikvergiftung haben wir noch auf eine andere Weise in dem Versuche, über welchen Tab. VII (S. 205) Auskunft gibt, der Prüfung unterzogen. Was die reine Arsenikwirkung auf den Lymphstrom anbelangt, so lehrt dieser Versuch, wie die früheren, die erhebliche Steigerung der Lymphbildung und der Concentration unter dem Einflusse des Giftes. Die Beschleunigung des Lymphstromes ist eher etwas grösser als in den beiden anderen Versuchen; der Concentrationszuwachs ist zwar sehr ausgeprägt, wiederum aber nicht gleicher Grössenordnung als wie bei den Lymphagogis, trotz der durch die Lymphvermehrung erwiesenen erhöhten Permeabilität. Als weiteres Prüfungsmittel der schon durch die Verhältnisse des Lymphstromes erwiesenen erhöhten Durchlässigkeit der Gefässe wandten wir ein zuerst von Orlow, dann von Cohnstein näher untersuchtes Verfahren an. Orlow[1]) hatte mit dem Blutplasma isotonische Flüssigkeiten in die Peritonealhöhle gebracht und gefunden, dass dieselben daraus resorbirt wurden, ohne dass eine merkliche Aenderung des Lymphstroms aus dem Brustlymphgange eintrat. Cohnstein[2]) hatte nach Infusion von 2 l Kochsalzlösung in die Bauchhöhle nur bei Massage des

1) W. N. Orlow, Einige Versuche über die Resorption in der Bauchhöhle. Pflüger's Archiv 1894, Bd. 59 S. 170.

2) W. Cohnstein, Ueber Resorption aus der Peritonealhöhle. Centralblatt f. Physiologie 1895, Bd. 9 No. 13 S. 401.

Tabelle VII.

Versuch 9. Hund 12 kg. 16 cg Morphiumnarkose.

Zeit	Lymphmenge in ccm	Lymphmenge pro Min. in ccm	Procentgehalt an festen Substanzen	Bemerkungen
9 h 37′ — 9 h 47′	2,7	0,27	4,37	
9 » 47′ — 10 » 2′	4,9	0,33	4,38	9 h 47′ — 52½′ 0,03 g Natr. ars. in die V. fem.
10 » 2′ — 10 » 17′	6,9	0,46	4,51	10 h 3½′ — 8′ 0,03 g Natr. ars. in die V. fem.
10 » 17′ — 10 » 27′	7,8	0,78	4,55	
10 » 27′ — 10 » 37′	10,8	1,08	4,59	10 h 28′ — 31′ 0,03 g Natr. ars. in die V. fem.
10 » 37′ — 10 » 44′	9,7	1,39	5,30	
10 » 44′ — 11 » 0′	19,4	1,21	4,73	10 h 48′ — 52′ Peritonealhöhle wird eröffnet, um in die Oeffnung eine Pipette einzuführen; 53′ — 59′ 20ccm einer 0,85 proc. Kochsalzlösung in die Peritonealhöhle.
11 » 0′ — 11 » 10′	9,4	0,94	4,54	
11 » 10′ — 11 » 30′	23,0	1,15	4,80	
11 » 30′ — 11 » 55′	27,5	1,80	4,79	
11 » 55′ — 12 » 10′	15,0	1,00	4,84	Thier starb um 1 h; bei der Section finden sich in der Bauchhöhle 90 ccm Flüssigkeit.

Leibes und Hochbinden der Hinterbeine Ansteigen der Lymphmenge, und bei Infusion der gleichen Menge nach 1½ Stunden Dauer des Versuches eine Abnahme der Concentration von 5,73 auf 5,42% beobachtet. Nach unseren Erfahrungen würde sich auch ohne den Versuchseingriff in Bezug auf die Concentration so ziemlich das Gleiche ereignen. Wir führten nur 200 ccm isotonischer Kochsalzlösung in die Bauchhöhle ein, von welcher im höchsten Falle 110 ccm resorbirt wurden. Es hat nun, wie die Versuchsergebnisse lehren, die Aufnahme dieser geringen Flüssigkeitsmenge in das Blut genügt, um die durch die Arsenvergiftung herbeigeführte Steigerung der Concentration der Lymphe von der erreichten Höhe herabzudrücken und längere Zeit auf einem niedrigeren Werthe festzuhalten. Es geht daraus hervor, wie wenig leistungsfähig die blosse Erhöhung der Permeabilität der Gefässwände in Bezug auf die Concentrirung der Lymphe ist, obwohl in dem vorliegenden Versuche die Arsenvergiftung fortfuhr, sich zu vertiefen. Die gute Durchlässigkeit der Gefässe wird ferner im vorliegenden Versuche durch die verhältnissmässig rasche Resorption der isotonischen Lösung erwiesen.

Uebrigens lehren zahlreiche Erfahrungen der Pathologie, dass schon ziemlich gewaltsame Eingriffe an den Gefässen und Geweben stattfinden müssen, um die Durchlässigkeit der Gefässe so weit zu erhöhen, dass sehr eiweissreiche entzündliche Transsudate entstehen.

Einen Augenblick müssen wir noch bei der Discussion der Bedeutung erhöhter Permeabilität der Gefässe verweilen, aus Anlass einiger anderen Beobachtungen, welche zu der gleichen Auffassung führen wie die bisher entwickelte. Heidenhain's Lymphagoga sollen nach Starling ihre merkwürdige Wirkung vermehrter Durchlässigkeit der Lebercapillaren verdanken, eine Hypothese, welche angesichts der vielen Vorgänge, die im lebenden Organismus sich als geknüpft an den Einfluss der Lymphagoga erwiesen haben, der schwächste Punkt der mechanischen Lymphtheorie ist. (Wir sehen im Augenblicke von den in unserer ersten und zweiten Mittheilung niedergelegten Beobachtungen über Anregung der Leberthätigkeit ganz ab.) Nun hatte Heidenhain seiner Zeit schon einen interessanten Versuch mitgetheilt, welcher beweisen sollte, dass die Wirkung der Lymphagoga ein Lebensvorgang sei; er hat nämlich gezeigt, dass nach zeitweiliger Verschliessung der Aorta die charakteristische Wirkung der Lymphagoga völlig ausbleibt. Daraus zog er den Schluss, dass durch Schädigung einer physiologischen Function die lymphtreibende Wirkung jener Substanzen unterdrückt worden sei und zwar glaubte er, gemäss seinen öfters erörterten Anschauungen, dass die Erregbarkeit der activ secretorischen Capillarzellen für jene Gifte durch die Anämie aufgehoben worden sei. Dieser, nach vielen Analogien, wenigstens was die Schädigung irgend eines physiologischen Vorganges anbetrifft, durchaus berechtigten Vorstellung setzte Starling[1]) die Muthmaassung entgegen, dass durch die lange Anämie Verhältnisse geschaffen worden seien, dass die Folgen der vermehrten Durchlässigkeit der Gefässe sich nicht ausbilden konnten. Eine Reihe von Beobachtungen nun, welche der Eine von uns gemeinsam mit

1) E. H. Starling, On the mode of action of lymphagogues. Journ. of Physiol. 1894, Vol. XVII p. 30.

Dr. J. P. Arnold aus Philadelphia gelegentlich einer anderen, demnächst zu veröffentlichenden Untersuchung gemacht hat, lehren im Gegentheil, dass die zeitweilige Verschliessung der Aorta der Ausbildung erhöhter Permeabilität der Gefässe ausserordentlich förderlich ist. Diese Thatsache ergab sich aus folgenden Erfahrungen: Nach Verschliessung der Aorta am Aortenbogen und Wiedereröffnung derselben genügte sehr oft eine geringe Menge von intravenös injicirter Kochsalzlösung, welche sonst spurlos am Organismus vorübergeht, um Transsudationen in den verschiedenen serösen Höhlen zu veranlassen. Es ist dies ein sicherer Beweis für die erhöhte Durchlässigkeit der Gefässe. Magnus hat in seiner citirten Arbeit die ungemein erhöhte Durchlässigkeit der todten Gefässe experimentell schlagend erwiesen. Wäre also wirklich die wesentliche Ursache der Wirkung der Lymphagoga in der vermehrten Permeabilität zu suchen, so müsste sich dies gerade nach zeitweiliger Verschliessung der Aorta offenbaren. Thatsächlich beweist also der negative Ausfall von Heidenhain's oben beschriebenen Experimenten, dass die Wirkung seiner Lymphagoga nicht zureichend durch die Annahme erhöhter Permeabilität der Gefässe erklärt werden kann.

Ueberblicken wir nochmals die Ergebnisse der Arsenversuche, so lehren sie jedenfalls, dass Arsen einen grossen Einfluss auf die Lymphbildung hat, dass seine Wirksamkeit aber trotz erweislicher, stark erhöhter Durchlässigkeit der Gefässe weit zurücksteht hinter derjenigen so viel harmloserer Mittel wie Krebsmuskel- oder Blutegelkopfextract. Es hat sich auf diese Weise durch die Anwendung des Arsens den früheren positiven Beweisen für die »physiologische Componente« der zuletzt genannten Mittel ein neuer Beweis zugesellt. Andererseits ergibt sich aus der Art und Weise, wie während einer tiefen Arsenvergiftung dem Organismus künstlich zugeführtes Wasser und Zucker aus dem Blute in die Lymphe übertritt, kein Anhaltspunkt für die Auffassung, dass eine active, secretorische Thätigkeit der Capillarendothelien regelnd hierbei eingriffe. Es ist vielmehr wahrscheinlich gemacht worden, dass diese Erscheinungen zur »physikalischen Componente« bei der Lymphbildung gehören; aber auch

nicht mehr wie wahrscheinlich, denn welche Gewähr besitzen wir dafür, dass das Arsen alle physiologischen Vorgänge, welche in Betracht kommen könnten, beseitigt habe?

Einiges über Lymphbildung nach dem Tode.

Mit unserem Hauptthema, dem Einflusse von Protoplasmagiften auf die Lymphbildung, steht die Untersuchung der Lymphbildung nach dem Tode scheinbar in einem nur losen Zusammenhange. Thatsächlich war auch der Zufall, dass gelegentlich eines nicht gewollten Vergiftungstodes ganz überraschende und für die Theorie der Lymphbildung bedeutungsvolle Erscheinungen zu Tage traten, die nächste Veranlassung für ein Eingehen nach dieser Richtung hin. Aber doch besteht auch ein mehr innerer Zusammenhang; denn der Tod des Organismus ist der mächtigste Zerstörer des lebenden Protoplasmas. Da diese Zerstörung aber eine ganz allmähliche ist, das Erlöschen der einzelnen Functionen für die verschiedenen lebenden Theile zeitlich ein ganz getrenntes sein kann, konnte auch daran gedacht werden, dass die Untersuchung der Lymphbildung nach dem Tode als eine Methode der Analyse sich brauchbar zeigen würde.

In Tab. VIII (S. 209) ist ein Versuch mitgetheilt, in welchem das Versuchsthier in Folge der schweren Chininvergiftung starb. In der 8. bis 4. Minute vor dem Tode waren dem $9^1/_2$ kg schweren Thiere 25 g Traubenzucker intravenös beigebracht worden, also pro Kilo 2,6 g. Trotz der Schwere der Vergiftung, welche nach Allem, was wir wissen, ein tiefes Darniederliegen der Kreislaufsverhältnisse bedingen musste, hob sich sofort, d. h. innerhalb der vier Minuten Injectionsdauer und den zwei darauf folgenden Minuten die ausfliessende Lymphmenge um das $4^1/_2$ fache. Dies mag hervorgehoben werden, weil von Seiten der Anhänger der Filtrationstheorie Gewicht darauf gelegt wird, dass die erste Folge der intravenösen Krystalloïdinjection eine anfängliche Verringerung des Lymphflusses sein müsse.[1]) Das Nichteintreten

1) W. Cohnstein, Ueber die Einwirkung intravenöser Kochsalzinfusionen auf die Zusammensetzung von Blut und Lymphe. Pflüger's Arch. 1895, Bd. 59 S. 508.

Tabelle VIII.

Versuch 10. Hund 9,5 kg. Morphiumnarkose.

Zeit	Lymphmenge in ccm	Lymphmenge pro Min. in ccm	Procentgehalt an festen Substanz.	Zucker in Procent	Bemerkungen
9 h 7′ — 9 h 20′	5,2	0,4	5,62		
9 » 20′ — 9 » 39′	7,6	0,4		0,192	9 h 20′—31′ 1 g Chinin mur. in die V. fem.
9 » 40′ — 9 » 46′	11,0	1,83		1,095	9 h 40′—44′ 25 g Traubenzucker + 0,3 g Chinin mur. in 80 ccm Salzlösung in die V. fem. 9 h 45′ 35 ccm Blut aus d. Art. fem. mit **0,707%** Zucker.
9 » 46′ — 9 » 50′	15,0	3,75		1,646	Tod des Hundes 9 h 48′.
9 » 50′ — 9 » 53′	9,5	3,17		1,875	
9 » 53′ — 9 » 59′	10,5	1,75		1,920	
9 » 59′ — 10 » 9′	10,5	1,05		2,031	
10 » 9′ — 10 » 24′	11,0	0,73		2,138	
10 » 24′ — 10 » 44′	12,0	0,60		2,165	
10 » 44′ — 10 » 54′	6,0	0,60	5,77		
10 » 54′ — 11 » 54′	26,5	0,44	5,80	2,237	
11 » 54′ — 12 » 54′	15,0	0,25	5,90	1,825	

dieser Verringerung, welche wir übrigens niemals beobachten konnten, liegt in diesem Falle mit aller erwünschten Deutlichkeit zu Tage. In den nächsten vier Minuten, innerhalb welchen das Thier stirbt, wächst die Beschleunigung bis über das 9fache. Wie aus unseren früheren Chininversuchen, geht auch aus diesem, vielleicht mit noch grösserer Schärfe, hervor, dass Chinin gegenüber der Lymphbeschleunigung durch Zuckerinjection machtlos ist. Dass dieses Versagen des Chinins aber der Filtrationstheorie zu gute kommt, erscheint uns wenig annehmbar angesichts des vorliegenden Versuchszustände. Auch hinsichtlich der Frage der Zuckerausscheidung ist dieser Versuch lehrreich; denn der Zucker verlässt mit der gewohnten erstaunlichen Raschheit die Blutbahn: schon in den ersten 6 Minuten wächst die Zuckerconcentration der Lymphe auf 1,095%, während in derselben Zeit die Zuckerconcentration des Blutes auf 0,707% offenbar wieder gefallen ist. Der Anstieg erreicht in den nächsten vier Minuten den Werth von 1,646%. Wiederum ist, wie in den

früheren Chininversuchen, jene merkwürdige Erscheinungsreihe, welche nach Heidenhain in dem Secretionsvermögen der Capillarendothelien wurzelte, unversehrt geblieben. Wir verweisen auf unsere am Schlusse der Chininversuche vorgetragenen Erörterungen über die Frage, woher es kommen möge, dass Chinin spurlos an jener »physiologischen Componente« vorübergehen könne, vorausgesetzt, dass eine solche in diesen Processen vorliegt.

Weit interessanter ist aber das Verhalten des Lymphstroms nach dem Tode. Drei Stunden lang nach dem Tode fliesst aus dem Brustlymphgang, ohne jede künstliche Mithilfe, ein ergiebiger Lymphstrom. Wohl als erster Eindruck drängt sich die Ueberzeugung auf, dass die Lehre von der unmittelbaren oder gar zwingenden Abhängigkeit der Lymphbildung vom Blutdruck, die neuere Filtrationstheorie, diesem Experimente gegenüber ganz und gar versagt.

Vor der weiteren Discussion des eben Gesagten erübrigt es noch, kurz die Verhältnisse der Zuckerconcentration in der postmortalen Lymphe zu erledigen. Zwei Stunden lang steigt die Zuckerconcentration der Lymphe an und erreicht ganz ungewöhnlich hohe Werthe. Zwei Gründe, glauben wir, liegen in den Versuchsbedingungen hiefür zur Erklärung vor: erstens der Wegfall der Zuckerausscheidung durch die Niere (bei Ausschaltung der Nierenfunction durch Unterbindung der Nierenarterien beobachtete Heidenhain das gleiche Verhalten), zweitens das vermuthliche Erlöschen einer Reihe von physiologischen Zellfunctionen, welche sonst zur rascheren Beseitigung des Zuckers aus der Lymphe beitragen würden. Da sich der Umfang, welche diese beiden Momente gewinnen, gar nicht bemessen lässt, darf nicht allzuviel Gewicht auf die Thatsache gelegt werden, dass lange Zeit aus dem zuckerärmeren Blute Zucker in die zuckerreichere Lymphe hinübergeschafft wird. Immerhin ist das postmortale Auftreten dieser Erscheinung sehr bemerkenswerth und kann gemeinsam mit den mannigfachen früher mitgetheilten Erfahrungen gegen die Annahme von dem secretorischen Vermögen der Capillarendothelien verwerthet werden: für sich allein beweist aus naheliegenden Gründen diese Erscheinung nichts dagegen.

Dass es sich bei diesem Versuche um Zucker allein handle und nicht etwa um andere postmortal gebildete reducirende Substanzen, haben wir dadurch zu beweisen versucht, dass wir eiweissfrei gemachte Lymphe vergähren liessen und nach der Vergährung keine Reduction mehr constatiren konnten; ausserdem stellten wir Phenylosazon dar.

Die nähere Betrachtung des vorliegenden Versuches lehrt, dass die Beschleunigung, wenn auch abnehmend, eine Stunde lang nach dem Tode anhält, und auch während der ganzen zweiten Stunde beträgt die Menge pro Minute immer noch ein klein wenig mehr als zu Anfang des Versuches vor der Chininvergiftung. Selbst in der dritten Stunde ist der Lymphfluss kein schlechter. Es erhebt sich die Frage, wie erklärt sich die Bildung der Lymphe und woher kommen die Triebkräfte zum Ausstossen derselben im vorliegenden Falle? Dass die tödtliche Chininvergiftung nichts damit zu thun habe, lehrt Versuch 11 in Tabelle IX. Sofort mit dem Tode stockt der Lymphstrom

Tabelle IX.

Versuch 11. Hund 12 kg. Morphiumnarkose.

Zeit	Lymphmenge in ccm	Lymphmenge pro Min. in ccm	Procentgehalt der festen Substanzen	Bemerkungen
9h 21′ — 9h 36′	2,4	0,16	4,87	
9 » 36′ — 9 » 53′	3,8	0,22	5,24	9 h 36′—51′ 1 g Chinin mur. in 80 ccm Salzlösung in d. V. fem.; am Ende leichte Convulsionen.
9 » 53′ — 10 » 5′	2,25	0,19	6,26	Lymphe wird während des Versuches blutiger; 10 h 4′ Tod.
10 » 5′ — 10 » 13′	6,8		6,15	Kein Ausfluss ausser durch Pumpen.

und lässt sich nur, wie das schon lange bekannt ist, durch Pumpen künstlich im Gange erhalten. Hingegen wurde in dem oben beschriebenen Versuche 8 (Tabelle VI) nach dem Vergiftungstode durch Arsen eine Stunde lang vollständiges Ausfliessen der Lymphe beobachtet, also in einem Falle, wo ein lymphtreibendes Agens angewandt worden war. Aber jener Lymphfluss verlangsamte sich, ganz anders wie in diesem Versuche, momentan ganz erheblich mit dem Tode und blieb an der Grenze des Versiechens. Das lymphtreibende Mittel in unserem Falle ist die vorausgegangene intravenöse Traubenzuckerinjection und

hierin liegt die grosse theoretische Bedeutung des Experimentes. Die Filtrationstheorie, deren plausibelste Seite — wenn auch durchaus nicht einwandsfrei — die mechanische Deutung der Lymphbeschleunigung nach intravenöser Krystalloïdinjection war, lehrt, dass durch die Salzinfusion der osmotische Druck des Blutes über die Norm steigt, in Folge dessen das Blut aus den Lymphspalten Wasser anzieht und nun durch den abnormen Flüssigkeitszuwachs der intracapillare Druck steigt; entsprechend den Filtrationsgesetzen filtrirt dann eine grössere Menge verhältnissmässig wasserreichen Blutplasmas. Beim todten Thiere kann von einer derartigen Erhöhung des Capillardrucks keine Rede sein; selbst wenn man den arteriellen Blutdruck mit Starling nicht als maassgebend für die Höhe des Capillarblutdrucks ansieht, wird man nicht annehmen dürfen, dass bei stillstehendem Herzen und arteriellem Nulldruck nach dem Tode längere Zeit ein Capillardruck bestehen kann, der fähig zu vermehrter Filtration sei. Wir behaupten, dass aus diesem Experimente folgt, dass die Lymphbeschleunigung nach Krystalloïdinjection nicht ihre Ursache in gesteigertem Capillardrucke habe. Nach der Widerlegung der Filtrationshypothese tritt die ursprüngliche Heidenhain'sche Erklärung in ihre Rechte wieder ein: »die injicirten Substanzen treten durch Diffusion schnell aus dem Blute in die Lymphräume und wirken hier wasseranziehend auf das Gewebswasser der Zellen, Fasern u. s. f.; das diesen entzogene Wasser fliesst zum Theile durch die Lymphkanäle ab.« Wenn diese Annahme richtig ist, so muss die Lymphbeschleunigung abhängen von der Zuckermenge, welche Gelegenheit hat, vor dem Tode in die Gewebsspalten überzutreten. Der Versuch bestätigt, dass diese Bedingung von dem grössten Einflusse ist. In Vers. 12 (Tab. X, S. 213) war das Thier schon eine Minute nach der vollendeten Traubenzuckerinjection gestorben; es kommt zwar zur sofortigen Beschleunigung und diese hält zehn Minuten nach dem Tode an, dann aber mindert sich der Ausfluss und hört drei Viertelstunden nach dem Tode ganz auf. Dem ersten Versuche hingegen vollkommen gleich verhält sich der letzte hier mitzutheilende in Tabelle XI (S. 213).

Tabelle X.

Versuch 12. Hund 7 kg. Morphiumnarkose.

Zeit	Lymphmenge in ccm	Lymphmenge pro Min. in ccm	Procentgehalt der festen Substanzen	Bemerkungen
1 h 0′ — 2 h 0′	6,8	0,11	4,66	2 h 10′ — 13′ 21 g Traubenzucker in die V. jugularis; sofortige Beschleunigung. 2 h 13′ Chloroform in das Herz. 2 h 14′ Tod constatirt.
2 » 14′ — 2 » 24′	10,0	1,0	4,65	
2 » 24′ — 2 » 40′	3,0	0,19		
2 » 40′ — 3 » 0′	1,0	0,5		

Tabelle XI.

Versuch 13. Hund 12 kg. Morphiumnarkose.

11 h 15′ — 11 h 55′	3,1	0,078	5,51	
12 » 9′ — 12 » 15½′	1,8	0,28	5,73	12 h 7′ — 9′ 30 g Traubenzucker in die V. jug. 12 h 15′ Chloroform in die V. jugul. **12 h 15½′ Tod.**
12 » 15½′ — 12 » 25′	4,6	0,49	4,71	
12 » 25′ — 12 » 30′	1,8	0,36	4,17	Während der ganzen Zeit starker Speichelfluss u. starkes Secerniren der Augendrüsen. 1 h 5′ noch lebhaftes Ausfliessen von Speichel und Lymphe, wenn auch langsamer als vorher.
12 » 30′ — 12 » 35′	2,8	0,56		
12 » 35′ — 12 » 40′	1,4	0,28		
12 » 40′ — 12 » 45′	1,2	0,24		

Hier verläuft alles so, als ob das Thier noch lebte. Das Maximum der Beschleunigung, das 7,2fache gegenüber dem Lymphflusse vor dem Versuchseingriffe, tritt 21 bis 26 Minuten nach Vollendung der Traubenzuckerinjection ein, zu einer Zeit, wo das Thier schon über eine Viertelstunde todt ist. Länger als drei Viertelstunden hält die sehr ausgeprägte Beschleunigung des Lymphstromes an. Auch die Concentrationsverhältnisse der Lymphe entsprechen den bekannten Erfahrungen bei den nämlichen Versuchen am lebenden Thiere. Die Erklärung für den geschilderten Gang der Ereignisse liegt in den Versuchsbedingungen deutlich zu Tage. Hier war nach vollendeter Zuckerinjection dem Zucker $6^1/_2$ Minuten Zeit geboten, um sich in den Gewebsspalten anzuhäufen; in diesem ersten Zeitraume findet ja bekanntlich die grösste Abnahme der Zuckerconcentration des Blutes statt. Die dargelegten Versuche beweisen, wenn wir sie zusammenfassend betrachten, dass die vermehrte Lymphbildung

nach Injection von Krystalloïden nicht eine Function des gesteigerten Blutdruckes ist, wohl aber nach Heidenhain in einfach physikalischer Weise durch die Anziehung der krystalloïden Substanzen zu dem Gewebswasser erklärt werden kann.

Die Triebkraft zum Ausstossen der vermehrt gebildeten Lymphe kann in unseren Versuchen auch nicht in dem Blutdrucke gesucht werden. Es kann durch die grundlegenden Arbeiten Ludwig's und seiner Schüler als gesichert betrachtet werden, dass unter physiologischen Verhältnissen der Blutdruck eine wesentliche Rolle bei der Mechanik des Lymphstromes spielt. Dass aber noch andere Momente mitwirken, lehren die vorliegenden Versuche. Dass die blosse Mehrbildung von Lymphe nicht nothwendiger Weise eine vermehrte Abfuhr derselben bedingt, beweisen zahlreiche Beobachtungen; Oedeme könnten nicht so hartnäckig bestehen, wenn mit der Bildung die Wegschaffung der Lymphe Hand in Hand ginge. Es liegt die Annahme nahe, dass in den vorliegenden Versuchen der osmotische Druck des Zuckers, wie er die Ursache der vermehrten Lymphbildung ist, auch diejenige des postmortalen Fliessens ist. Aber neben dieser Annahme sind noch andere Möglichkeiten denkbar, die aber hier nicht weiter discutirt werden mögen.

Nur ein letzter wichtiger Punkt bedarf im Anschlusse an die mitgetheilten Beobachtungen der näheren Berücksichtigung. Im letzten Versuche war der postmortale Lymphstrom von einer lebhaften postmortalen Drüsensecretion begleitet. Die Speichelsecretion nach dem Tode ohne jeden Blutstrom ist, neben Ludwig's klassischem Speicheldruckversuch, die Fundamentalthatsache, auf welche sich die allgemein anerkannte Lehre stützt, dass die Speichelsecretion kein Filtrationsprocess sei. Der vollkommene Parallelismus der beiden Vorgänge im letzten Versuche weist darauf hin, dass Drüsensecretion und Lymphbildung Processe gleicher Grössenordnung sind und nicht etwa der letztere ein einfacher Filtrationsvorgang; er macht es auch wahrscheinlich, dass die »physiologische Componente« bei der Lymphbildung zum guten Theile in der Thätigkeit der specifischen Zellen und nicht der Capillarendothelien gegeben sei.

Wenn Lymphbildung und Drüsensecretion einigermaassen analoge Processe sind, so wird dadurch verständlich, warum wir so wenig über die Triebkräfte des Lymphflusses wissen; diejenigen der Secretion sind ja gleichfalls noch nicht entwickelt.

Wir fassen die Ergebnisse dieser Untersuchung in Folgendem zusammen:

1. Chinin hat auf diejenigen Vorgänge, welche nach intravenöser Zuckerinjection am Lymphstrome in Bezug auf Menge und Concentrationsverhältnisse der festen Substanzen, sowie besonders des Zuckers zur Beobachtung kommen, keinen erkennbaren Einfluss.
2. Die Unwirksamkeit des Chinins in dieser Beziehung gestattet nicht mit Bestimmtheit, eine »physiologische Componente« bei dieser Art der Lymphbildung auszuschliessen, da diejenigen Stoffwechselvorgänge, welche im Organismus zur CO_2-Bildung führen, nicht nachweisbar gestört werden.
3. Da bei tiefer Chininvergiftung die Gefässe in Mitleidenschaft gezogen werden sollen, sprechen die unveränderten Ausscheidungsverhältnisse des Zuckers in die Lymphe nicht zu Gunsten eines Secretionsvermögens der Capillarendothelien.
4. Die Wirkung der »Lebergifte« oder von Heidenhain's »Lymphagoga erster Art« werden durch tiefe Chininvergiftung unterdrückt oder gehemmt. Hiermit ist ein neuer Beweis dafür gegeben, dass diese Mittel eine »physiologische Componente«, bestehend in erhöhter Leberthätigkeit als Ursache der Lymphbildung, besitzen. Damit steht die anderweit bekannte Thatsache im Einklange, dass Chinin diejenigen Processe, welche zur Harnstoff- und zur Glykogenbildung führen, hemmt. Die Wirkung der Lebergifte kann nicht ausschliesslich auf vermehrter Durchlässigkeit der Lebercapillaren beruhen. Die zum Mindesten nicht verminderte Durchlässigkeit der Gefässe bei der Chininvergiftung lässt sich experimentell nachweisen.

5. Arsen, ein »typisches Capillargift«, bewirkt den Ausfluss einer vermehrten und höher concentrirten Lymphe. Obwohl aber die Schädigung der Eingeweidecapillaren viel grössere sind als diejenigen weit schwächerer Mittel, wie Krebsmuskel- und Blutegelkopfextract, ist der Umfang der Lymphbildung durch Arsen viel geringer als bei den letztgenannten. Hieraus folgt wiederum, dass blosse erhöhte Permeabilität der Gefässwände die Wirkungsweise der Lymphagoga nicht ausreichend erklärt.
6. Die Zuckerausscheidung aus dem Blute in die Lymphe nach intravenöser Traubenzuckerinjection verhält sich wie beim unvergifteten Thiere, wesshalb eine active Betheiligung der Capillarendothelien hierbei unwahrscheinlich gemacht wird.
7. Da sich auch bei tiefer Arsenvergiftung durch geeignete Eingriffe wesentlich beschleunigter Lymphstrom erzielen lässt, können Begleiterscheinungen der tiefen Arsenvergiftung nicht der Grund sein, warum trotz erhöhter Permeabilität der Gefässwände nicht so machtvolle Wirkungen am Lymphstrome auftreten, wie durch die Lebergifte (Lymphagoga).
8. Zeitweilige Aortenverschliessung sowie Tod der Capillaren führen zu experimentell nachweisbarer, ungemein vermehrter Durchlässigkeit der Gefässe; Heidenhain's Nachweis, dass Aortenverschliessung die Lymphagoga unwirksam macht, beweist gleichfalls, dass diese Substanzen nicht bloss durch Erhöhung der Gefässdurchlässigkeit wirken können.
9. Lange Zeit nach dem Tode dauert ein beschleunigter Lymphstrom in Folge von intravenöser Zuckerinjection an; die Beschleunigung kann ihren Maximalwerth erst eine Viertelstunde nach dem Tode erhalten. Bedingung für das Eintreten eines länger andauernden postmortalen Lymphstromes ist, dass zwischen der Vollendung der Zuckerinjection und dem Tode vier bis sieben Minuten vergehen. Diese Thatsachen beweisen, dass die Lymph-

bildung nicht eine Leistung des Blutdruckes ist, hingegen wird Heidenhain's Erklärung der Lymphbeschleunigung durch intravenöse Krystalloïdinjection aus der Anziehung der Salze zu dem Gewebswasser den Thatsachen gerecht.

10. Der vollkommene Parallelismus der postmortalen Speichelsecretion und der postmortalen Lymphbildung beweist nicht allein die Unabhängigkeit beider Vorgänge vom Blutdrucke, sondern weist auch darauf hin, dass beiden physiologische Processe ähnlicher Art zu Grunde liegen.

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THE TOXICOLOGY OF TELLURIUM COMPOUNDS, WITH SOME NOTES ON THE THERAPEUTIC VALUE OF TELLURATES.

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A. Action on Plants and Microorganisms.—The earliest as well as most important researches on the biological influence of tellurium compounds were conducted on domestic animals and on man. It was not until 1885 that the results of a study of their action on plants was announced. Knop, in that year, after an investigation of the influence of various substances on growing plants (maize) by the water-culture method, reported that telluric acid[1] to the amount of 0.05 to 0.1 gm. per litre of nutrient fluid, (0.005–0.01%), exerted no observable influence on their development, although analysis of the plants showed that tellurium had been absorbed.

Bokorny, a few years later, working with tellurous oxide and potassium tellurite, found that aqueous solutions containing only a trace of the very insoluble oxide had no effect whatever on algae and infusoria, even after 5 days' treatment, and that 0.02% solutions of potassium tellurite (containing, also, 0.1% of dipotassium phos-

[1] Tellurium was discovered in 1782 by Müller von Reichenstein, and identified and named (from *tellus*, the earth) by Klaproth in 1798. The metal is silver-white, of markedly crystalline structure, with strong metallic lustre. Its atomic weight is still uncertain, but closely approximates 128. Tellurium is very nearly related chemically to sulphur and selenium. Its chemical qualities have made it a difficult problem from the time of its discovery, and at first it was called *aurum paradoxum* and *metallum problematicum*. It is one of the rarer elements and occurs in nature mostly as telluride in combination with bismuth, lead, mercury, silver, and gold. The following formulae show the composition and relationships of the tellurium compounds referred to in this paper:

Tellurous oxide	TeO_2.
Telluric oxide	TeO_3.
Tellurous acid	H_2TeO_3.
Telluric acid	H_2TeO_4.
Sodium tellurite	Na_2TeO_3.
Sodium tellurate	Na_2TeO_4.
Hydrogen telluride	H_2Te.
Methyl telluride	$(CH_3)_2Te$.
Ethyl telluride	$(C_2H_5)_2Te$.
Tellurium tartrate	$Te\ (C_4H_5O_6)_4$.

phate) were likewise without toxic influence, although the algae had been kept in the fluid for a week. Under the microscope the cells were seen to be perfectly normal in all outward aspects. Even with a 0 1% solution of potassium tellurite (containing a trace of potassium hydroxide), only one form of spirogyra seemed to be affected. All of the rest vegetated normally, even at the end of a week of treatment. Continuing his experiments, Bokorny, in the following year, reported that when various algae, such as Spirogyra communis, S. nitida, conferveae, diatomaceae, etc., and also infusoria, were placed in 0.1% solution of telluric acid and kept there in diffused light for some time, little, if any, influence was exerted. At the end of 48 hours the Algae remained perfectly normal, and the infusoria swam about in very lively fashion. Even after 14 days some of the algae were still growing, in spite of the fact that the faintly acid solution contained no mineral or other nutrient material. Potassium tellurate (slightly alkaline in reaction), in like quantity, was just as innocuous.[2]

Scheurlen, very recently, wishing to grow *bacillus anthracis* in pure cultures, and in the absence of atmospheric oxygen, sought a medium which, containing loosely bound oxygen in oxyhemoglobin-like combination, would be almost as favorable to their growth as blood itself. Having previously found that selenious acid on warming with organic substances is reduced and red selenium deposited, he experimented with sodium selenite and also with sodium tellurite, which on similar treatment yields grayish black metallic tellurium. He found that not only *B. anthracis* but also all of the growing bacteria he worked with were colored by reduced metal in the presence of sodium salts of these acids. The bacteria themselves were colored, not the nutrient medium. They were grown on 10 cc. of a meat infusion peptone agar with 1 to 3 loopfuls of a 2% solution of the salts.

These results led directly to the detailed work conducted by Klett, who studied the growth of numerous species of bacteria and some moulds under the influence of selenium and tellurium compounds, and found that the development of various forms, such as *staphyl-*

[2] Further reference to effects on lower animals is made farther on in the reviews of Hofmeister's and Czapek and Weil's work.

ococcus pyogenes aureus and *B. mesentericus vulgatus*, as well as the various moulds, was not materially hindered by slight quantities of sodium tellurite, although several others, such as *B. fluorescens liquefaciens*, were strongly retarded in growth by only traces of the tellurite, which seems to be more inhibitory than the selenite. Yet a few, such as the bacillus of malignant edema and of symptomatic anthrax, which are markedly arrested in growth by selenite, not only reduce tellurite, but appear to continue their development in the presence of a larger proportion of the latter salt. Most of the experiments were made on 10 to 12 cc. of nutrient medium (gelatin, agar-agar), containing 1 to 3 loopfuls of 2% solution of the tellurite. Increasing amounts of tellurite wrought more destructive effects, of course. The colonies in all cases, as in Scheurlen's experiments, were colored grayish black by metallic tellurium, the intensity of the coloration having been proportional to growth. Grayish particles were deposited within the bacteria. Since the colonies only were pigmented by the metal and the surrounding medium was left entirely colorless, Klett concluded that the reduction took place in the protoplasm of the bacterial cell and not outside the cell by secondary action of metabolic products. For this reason, then, he considers tellurites, with selenites, the most satisfactory reagents for detecting and determining accurately reducing action on the part of bacteria. It was observed, further, that the oxygen set free from tellurite during the reduction could not be utilized by aerobic bacteria in anaerobic environment, nor was the presence of tellurite favorable to the growth of anaerobic forms. Klett found, also, that tellurite, in the quantities used, did not decrease the virulence of such forms as *B. anthracis*. Sodium tellurite was the only tellurium compound tried in this connection. Sodium selenate in slight quantity was found to have little or no effect on the growth of bacteria and was not reduced. Klett appears to have concluded, from analogy, that tellurates, also, would not be reduced by them.[3]

B. Effect on Cold-Blooded and on Domestic Animals.—Chr. Gmelin appears to have been the first to give

[3] The author is greatly indebted to Dr. P. H. Hiss for the references to the work of Scheurlen and Klett, and for suggestions in connection with this review of their results.

special attention to the action of tellurium compounds in the animal body. Early in the last century he experimented with tellurous acid on a dog and a rabbit. The former he gave 3 grains (0.2 gram) in a single dose; the latter, 14 grains (0.9 gram) in the course of three days. The dog lost its sprightliness at first and also its appetite, but in a few days recovered both. The rabbit's appetite remained normal throughout the experiment, but on the fourth day it died. On post-mortem examination of the poisoned animals Gmelin noted that a peculiar garlicky odor proceeded from the abdominal cavity; that the mucous membrane of the stomach and intestines was much swollen and covered with a thick layer of tough mucus; and that from the pylorus to the rectum the walls of the intestines were very black. The liver was covered with minute red spots, the blood-serum colored violet, the gallbladder widely distended and the heart full of coagulum.

A more extended series of experiments was next carried out by Hansen, who, working in Wöhler's laboratory, found that 0.3 gm. of potassium tellurite, introduced directly into the stomach of a medium-sized dog, was followed almost immediately by an unpleasant, garlicky odor in the breath, similar to that which Gmelin had noted on opening the bodies of the poisoned animals and which Wöhler and his pupils had attributed to ethyl telluride. Twenty minutes after dosage repeated vomiting ensued. The symptoms noted by Gmelin (languor and loss of appetite) were also observed and recovery was not long delayed. The same dose twice on the following days, morning and afternoon, induced identical results, while the odor in the breath became stronger each day and persisted long after the conclusion of the experiment. The vomit and feces were slimy and black with tellurium granules.

In a second experiment on a dog of average size, 0.5 gm. of tellurous acid *per os* on two succeeding days caused no toxic symptoms, although the odor of the breath became more and more marked, and the feces were blackened by metallic tellurium. On the third day, 0.7 gm. of acid potassium tellurite induced vomiting of grayish-black slimy. material in addition to the previous results, and the odor of the breath rapidly grew stronger. On the fourth day another dose of 0.7 gm. of the tellurite caused vomiting, and considerable thick mucus ran

from the mouth. On the seventh day 0.5 gm. of the same potassium salt, in solution, was injected into the jugular vein. Convulsions resulted at once and death followed in four minutes. The body cavity gave off the characteristic odor and the alimentary tract as well as the kidneys and all other glands, except the spleen and parotids, were colored bluish-black. The liver was not covered with the inflammatory spots, nor was the blood-serum colored violet, as Gmelin had previously found. The lungs, brain and spinal cord retained their normal appearance. The pigmentation of the glands, etc., was caused by deposition of microscopic granules which were shown to consist of tellurium. The peritoneal cavity contained a small quantity of serous fluid, but neither hyperemia nor inflammation was observed. The wall of the urinary bladder was bluish in color and the urine, acid in reaction, contained the odoriferous compound. The right side of the heart and the vena cavae were swollen with blood. In the crystalline lens of each eye, as reported by Hansen's friend, Dr. Schrader, there was a deposit of chalky granules of varying size. They were least in quantity in the center. The cataract was greatest in the left eye. The humours of the eye gave off the odor of garlic. Tellurium was separated from the urine, liver, stomach and intestines. Two additional experiments on dogs gave results that were identical with the above in practically all particulars. The blood-serum was normal in color in each case.

Hansen concluded his paper with the opinion that the pigmentation of the contents of the gastrointestinal tract was due to deposition of tellurium by a process of reduction and that direct absorption of the metal through the intestinal wall was indicated by the bluish-black color of the mucous membrane. He suggested, further, that the violet color of the blood-serum, noted by Gmelin, was due to the presence of absorbed metal in suspension, and that it was not observed in his own experiments because there had been time in each for the tellurium to be deposited in the tissues.

Kletzinsky,[4] also, in experiments on animals noted that administered tellurium was eliminated, in part, in the urine. Rabuteau, 15 years after Hansen's results had

[4] Kletzinsky: Ueber die Ausscheidung der Metalle in den Secreten, *Wiener med. Wochenschr.*, 1858, viii, 355.

been recorded, found tellurium to be exceedingly poisonous and considered it very similar in its action to selenium, although stronger. This deduction was based on the results of only one experiment, however, with sodium tellurite. Following an intravenous injection of 0.08 gram of that substance in a dog, vomiting ensued within 2 hours, after which profound dyspnea set in, with anesthesia, opisthotonus, and finally death from asphyxia in 4 hours. Postmortem examination 12 hours after death showed marked congestion and ecchymosis of the whole of the intestinal canal; also of the liver, spleen, lungs and especially the kidneys. The latter were almost black as a consequence and the tubuli were studded with fat globules. In the heart the right side was filled with blood, the left side on the contrary was empty.

The contents of the right side of the heart, and also of the larger bloodvessels, held a multitude of small prismatic crystals of unknown chemical composition—0.002 to 0.004 mm. in width and from 5 to 10 times as long—which, in the opinion of Rabuteau, presented a mechanical obstacle to the movement of the blood and thus eventually caused the death of the animal in asphyxia. These crystals were apparently identical with those Rabuteau reported he had found under similar conditions after intravenous injections of sodium selenite and administrations of the same *per os*. They were not produced, he says, by selenates—only by selenites and tellurites. Rabuteau states, further, that they were more numerous than the corpuscles. He says nothing about their color, but his sketch of them suggests that they may have been hemoglobin or some derivative of it. Radziejewski[5] seems to entertain this opinion.

It should be remarked, in passing, that Chabrié and Lapicque[6] were unable to find these crystals in the blood of animals poisoned with sodium selenite and, also, that Czapek and Weil, whose work with tellurium is summarized farther on, obtained the same negative result, both with selenites and tellurites, after intravenous injections. Rabuteau's observations in this connection have never been confirmed. Consequently, his theory that death after injection of tellurites results from a

[5] Radziejewski: In abstract of Rabuteau's paper, *Cent. f. d. med. Wiss.*, 1869, vii, 446.

[6] Chabrié et Lapicque: Sur l'action physiologique de l'acide sélénieux, *Compt. rend.*, 1890, cx, 152.

"mechanical poisoning," which produces asphyxia, cannot be accepted. Rabuteau makes no reference whatever to the work of Hansen, or any of his predecessors, and says nothing definite about odor in the expired air of the dog to which he had given tellurite.[7]

Czapek and Weil, in perhaps a more thorough research than any of the preceding, learned that, in its toxicological influence, tellurium behaves very much as does its close chemical relative, selenium, although the symptoms it induces appear later and are, for the most part, weaker—just the reverse, in the latter respect, of Rabuteau's deduction. Sodium tellurite, in quantities of 0.002 gm., under the skin, caused the death of frogs within 48 hours; 0.01 gm. of sodium tellurate was required to produce the same result. In cold-blooded animals these quantities of tellurium gradually brought about paralysis of the central nervous system and death. The heart was arrested in diastole, apparently because of paralysis of the so-called excito-motor ganglia. Atropin did not restore the beats, and the heart-tissue itself remained susceptible to mechanical and electrical stimulation. The garlic odor was detected about the animal in most of these cases. Muscular fibrillations were almost always observed in frogs into which tellurium had been injected, but neither clonic nor tetanic convulsions followed its introduction in the quantities employed.

In warm-blooded animals these same observers found that 0.02 gm. of sodium tellurite, and 0.05 gm. of sodium tellurate, per kilo of body-weight, gave very toxic effects.[8] Dogs very soon became restless. Vomiting quickly ensued, followed by diarrhea, weakening of the reflexes, somnolence, unconsciousness, general paralysis, stoppage of respiration, and death after convulsions. Within five minutes of the time of administration of the poison, the garlic odor in the expired air was intense. There was no muscular fibrillation as in the case of frogs, and, with the exception of the spasm just before death, no clonic or tetanic convulsions. In all cases a lowering of blood-pressure followed the in-

[7] The odor caused by selenates Rabuteau mistakenly ascribed to hydrogen selenide instead of methyl selenide. Hofmeister, whose experiments are referred to on a subsequent page, assumes that the usual odor was recognized by Rabuteau, and, from analogy apparently, that it was referred in error to hydrogen telluride.

[8] We are left to infer the manner of introduction of tellurium in these experiments. It seems to have been both by way of the mouth and under the skin.

jection of tellurium salts. This was due, not to central influences, but to direct peripheral action on the blood-vessels, resulting in impaired tonic contraction, for the vasomotor center remained sensitive to stimulation and the vagi were able to carry impulses. The abdominal capillaries, particularly, were very greatly distended.

The blood from animals poisoned with tellurium was dark-colored and had a distinct garlic odor. Spectroscopically it was normal and the corpuscles showed no change. Czapek and Weil could not confirm Rabuteau's observation in this connection. Postmortem examination showed profound changes in the intestinal mucous membrane, in which edema, congestion, and extravasations were especially prominent. Desquamation of the villi was also observed in most cases. Destructive changes were the rule in the tubules of the kidneys. The urine was bloody now and then, and frequently tellurium could be detected in it. Nearly all of the body parts, in the cold as well as warm-blooded animals experimented on, were colored grayish by metallic tellurium, but no deposit of the metal in granules was observed, on microscopic examination, in any of the tissues. It seemed to be in solution. The muscles of the poisoned animals retained their susceptibility to stimulation.

Tellurium was found to differ from selenium, in toxicity, mainly quantitatively.[9] Czapek and Weil concluded that the difference between the two lies in the different modes of elimination. Tellurium salts are less toxic, they think, because the tellurium is quickly transformed by reduction to the metallic state and so is rendered comparatively passive at once. The results of their experiments indicate that in its toxic action tellurium behaves much as do selenium, arsenic and antimony.[10]

Although the garlic odor in the breath and about the organs of animals to which tellurium salts had been administered was thought at first to be due to ethyl telluride, its resemblance to methyl telluride, when that substance was first made, satisfied Wöhler and his pupils that it resulted from a formation of that organic

[9] Also in having anidrotic action. See footnote further on, where additional results of Czapek and Weil's work are given.

[10] It is interesting to note, in this connection, that tellurium is believed by some chemists to be in reality a mixture of elements, containing an antimony arsenic-like body. Brauner calls one of the presumed constituents of the tellurium complex, *austriacum*, which appears to be the *dwitellurium* predicted by Mendeléeff.

compound. This conclusion was generally accepted for some time. Hofmeister, in some very exact experiments, finally determined in a chemical way that the methyl synthesis, assumed by previous investigators, really does take place when tellurium is administered and that the garlic odor arising as a consequence is caused by methyl telluride.[11]

In experiments on warm and cold-blooded animals he confirmed the observations of previous workers that the various body parts take on the same odor, and showed that it is strongest, or in other words the methyl synthesis is relatively greatest, in the testes and the lungs, and pronounced in the blood, liver and kidneys. He found that when the organs of an animal into which sodium tellurite had been injected intravenously, are put in a warm place (at 36° C.), the smell of methyl telluride is intensified about those having that odor to begin with and is gradually made distinct in others. Under the same conditions, blood loses it, however. Time and intensity vary, of course. These facts show that the cells of the glands are able to absorb tellurium and that they also have the power, at the body temperature, of forming methyl telluride from it. This substance is formed also by minced fresh organs from dogs and rabbits when they are treated with the same substance at the body temperature. Hofmeister proved that this synthesis, with production of the characteristic odor, takes place, also, in frogs, fishes, crabs, and even in earthworms, when small quantities of tellurite are given them.[12] The tellurium was deposited in the animals experimented on in large part in metallic form in many parts of the body, the reduction, judging from the discoloration, varying considerably.

In the body of a dog weighing 850 gms., into which 0.04 gm. of sodium tellurite had been injected intravenously, and which after bleeding to death had been

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[11] Sodium tellurate, 0.03—0.06 gram, was injected subcutaneously into dogs and cats. As soon as the garlic odor became evident in the expired air, the latter was passed through saturated solution of iodine in potassium iodide for 20 to 48 hours. The solution decomposed the methyl telluride, but retained each group and from it methyl was separated in the form of methyl sulphide by treatment with sodium sulphide. Tellurium after evaporation of the solution and treatment with nitric and hydrochloric acids, was precipitated in metallic flakes with sodium sulphite.

[12] Of the other influences of tellurium salts on these animals, Hofmeister says nothing except that injection of sodium tellurite into the soft parts of crabs is followed by paralysis and death.

kept at normal temperature for four hours, practically all parts were pigmented by tellurium except cartilage, bone and the white matter of the nervous system. When dosage was not too great, however, it was found that in the lungs and testes the tellurium, instead of having been deposited was transformed wholly into methyl telluride, which accounts for the fact that these organs are rarely colored by the bluish-black metallic deposits usually found in practically all of the glands. The long-continued elimination of methyl telluride in the breath, Hofmeister shows, is due to *gradual* synthetic transformation of the tellurium which had been deposited in the tissues in metallic form soon after its introduction. He suggests that the reduced tellurium is slowly transformed into the soluble sodium tellurate by the action of the alkaline tissue fluids before it reaches the lungs, and that it is there changed to the methyl compound. In this way he explains the persistence of the odor in the breath.

Hofmeister was unable to determine the specific source of the methyl for this synthesis, but, as the liberation of methyl groups, and also their incorporation in other substances like cholin and creatin, seem to be intermediate processes in general metabolism, he concluded, from his experiments, that the tellurium unites with methyl groups set free in some manner in the cells. He showed that this conversion of tellurium to methyl telluride, and the process of reduction of tellurium compounds, may take place quite independently of each other, for when fresh normal glands after maceration are warmed a few minutes, at 50 to 55° C., and then treated with sodium tellurite, their power to reduce is undiminished, although no methyl telluride is formed by them. The synthetic process is entirely prevented, also, after treatment of the tissues with solutions of various chemicals—even physiological salt solution.

Beyer, following the general suggestions of Ludwig, demonstrated, in some transfusion experiments on perfectly fresh kidneys with oxygen free and arterial blood containing sodium tellurate, that the methyl synthesis does not take place in the absence of oxygen, although reduction to the metallic state occurs in the cells quite independently of the character of the transfused blood. He sought also, by histological methods,

to determine just where in the tissues the reduction of tellurium from its salts occurs. He injected small quantities of sodium tellurate, dissolved in physiological salt solution, into the jugular veins of dogs and rabbits, and found that granular metallic tellurium was deposited only in form elements; in nerve and glandular cells, leukocytes and striated muscle especially. Endothelium, unstriated muscle, nerve and connective tissue fibers, on the other hand, were found to have no affinity for tellurium. The deposit of metallic element in the cells did not appear to cause their degeneration. Destruction occurred only occasionally. The cells, for the most part, seemed to have the power of gradually removing the foreign material without loss of normal function, and even when quite full of the deposit behaved toward all the various staining reagents exactly as normal cells do. Even three weeks after injection of tellurate, while the breath still smelled strongly of methyl telluride, Beyer found metallic tellurium in the glandular cells. Its transformation must, therefore, have been gradual, as Hofmeister has shown was the case in other connections.

Increasing amounts of sodium tellurate injected into the blood of rabbits induced clonic convulsions, respiratory paralysis and death. The blood became laky. Lakiness was not produced by tellurate in rabbit's blood outside the body, which fact suggests that a tellurium transformation product caused it in Beyer's experiments. Intravenous injections, in dogs, of quantities of sodium tellurate ranging from 0.025 to 0.04 gm. per kilo of body-weight were quickly followed by death in some cases; at other times, by vomiting and loss of appetite, with recovery in several days. These quantities also brought about general paralysis; sometimes only of the hind legs and masseters, but usually also of the intercostals, making respiration very labored. Fatty degeneration of the hepatic cells and destructive changes in the uriniferous tubules also resulted. The lymphatic vessels of the liver were found to be much enlarged and other structural changes were observed. In one case lymph from the thoracic duct had a grayish color, due to suspended tellurium.

The urine under these same conditions was turbid, greenish brown to a dark green in color, and gave off the odor of methyl telluride. It contained metallic

tellurium, crystals of urocanic acid and triple phosphate; also, blood-corpuscles, albumin and bile pigment. The latter appeared in the blood-serum also. From the urine of a dog, collected during the first 24 hours after intravenous injection of 0.75 gm. of sodium tellurate (0.27 gm. Te), Beyer separated 0.062 gm. of metallic tellurium. From the urine of the second day, 0.081 gm. There was only a trace in that of the third. None in the fourth. More than one-half of the tellurium administered was, therefore, eliminated through the kidneys.

Until recently, a brief and imperfect experiment by Beyer, on the excretion of urea after intravenous injection of sodium tellurate, had been the only one to suggest the metabolic influence of tellurium. Beyer found that the normal amount of urea eliminated in the urine of a healthy dog, during three preliminary days, was 9.45, 10.41 and 7.62% respectively, an average of 9.16%. After injection of 0.75 gm. of sodium tellurate into the jugular vein, the urea in the urine on five successive days was 1.79, 6.06, 8.50, 7.98, 9.00%, an average of 6.67%. This marked falling-off in the amount of urea was due mainly to the refusal of the dog to eat on the first and second days of the tellurium period, and as Beyer does not give any analytic data regarding the food, it is impossible to attach any special importance to his results in this connection.

The author, very ably assisted by Mr. L. D. Mead, recently completed a series of experiments on dogs in which an attempt was made to ascertain, among other things, the effects of continued dosage of tellurium compounds. It was found that nontoxic doses of tellurium (in quantities several times as great as therapeutic doses and in the forms of oxide, tellurite, tartrate and tellurate) did not materially affect metabolism in dogs brought to a state of nitrogenons equilibrium, even when dosage was continued for a week. These substances appeared to stimulate proteid catabolism only slightly. They increased somewhat the weight of dry matter in the feces and diminished, in small degree, the absorption of fat. The urine was unaffected in volume, specific gravity, and reaction, but became dark brown in color during the dosage periods.

Excesive doses retarded gastric digestion; induced violent vomiting, loss of appetite and somnolence. They

caused, besides, inflammation and disintegration of the mucous membrane of the gastrointestinal tract and, also, intestinal hemorrhage. Introduced under the skin, tellurium (tartrate) caused restlessness, tremor, weakening of the reflexes, somnolence, diarrhea, paralysis, unconsciousness, stoppage of respiration and death, in convulsions from asphyxia. At the point of injection much of the tellurium was deposited in metallic form, but it was also distributed in large quantity to most of the organs and tissues.

It was found, also, that tellurium compounds, even in small proportion, markedly arrested the secretion of acid in the stomach—the direct cause, probably, of the indigestion brought about, not only in dogs but, as will be pointed out later, by tellurium compounds in man, also. Intestinal putrefaction was not influenced in any degree. The action of trypsin and pepsin outside the body was not very perceptibly diminished by quantities of tellurium compounds under 0.6%. Zymolysis was almost unaffected in the presence of as much as 1.25% of some of the salts. Ptyalin was more easily affected, even by the faintly alkaline tellurate. Trypsin appeared to be least sensitive to destructive influence, acting rapidly in the presence of even 2.5% of tellurite.

Tellurium was eliminated in metallic form in the feces; as methyl telluride in the breath, urine, feces, and epidermal secretions; in a soluble form, in small quantity, in the urine and in the bile. The urine was colored brown to yellowish green after heavy dosage with tellurium compounds, but return to normal coloration was rapid after administration had been discontinued. Albumin and bile pigment, besides tellurium, were the abnormal constituents of the urine found after subcutaneous injections. Toxic quantities given by the mouth caused the appearance of coagulable proteid, but no bile pigment, in the urine.

C. Influence on Man. (*a*) *General.*—Berzelius,[13] who led the way for so long in chemical studies of tellurium, found, from personal experience, that hydrogen telluride is irritant in its action and more poisonous in effect than the corresponding compound of sulphur. Berzelius and Kölreuter[14] have reported that the oxides of

[13] Th. Husemann und A. Husemann: Handbuch der Toxikologie, 1862, 773.
[14] L. Gmelin: Handbook of Chemistry (Watts), 1850, iv, 398, 399, 402, 403. Also *Ibid.*, 1856, x, 309, and Berzelius: Traité de Chimie, 1846, ii, 225, 230.

tellurium, as well as a number of salts of telluric and tellurous acids, have a very unpleasant metallic taste resembling that of compounds of antimony and that some have a nauseating action and are strongly emetic.

Wöhler, at the time of his discovery of ethyl telluride,[15] referred to the disagreeable odor of that substance, and stated that it is very poisonous. At that time, and subsequently, while engaged in his chemical researches on ethyl telluride, Wöhler observed that his sweat and breath took on an odor closely resembling that of the substance he was working with.[16] One night, while perspiring very freely, the garlic odor in his sweat became so great that he himself could hardly bear it. It persisted in his breath for weeks. These facts led Wöhler to suggest the physiological researches made in his laboratory by his pupil, Hansen.

The latter was the first to experiment systematically on man with tellurium compounds. For 7 successive days he himself took neutral potassium tellurite an hour before dinner. On the first 4 days 0.04 gm., on the 2 following days 0.05 gm., and on the last day 0.08 gm.—a total of 0.34 gm. During the first two days very unusual sleepiness was the main symptom. Later it disappeared. At the beginning there was increased appetite, but later the appetite was reduced. After dosage on the last day there was a sense of oppression in the cardiac region, also nausea and abundant salivation. The tongue was heavily coated with a white deposit, and there was complete loss of appetite. The gastric symptoms did not disappear completely until after a lapse of 2 weeks, and the alliaceous odor of the breath continued 7 weeks.

The characteristic odor of the breath was noticed within a few minutes after the first dose had been taken, and soon became so strong and so obnoxions to others that his own seclusion was necessary for their comfort. At that time the odor was attributed to a volatile compound of tellurium identical with or similar to ethyltelluride. Hansen was unable to separate any tellurium from the urine; not even from that passed during the first 24 hours after the last dosage. Experiments on his friend, von Röder, who took 0.04 gm. of acid potassium tellurite before dinner one day, and nearly 0.05

[15] Wöhler: Telluraethyl, *Ann. d. Chem. u. Pharm.*, 1840, xxxv, 112.
[16] Gorup-Besanez: Lehrbuch der physiol. Chemie, 1878, 552.

gm. at the same time the next, presented essentially the same results. Hansen refers to Wöhler's previous experience and says that during these later experiments in the latter's laboratory Wöhler observed the same phenomena, with regard to himself, a second time.

Heeren,[17] also working under Wöhler's direction, on the chemical nature of various compounds of ethyl and methyl tellurides, noted that the garlic odor of the breath was especially strong in his own experience when methyl telluride or any of its derivatives was under examination. He states that even when these products are merely touched with the fingers their characteristic odor is carried to all parts of the body and in a few days the breath also acquires it, the odor quickly becoming so obnoxious that, as he puts it, "one must avoid all social life for months, so as not to annoy others."

Sir J. Simpson records a case[18] in which a student inadvertently swallowed a dose of tellurium, which was followed by the evolution of such a persistent odor that for the remainder of the session he had to sit apart from his fellow students.

Prof. Victor Lenher, who for several years has been engaged in chemical studies of tellurium, greatly favored the author with a statement of his toxicological experiences for use in this connection After inhalations of the volatile tellurous oxide, which he formed repeatedly in preparing metallic tellurium by the fusion method, Prof. Lenher's breath and the excretions from his skin took on the usual garlic odor. Metallic taste was noted and nausea also frequently experienced. The odor of the breath in one case persisted for about a year. General depression followed continuous inhalation of the oxide, and in one instance a prolonged period of somnolence resulted, an experience similar to Hansen's after ingestion of tellurite. Severe constipation was also a marked symptom following tellurium inhalation. At no time could Prof. Lenher detect any tellurium in his urine, not even during the periods of his worst experiences.

The author has found in his own experience that when the methyl telluride which had been exhaled by

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[17] Heeren: Ueber Telluräthyl und Tellurmethyl-Verbindungen, *Chem. Centralbl.*, 1861, vi, 916 (N. F.)

[18] Quoted from Blyth: Poisons, their Effects and Detection, 1885, 559.

the dogs he experimented with was taken into his own lungs, an alliaceous odor of the breath and excretions from the skin soon became noticeable and continued persistently. Also, that such inhalation was accompanied frequently by short periods of drowsiness and nausea.

(*b*) *The cause of "bismuth breath" and the minimal quantity of tellurium that will produce it.* As early as 1875 tellurium had been suspected in commercial preparations of bismuth.[19] The evidence on this point at that time was not of an analytical character, but was based upon the observation that people to whom certain bismuth preparations had been administered suffered from fetid breath. The presence of tellurium in bismuth preparations has since been repeatedly shown,[20] and their medicinal use implies frequent incidental action of this tellurium impurity.

Reisert, in 1884, after an investigation of the cause of the so-called bismuth breath, ascertained that it was due, as had been supposed by some, to the minute tellurium impurities often found in the commercial bismuth compounds used in medicine, and not to arsenic or bismuth itself, as had been assumed by others.[21] He not only demonstrated, in some experiments on himself and friends, that the "bismuth breath" did not follow dosage with chemically pure bismuth sesquioxide, or arsenious oxide, but also determined the minimal amount of tellurium which would produce the alliaceous odor in the breath. He found that as little as 0.000,000,5 gm. of tellurous oxide, given in solution to men, was followed by the smell of garlic in 75 minutes, and that it continued for about 30 hours; 0.000,000,3 gm., given to three different individuals, failed to produce a detectable quantity of the odor. In one experiment, three doses of 0.005 gm. each were taken on the same day at intervals of 3 hours. "In 15 minutes after the first dose the breath had a strong garlic-like odor, and in an hour a metallic taste was observed. An hour

[19] Blyth: A Manual of Practical Chemistry, 1879, 428.

[20] Lately again: *Druggists' Circular and Chemical Gazette*, 1894, xxxviii, 256, referring to observations of Janzon in *Pharm. Zeitschr.*

[21] The author is greatly indebted to Prof. John Marshall for calling his attention to Reisert's work. It seems that subsequent foreign investigators of the behavior of tellurium in the animal body were unaware of Reisert's results. It is probable, however, that Kunkel refers to these results when he says, "The odor (of methyl telluride) has been detected in the feces of man over two months, and in the breath more than a half year, after the last dose of tellurium." *Handbuch der Toxikologie*, 1899, 365.

after the second dose the urine and sweat had the garlic-like odor, which was also observed in the feces, 4 days later. The metallic taste was observed for 72 hours; and the garlic-like odor in the urine for 382 hours, in the sweat for 452 hours, in the feces for 79 days, and in the breath it was still present, though very faintly, after 237 days."

Reisert passed his breath through a tall column of distilled water for several hours, in the hope of catching the odoriferous compound which seemed to be eliminated from the lungs in appreciable quantity, but analysis of this water afterwards gave negative results. He assumed, therefore, that the quantity of substance responsible for the odor was too small to be detected by known chemical means and suggested that the "physiological test" is much more delicate than any purely chemical one for this purpose.[22] Reisert concluded his paper with the remark that idiosyncrasy did not seem to have any influence in his experiments, since the breath of every one to whom the tellurous oxide had been administered, in quantities not less than 0.000,-000,5 gm., was affected with the alliaceous odor.

(*c*) *Antihydrotic Action and Therapeutic Use.*—Neusser was the first to show that tellurium compounds are of therapeutic value. In about fifty clinical experiments, on as many consumptives, he observed that the night-sweats were very perceptibly reduced after administrations of potassium tellurate in daily doses of 0.02 to 0.06 gm.[23] In a majority of cases 0.02 gm. was sufficient, although cumulative dosage was necessary at times to effect continued results. He noted, also, that these amounts did not cause any particularly toxic symptoms, although mild dyspepsia (eructations, coated tongue,

[22] Reference has already been made to Hofmeister's method for separating tellurium eliminated in the form of methyl telluride in the expired air. This was not applied, of course, until after Reisert's work had been reported. Reisert knew, however, that Wöhler and his pupils attributed this odor to methyl telluride, but he failed to use adequate means for the retention and chemical detection of such a volatile compound.

[23] In order to test the anidrotic action of tellurium, Czapek and Weil, whose work has already been reviewed, made careful experiments in this connection on kittens with results that entirely confirmed Neusser's original observation. Moderate nontoxic doses (presumably of tellurates) were given and before any of the usual sickening influences had manifested themselves the moisture on the soles of the hind paws became less and less, until they were quite dry, when even the strongest electrical stimulation of the peripheral end of the divided sciatic nerve was insufficient to call forth secretion; after the tellurium had reached its fullest effect, pilocarpine, however, was able to induce secretion. These investigators were unable to determine any pathological changes in the structure of the sweat glands and concluded that the interference with secretion was a direct peripheral action of the tellurium and not one upon the central nervous system.

loss of appetite) was produced now and then by the use of the largest dose. In some cases there appeared to be stimulation of appetite at first and, in quite a number of instances, Neusser received the impression that slight narcotic action had been manifested. The breath of each individual experimented on always quickly assumed the characteristic alliaceous odor even with the smallest quantities of the tellurate. This was the only undesirable feature that occurred regularly. Neusser stated that the odor was not noticed by the patients themselves except in a few cases. Sulphurous and camphoraceous odors in eructations were sometimes complained of. His experiments were conducted on patients in advanced stages of phthisis, but with none of these was any favorable influence of the tellurium observed on the disease itself.

Pohorecki, following Neusser's lead, confirmed, in a large number of clinical experiments, the latter's results in practically all particulars. He reported that increased appetite and better general nutrition resulted from dosage with 0.01—0.02 gm. of potassium tellurate in the earlier stages of phthisis. Anidrotic action was manifested in fifteen minutes to an hour, and continued five to seven hours. The garlic odor of the breath could be detected fifteen minutes after administration and continued four to eight weeks. Even in people who were perfectly well it was observed that potassium tellurate greatly hindered the secretion of sweat.

Combemale and Dubiquet found that sodium tellurate in daily doses of 0.02 to 0.05 gm. had a pronounced antidiaphoretic action and was more effective in this respect than even camphoric acid. Anidrosis was obtained not only with patients suffering from phthisis, but also in other cases in which sweating is often profuse (rheumatism, dyspepsia, etc.). Administration of sodium tellurate was followed by diminished perspiration in 18 of 20 cases. In 6 of the 18 it was arrested completely. 0.02 gm. was found to be the minimal dose which would induce anidrosis ; 0.05 gm. the most effective quantity. Repeated dosage with this amount for a few days brought about the result, if it was not manifested immediately after the first administration. These observers, unlike Neusser in his experiences with the potassium salt, did not find that any gastrointestinal disturbances were set up and report the alliaceous odor

of the breath in but a few instances as the only objectionable feature following its administration in the doses indicated and for reasonable lengths of time.[24] Combemale and Dubiquet consider sodium tellurate the very best anidrotic agent and prefer it as a result of their experiments to camphoric acid, white agaric, atropin, phosphate of lime, etc. Combemale favors the view that excessive sweating, in such disorders as phthisis, is due to the action of ptomaines elaborated by the specific germs of the disease and he supposes that sodium tellurate exerts an antihydrotic influence by rendering these soluble septic products innocuous. He presents nothing, however, in direct evidence to substantiate this deduction. His theory would not explain the reduced sweating in perfectly well people, which Pohorecki observed after administration of potassium tellurate.

Mr. Mead and the author have shown, as has already been pointed out, that tellurates, in quantities not excessive and yet much greater than the therapeutic doses in man, exerted no particularly deleterious effects on the nutritional processes in dogs, even when dosage was continued for a week, although proteid catabolism seemed to be slightly stimulated after a time, and secretion of acid in the stomach retarded. The alliaceous odor imparted to the breath appears, therefore, to be the chief objectionable feature constantly following the use of therapeutic amounts of tellurates.

BIBLIOGRAPHY.

Chr. Gmelin. 1824. Versuche über die Wirkungen des Baryts, Strontians, u. s w., auf den thierischen Organismus. Tübingen, 43.

Hansen. 1853. Versuche über die Wirkung des Tellurs auf den lebenden Organismus. *Ann. d. Chem. u. Pharm.*, lxxxvi, 208.

Rabuteau. 1869. Recherches sur les propriétés et sur l'élimination des composés oxygénes du sélénium et du tellure. *Gaz. hebd. de Méd. et de Chir.*, xvi, 194; 241.

Reisert. 1884. The so-called bismuth breath. *American Journal of Pharm.*, lvi, 177.

Knop. 1885. Ueber die Aufnahme verschiedener Substanzen durch die Pflanze, welche nicht zu den Nährstoffen gehören. *Botan. Centralbl.*, xxii, 35.

Neusser. 1890. Ueber tellursaures Kalium als Mittel gegen die Nachtschweisse der Phthisiker. *Wien. klin. Wochenschr.*, iii, 437.

Pohorecki. 1891. Ueber den Einfluss des Kali telluricum auf die Schweisse der Phthisiker. *Jahresber. ü. d. ges. Med.*, xxvi, (i), 398.

Combemale et Dubiquet. 1891. Le tellurate de soude comme médicament antisudoral. *Sem. medic.*, xi, Annexes, 24.

[24] The reported absence of the garlic odor in the breath in a large majority of these cases is in direct disagreement with the results of Reisert's quantitative experiments and the observations of all previous and subsequent investigators, except Rabuteau, each of whom has found that it *invariably* follows the introduction of very small quantities of tellurium compounds both in man and lower animals.

Combemale. 1891. Recherches cliniques sur deux agents antisudoraux ; l'acide camphorique et le tellurate de soude. *Bull. gen. de. Therap.*, cxx, 14.

Czapek und Weil. 1893. Ueber die Wirkung des Selens und Tellurs auf den thierischen Organismus. *Archiv. f. exp. Path. u. Pharm.*, xxxii, 438.

Bokorny, (*a*). 1893. Ueber die physiologische Wirkung der tellurigen Säure. *Chem. Zeitung*, xvii, (ii), 1598 ; (*b*). 1894. Toxikologische Notizen über einige Verbindungen des Tellur, Wolfram, u. s. w., *Ibid.*, xviii, (ii), 1739.

Hofmeister. 1894. Ueber Methylirung im Thierkörper. *Archiv. f. exp. Path. u. Pharm.*, xxxiii, 198.

Beyer. 1895. Durch welchen Bestandtheil der lebendigen Zellen wird die Tellursäure reducirt? *Archiv f. Anat. u. Physiol.*, Physiol. Abth'l'g, 225.

Scheurlen. 1900. Die Verwendung der selenigen und tellurigen Säure in der Bakteriologie. *Zeitschr. f. Hyg. u. Inf.-krank.*, xxxiii, 135.

Klett. 1900. Zur Kenntniss der reducirenden Eigenschaften der Bakterien. *Zeitschr. f. Hyg. u. Inf.-krank*, xxxiii, 137.

Mead and Gies. 1901. Physiological and toxicological effects of tellurium compounds, with a special study of their influence on nutrition. *Amer. Jour. of Physiol.*, v, 104.

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TELLURIUM TOXICOLOGY.

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PHYSIOLOGICAL AND TOXICOLOGICAL EFFECTS OF TELLURIUM COMPOUNDS, WITH A SPECIAL STUDY OF THEIR INFLUENCE ON NUTRITION.[1]

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ABOUT two years ago Professor Victor Lenher was engaged at this University with extended studies of the properties of tellurium and its compounds.[2] The ill effects which Professor Lenher experienced from involuntary inhalations of volatile products formed in preparing tellurium impressed him with the desirability of a systematic study of its physiological effects. He generously offered to furnish Dr. Gies with pure tellurium preparations for such an investigation. We wish to thank Professor Lenher for the suggestion

[1] A preliminary account of some of the experiments referred to in this paper was given in abstract in the Proceedings of the American Physiological Society. This journal, 1900, iii, p. xx.

[2] See Journal of the American Chemical Society, 1899, xxi, p. 347; 1900, xxii, pp. 28, 136.

which led to these experiments, and for the costly material without which they would not have been possible. We are also greatly indebted for valuable facts communicated by Professor Lenher from his large chemical experience.

I. Influence on Metabolism.

With the exception of a brief and very imperfect experiment by Beyer (13),[1] on the excretion of urea after intravenous injection of sodium tellurate, no special study has ever been made of the influence of compounds of tellurium on the nutritional processes in the body.[2] Neusser (6) was the first to note that potassium tellurate induces anidrosis. In about fifty clinical experiments, on as many consumptives, he observed that the night-sweats were very perceptibly reduced after administrations of potassium tellurate in daily doses of 0.02–0.06 gm. Subsequent investigators, principally Pohorecki (7), Combemale and Dubiquet (8) and Czapek and Weil (10) confirmed this observation of the physiological action of tellurates, and Combemale (9) even expressed the conviction that sodium tellurate is one of the very best antisudorific agents. Consequently, both potassium and sodium tellurates have been employed for the purpose of arresting sweating, particularly the colliquative sweats of phthisis.[3] Further, tellurium is repeatedly found, in small quan-

[1] The numerals in parentheses correspond with those preceding the references in chronological arrangement at the end of this paper.

[2] Tellurium was discovered in 1782 by Müller von Reichenstein and identified and named (*tellus*, the earth) by Klaproth in 1798. The metal is silver-white, of markedly crystalline structure, and possesses a strong metallic lustre. Its atomic weight is still uncertain, but closely approximates 128. (See note, page 148.) Tellurium is very nearly related chemically to sulphur and selenium. Its chemical qualities have offered difficulties from the time of its discovery, so that at first it was called *aurum paradoxum* and *metallum problematicum*. It is one of the rarer elements and occurs in nature mostly as telluride in combination with bismuth, lead, mercury, silver, and gold. The following formulæ show the composition and relationships of the tellurium compounds referred to in this paper:

Tellurous oxide, TeO_2.	Sodium tellurate, Na_2TeO_4.
Telluric oxide, TeO_3.	Hydrogen telluride, H_2Te.
Tellurous acid, H_2TeO_3.	Tellurium tartrate, $Te(C_4H_5O_6)_4$.
Telluric acid, H_2TeO_4.	Methyl telluride $(CH_3)_2Te$.
Sodium tellurite, Na_2TeO_3.	Ethyl telluride $(C_2H_5)_2Te$.

[3] Cerna: Notes on the newer remedies, 2d ed., 1895, pp. 164 and 185. See also, New York medical journal, 1891, liii, p. 370, on camphoric acid and tellurate of sodium as anidrotics, referring to the recommendations in La province médi-

tity, in commercial bismuth preparations,[1] and their medicinal use implies frequent incidental action of this tellurium impurity. In view of these facts, we have attempted first of all in our experiments to determine the influence of small non-toxic quantities of tellurium on metabolism, as measured especially by fluctuations in the excretion of nitrogen.

Conduct of the Experiments.

Animals and Environment. — The experiments were performed on full-grown dogs weighing from 10 to 16 kilos. The general methods were those outlined in the report of some previous investigations made by Dr. Gies under Professor Chittenden's supervision.[2] The animals were confined in a suitable cage, well adapted for the collection and separation of fluid and solid excreta. The cage was open at the top so as to permit of free circulation of air, and was kept in a comfortable room with a constant temperature.

Character of Diet. Feeding. — The animals received regularly a mixed diet of hashed lean meat, cracker dust, lard, and water. Former experience proved this to be a very acceptable, digestible and nutritious mixture. The hashed meat was prepared by a method similar, in general, to that previously described by Dr. Gies.[3] The hash was preserved *frozen* with results which were satisfactory throughout all the experiments. Commercial cracker dust, containing only 1.51 per cent of nitrogen, afforded the carbohydrate element of the diet. This was kept entirely dry in large quantity in well stoppered bottles. The lard employed was perfectly fresh. Ordinary river water was used. Neither lard nor water contained appreciable quantities of nitrogen.

The daily mixed diet was given regularly in two equal portions, in the morning at nine and in the evening at six o'clock. The water was stirred with the other ingredients, until the whole mixture had the consistency of very thick soup. This mixture, while not very appetizing in appearance, possessed an agreeable odor and was always lapped eagerly by all the animals in the normal periods. The food

cale. Tellurates have not, however, come into general employment, because of the obnoxious odor imparted to the breath after their administration. See page 130.

[1] See Ekin and Brownen: American journal of pharmacy, 1876, xlviii, p. 133 (Abstr.). Blyth: Poisons, their effects and detection, 1885, p. 559. Janzon: Druggists' circular and chemical gazette, 1894, xxxviii, p. 256 (Abstr.).

[2] Chittenden and Gies: This journal, 1898, i, p. 4. [3] *Ibid.*, p. 5.

was presented in a common glass crystallization dish, a receptacle especially suited for the licking up of last traces.

Dosage, Weighing, etc. — The daily doses of tellurium were also divided equally. Each half was enclosed in a capsule made of a small portion of the weighed hash. This was always quickly swallowed, in eager anticipation of the rest of the meal, which followed immediately, so that the tellurium entered the stomach almost simultaneously with the main portion of the food.

In the records of the experiments each period of twenty-four hours ended at 9 o'clock in the morning, when the first food of the new day was given. The animal was weighed just before that hour. The daily analytic data are for the twenty-four hours ending at 9 A. M. The figures representing weight are therefore for the weight at the *end* of each experimental day.

Collection of Excreta. — It was found in the experiments already alluded to[1] that diurnal variations in the elimination of urine were practically neutralized at the end of a week or ten days. Consequently, in these experiments, in which the periods were of from seven to ten days' duration, it was unnecessary to remove any urine with a catheter. We collected the urine as it was excreted naturally and thus avoided the disturbances which may arise from catheterization. At the end of each day the interior of the cage was thoroughly sprayed, and rubbed with a stiff test-tube brush. After the physical qualities of the combined 24 hours' urine had been noted, the cage washings were used in making up the daily volume of urine to a litre, in preparation for analysis. Powdered thymol was added in order to prevent bacterial changes. This was at times particularly desirable, for not all the analyses could be begun on the day of collection.[2]

No special indigestible substance was introduced with the food to mark off the fæces. As the elimination of solid excreta from the dog is quite regular under normal conditions, and also when equilibrium is maintained, it seemed best to refer the excrementitious matter from the intestines to the period of their collection. While this course permits of error, only unimportant influences on character and elimination would be hidden under these conditions. The

[1] CHITTENDEN and GIES: This journal, 1898, i, p. 4.

[2] Some of this urine remained in the laboratory for almost two years, without undergoing any change in nitrogen content. A very thin scum formed during that time and the urine became a little darker in color.

inaccuracies of deduction resulting from this procedure certainly could not have been material in our work, since the figures for the nitrogen in the fæces of whole periods, to be given farther on, are essentially the same for each period in a group.[1] The fæces were thoroughly desiccated over the water bath on a weighed dish immediately after collection, then weighed, thoroughly ground, preserved in dry, well-stoppered bottles, and analyzed at convenient intervals.

An appreciable quantity of hair falls from most dogs during such an experiment. This was collected daily, combined for each period, and the nitrogen content determined. It will be observed, in the tables giving analytic data, that the nitrogen thrown off in this way is so considerable that it must be taken into account in equilibrium experiments. From long-haired dogs the loss of hair is especially marked. The nitrogen eliminated in this way is not the same for each period, as our results will show.[2]

Analytic methods. — Nitrogen of the food and excreta was determined by the Kjeldahl process, in all except the last experiment. Oxidation was accomplished with sulphuric acid aided by copper sulphate.[3] In the urine of the last experiment nitrogen was estimated by the hypobromite method with Marshall's apparatus.[4] Urea was calculated from the nitrogen thus obtained (1 c.c. N = 0.00282 gm. urea). Total sulphur and phosphorus were determined by the usual fusion methods;[5] phosphoric acid by Mercier's modification of Neubauer's method;[6] total and combined sulphuric acid gravimetrically by customary methods, the former with Salkowski's precaution,[7] the latter by Baumann's process;[8] uric acid by Ludwig's well-known silver method;[9] fat (ether-soluble matter) in the fæces by extraction with anhydrous ether in the Soxhlet apparatus in the usual manner. The total solids in the urine were calculated from the volume and the specific gravity ("Christison's formula") with the aid of Haeser's coefficient.[10] Indoxyl was estimated quali-

[1] See tables giving quantitative elimination of fæces, composition, etc., under similar conditions: CHITTENDEN and GIES, *loc. cit.*, p. 37.

[2] See also, *Ibid.*, pp. 24 and 33.

[3] MARCUSE: Archiv für die gesammte Physiologie, 1896, lxiv, p. 232.

[4] MARSHALL: Zeitschrift für physiologische Chemie, 1887, xi, p. 179.

[5] Given in detail by CHITTENDEN and GIES: *loc. cit.*, page 7.

[6] NEUBAUER and VOGEL: Analyse des Harns, zehnte Auflage, 1898, p. 731.

[7] *Ibid.*, p. 721.

[8] *Ibid.*, p. 724.

[9] *Ibid.* p. 820.

[10] *Ibid.*, p. 703.

tatively with the Jaffe-Stokvis test.[1] The specific gravity of the urine was ascertained with the ordinary urinometer. The reaction to litmus was taken. When the urine was amphoteric, the stronger reaction was recorded. The quantities of which analyses were made were those customarily employed.

Tellurium was determined quantitatively in the following manner: Solid excreta, after fine division in a mortar, and also concentrated urine, were treated with strong hydrochloric acid and potassium chlorate over the water bath until completely disintegrated and almost perfectly dissolved. After that had been accomplished the fluid was kept on the bath until it was entirely freed of chlorine gas. It was then concentrated to 400-500 c.c. and filtered. The clear acid filtrate was next saturated, while warm, with sulphur dioxide gas and allowed to stand for 24 hours. The bluish black metallic tellurium which had separated in this process was then filtered on a weighed paper, washed with dilute acid, dried at 110° C to constant weight, and determined gravimetrically.[2]

First Experiment; with Tellurous Oxide.

The animal used in this experiment was a long-haired bitch weighing approximately 15 kilos. A preliminary period of six days sufficed to bring her into nitrogenous equilibrium. The daily diet throughout the experiment was 250 gms. of prepared meat (9.099 gms. N), 50 gms. of cracker dust (0.755 gm. N), 40 gms. of lard, and 700 c.c. of water, containing a total of 9.854 gms. of nitrogen. The experiment continued twenty-four days, and was divided into three periods: a fore period of seven days during which normal conditions prevailed; a longer period of ten days during which doses of tellurous oxide, averaging nearly 0.1 gm., were given twice daily; and an after period, equal in length to the first, during which no tellurium was administered. During the tellurous oxide period of ten days a total of 1.6 gm. of the oxide was retained after ingestion, or 0.16 gm. per day. The smallest dose was 0.05 gm. in half of the food for the day; the largest was 0.5 gm. in the same quantity of food.[3]

[1] Neubauer und Vogel: Analyse des Harns, zehnte Auflage, 1898, p. 166.

[2] This method, Professor Lenher assures us, gives accurate quantitative results. The methods employed by Hansen, Kletzinsky and Hofmeister were much the same. Kletzinsky: Wiener medicinische Wochenschrift, 1858, viii, p. 355.

[3] The daily dose of tellurate, in therapeutic use, recommended by Neusser, Pohorecki and Combemale and Dubiquet, varies from 0.01 to 0.06 gm.

On the first day two doses of 0.25 gm. were given. A few minutes after the first dose was administered, the characteristic alliaceous odor became quite noticeable in the expired air and it increased steadily during the rest of the day. On the following morning the odor in the room was of sickening intensity. No special change except languor and sleepiness had been noticed in the animal itself up to this point. The dose in the morning meal (second day) was raised to 0.5 gm. But this was clearly a mistake, for, although the food with its contained tellurium oxide was eaten eagerly and quickly, the whole meal was vomited in less than ten minutes afterward.[1] The vomit was collected quantitatively. The evening portion of food contained only 0.25 gm. The dog ate it very slowly, but before swallowing all of it, vomited violently what had just been eaten. This vomited material was also gathered quantitatively and added to that collected in the morning. The uneaten portion of the evening meal was mixed with the vomit of the day, and the whole thoroughly desiccated on the water bath for determination of its nitrogen content, which was found to be practically equivalent to that of the day's food.[2] The dog was sick throughout the second day. The urine, 220 c.c., was coffee colored.[3] It contained no granular tellurium, although some was held in solution. Bile pigment, albumin, and sugar were also absent.

At this stage of the experiment it was obvious that the animal had been thrown completely out of physiological equilibrium.[4] The quickest way to restore the equilibrium seemed to be to feed the dog an extra amount of food equivalent to the previous day's meal.

[1] In a few preliminary experiments on two other dogs of about the same size it was found that 0.75 to 1.0 gm. of the oxide administered in the same manner caused vomiting, but that 0.5 gm. did not. We had hoped, therefore, that this dose would be safely ingested, at least once, so that we should be able to determine very definitely what metabolic influence tellurium might exert under conditions approximately toxic; and yet not toxic enough to vitiate the experiment. It will be seen that this was practically accomplished, in the case of this particular dog.

[2] It contained 10.335 gm. of nitrogen. The food contained 9.854 gm. The difference (0.481 gm.) was doubtless due to the nitrogen of the mucus, etc., thrown from the stomach.

[3] Somewhat darker than No. 8 in Vogel's well known scale of urine tints. See TYSON: A guide to the practical examination of urine, 1896, 9th ed., frontispiece.

[4] The dosage period was lengthened to ten days on account of this occurrence. See CHITTENDEN and GIES, *loc. cit.*, page 9, for an account of similar experiences, with favorable outcome.

This amount was given in two equal portions on the third day, with the gratifying results shown in the tables for this experiment. Although cumulative action of the tellurium had been manifested, the dog's appetite did not seem to be at all impaired at this time. The food on the third day contained 0.25 gm. of the oxide. During the remaining seven days of the oxide period, the dosage was kept as high as was deemed expedient. On the evening of the fourth day the animal was again nauseated, although the food with its dose of 0.125 gm. of tellurous oxide was finally eaten and none thereafter vomited. For the rest of the period the daily amount—0.1 gm.—gave no special trouble. The dog was very stupid on the third and fourth days of the dosage period, and manifested a constant tendency to sleep. On the fifth day it was more lively and toward the end of the period was entirely normal. At the close of the experiment 0.5 gm. of the oxide in the usual quantity of food induced vomiting within an hour.

The color of the urine throughout the tellurous oxide period was considerably darker than normal, but this difference was less and less perceptible after the day of the greatest dosage. Only now and then could the odor of methyl telluride be detected. Indican was present in samples of each period. Bile pigment, sugar, coagulable proteid and abnormal sedimentary material were absent. Tellurium in small quantity could be detected in the urine during the first half of the period. The fæces were not greatly changed; they were somewhat more bulky, contained more mucus, and were bluish-black instead of brown, as in the fore period, and late in the after period. Occasionally the odor of methyl telluride in the fresh fæces was recognized, though usually it was lost in that of the normal fæcal aromatic compounds. The alliaceous odor in the dog's breath was most marked at about the middle of the experiment, when it began to diminish, although, so long as the animal remained under observation — for almost five weeks after the last dosage — it was very marked. The shed hair gave off distinctly the odor of the methyl compound, yet we were unable to separate any tellurium from it.

The accompanying tables, pp. 112 and 113, give the various analytical results and other data of the first experiment.[1]

[1] The first three metabolism experiments were performed before Mr. MEAD had been invited to assist in this research, and during the year when the routine labor connected with the equipment of the Department of Physiological Chemistry and its organization for regular work was most exacting. Hence it was im-

FIRST EXPERIMENT.

Date. 1898 Dec.	Body weight. K.	Food. Nitrogen. Grams.	TeO_2. Gms.	Urine. Vol. c.c.	Sp. gr.	Reaction litmus.	Nitrogen. Grams.	Phosphorus. Grams.	Sulphur. Grams.	Uric acid. Grams.	Fæces. Dry weight. Grams.	Nitrogen. Grams.	Ether-soluble matter. Grams.	%
Fore Period.														
1	14.9	9.854	..	578	1017	Acid	10.421							
2	14.8	9.854	..	590	1016	Acid	10.013	1.321	....	....	10.57	0.698	2.915	27.6
3	14.9	9.854	..	782	1013	Acid	9.403	....	1.794					
4	15.0	9.854	..	651	1013	Acid	8.921	....	....	0.242				
5	14.8	9.854	..	720	1013	Acid	9.410	2.005	....	....	21.82			
6	15.0	9.854	..	665	1014	Acid	8.960	....	....	....	....	1.676	9.711	26.9
7	15.0	9.854	..	639	1012	Acid	8.231	1.198	2.529	0.199	14.28			
Tellurous Oxide Period.														
8	15.0	9.854	0.50	711	1013	Acid	8.982							
9	14.3	(9.854)	(0.75)	220	1035	Alkaline	3.654	0.982						
10	15.1	19.708	0.25	865	1014	Acid	13.117	....	1.601	....	22.14			
11	15.0	9.854	0.25	731	1014	Acid	10.031	....	....	0.284	....	2.692	13.332	33.8
12	14.9	9.854	0.10	795	1015	Acid	12.831	2.334	....	....	17.28			

13	15.0	9.854	0.10	650	1016	Acid	9.992							
14	15.0	9.854	0.10	709	1014	Acid	9.674	....	2.834					
15	15.1	9.854	0.10	640	1015	Acid	8.361	....	....	....	28.96			
16	15.0	9.854	0.10	690	1014	Acid	8.904	....	....	....	....	2.462	13.976	33.4
17	15.1	9.854	0.10	704	1013	Acid	9.206	3.368	1.842	0.328	12.86			
After Period.														
18	15.0	9.854	..	692	1014	Acid	9.002							
19	14.9	9.854	..	708	1013	Acid	9.434	....	1.196	....	21.38			
20	15.0	9.854	..	660	1014	Acid	8.831	1.892	....	....	....	1.897	12.320	32.8
21	15.1	9.854	..	670	1015	Acid	9.324	....	1.219	....	16.21			
22	15.0	9.854	..	715	1013	Acid	9.238							
23	15.1	9.854	..	635	1015	Acid	8.597							
24	15.1	9.854	..	693	1014	Acid	8.768	2.805	1.821	0.468	26.74	1.394	7.790	29.1
Daily Averages for each of the Three Periods.														
Fore period (7 days)		9.854	..	661	..	....	9.337	0.646	0.617	0.063	6.66	0.339	1.804	27.1
Tell. oxide period (10 da.)		9.854	0.16	672	..	....	9.475	0.668	0.628	0.061	8.12	0.515	2.731	33.6
After period (7 days)		9.854	..	682	..	....	9.028	0.671	0.605	0.067	9.19	0.470	2.870	30.1

The tables show at a glance that during this experiment tellurium had no material influence on the weight of the animal, that the volume and reaction and specific gravity of the urine were not particularly altered, that the quantities of phosphorus, sulphur and uric acid excreted were uniformly the same, and that the nitrogen elimination was but little affected. The following summary gives the quantitative and the percentage distribution of nitrogen for each period:

Total nitrogen.	Fore period. Grams.	Tellurous oxide period. Grams.	After period. Grams.
Nitrogen of food	68.978	98.059[1]	68.978
Nitrogen of urine	65.359	94.752	63.194
Nitrogen of fæces	2.374 } 68.787	5.154 } 101.138	3.291 } 67.669
Nitrogen of hair	1.054	1.232	1.184
Nitrogen balance	+ 0.191	− 3.079	+ 1.309
Ratio to nitrogen ingested.	Per cent.	Per cent.	Per cent.
Nitrogen of urine	94.8	96.6	91.6
Nitrogen of fæces	3.4	5.3	4.8
Nitrogen of hair	1.5	1.3	1.7
Nitrogen balance	+ 0.3	− 3.2	+ 1.9

[1] Quantity remaining after subtraction of the nitrogen of the vomit. See footnote, p. 110.

possible to make daily detailed analyses of each 24 hours' urine, and Dr. GIES had to be content, in some cases, with results obtained from the urine of several days combined. The totals and daily averages were, of course, in no wise affected by this. Thus, throughout the three periods of the first experiment, the data for phosphorus, sulphur, and uric acid are for urine passed during several days. The figures are recorded on the last day of each separate combination. The dry weight of the fæces is recorded on the days of elimination. The 0.75 gm. of tellurous oxide given on Dec. 9 is not included in the total for the period (1.6 gm.), because practically all of it was ejected in the vomit. See pages 110 and 111 for references to the latter and the variations in quantity of food on Dec. 9 and 10. The average daily weight of hair shed was 1.24 gm. in the fore period (0.150 gm. N), 0.99 gm. in the tellurous oxide period (0.123 gm. N), and 1.35 gm. in the after period (0.169 gm. N).

These results show that in spite of the relatively large doses of tellurous oxide (quantities greater than therapeutic doses for man), given repeatedly during a period of ten days, the animal remained approximately in nutritive equilibrium. They also show that the immediate ingestion of food equal to that vomited, sufficed to restore promptly the balance that had been disturbed on the second day of the oxide period. It should, of course, be remembered, in considering the effect of quantity in this connection, that tellurous oxide is a comparatively insoluble substance — insoluble in water and dilute acids, soluble in dilute alkaline fluids; also that its reduction to the metallic state quickly follows ingestion and that its absorption is therefore comparatively slow and very incomplete. The odor of methyl telluride in the breath proved that some tellurium had been absorbed, but much of the tellurium was eliminated in the fæces in metallic form, a fact which will be referred to again.

The slightly increased elimination of nitrogen during the second period cannot be attributed solely to the influence of tellurium, because of the lack of food on the second day, and the excessive amount of food on the third day of that period. It is very well known that unusual amounts of ingested proteid stimulate nitrogenous catabolism and cause immediate increase in the output of urea; also, that when no food is eaten proteid catabolism, although diminished, still continues. In this experiment we could not well avoid a combination of both circumstances. The animal had been brought into nitrogenous equilibrium. On the second day of the tellurium period, however, when no food was retained, proteid catabolism continued at the expense of the body proteid. On the third day much of this lost proteid was made up from that ingested, but undoubtedly a good proportion of the nitrogen of the double quantity of food on this day was quickly passed into the urine. Nitrogenous equilibrium was probably very soon restored, but the small balance of 3 gms. in favor of excreted nitrogen was doubtless largely due to enforced irregularity in the feeding on the second and third days of the period.

The fæces, also, it will be seen, were not greatly altered chemically, although they were considerably increased in quantity. The percentage of nitrogen rose somewhat during the second period, but this increase was probably due to the greater quantity of mucus eliminated, to which we have already drawn attention, and was not a result of impaired digestion. There seems to have been a slight interference with the absorption of fat, since the quantity of

ether-soluble matter is somewhat increased in the second and third periods. This fact seems to harmonize with the cause assumed for increase in the fæcal nitrogen, for since tellurium is deposited in the mucous membrane of the stomach and intestines, and thereby increases the number of cells and the quantity of mucus thrown into the canal, it can be safely argued, that it may in some measure interfere with absorption. However, this increase in the quantity of ether-soluble matter, in the fæces, like the increase of nitrogen, is so slight that little importance can be attached to it.

We have already called attention to the bluish-black appearance of the fæces after administration of tellurous oxide. The color is due to metallic tellurium present in fairly large proportion. Since only traces of tellurium were present in the urine early in the oxide period, and none could be separated from a little more than 10 gms.

Periods.	Grams.			Per cent.	
	Fæces.	Ether-sol. matter.	Nitrogen.	Ether-sol. matter.	Nitrogen.
Fore	46.67	12.626	2.374	27.1	5.1
Tell. oxide	81.24	27.308	5.154	33.6	6.3
After	64.33	20.110	3.291	30.1	5.1

of hair shed during the same time, it seems very probable that the comparatively small quantity of tellurium which succeeded in getting through the walls of the intestine was finally converted into methyl telluride and that it was all being gradually eliminated in that form through the lungs. The largest proportion left the body in the fæces.[1]

Second Experiment; with Tellurous Oxide.

Although the analytic results of the first experiment indicated that there had been but slight stimulation of catabolism, we felt it desirable to make a second trial with tellurous oxide. In this second experiment we sought to avoid the vomiting which in the first had temporarily upset the equilibrium, while at the same time we aimed to keep the dose as large as possible in order to determine the maximum influences. We used a dog weighing approximately 10.5 kilos. Equilibrium was established in eight days. The diet consisted of

[1] See analytic results, Exp. 1, page 135.

175 gms. of prepared meat (6.121 gms. N), 40 gms. of cracker dust (0.604 gm. N), 30 gms. of lard, and 450 c.c. of water; it contained a total of 6.725 gms. of nitrogen. The experiment lasted three weeks and was divided into three periods of equal length. Throughout the second week tellurous oxide was given as before, in two equal doses averaging 0.21 gm. per diem; and each day there was retained 0.05 gm. more than in the previous experiment. The largest single dose was 0.15 gm., the smallest 0.05 gm.

On the fifth day, when a total of 0.3 gm. was given, the dog ate with reluctance and it was only after considerable coaxing and petting that all was swallowed. Loss of appetite had also been shown, during the previous day, when an equal amount of the oxide had been administered. We assumed, therefore, that increased dosage on the following day would cause vomiting, so the daily quantity given with the food was reduced. It was evident, however, that for that particular time we had administered the maximum quantity that could be borne without toxic manifestation. Loss of appetite was evident to the end of the period in spite of reduced dosage, but appetite quickly returned when the oxide was discontinued. Within an hour after the first dose had been swallowed the garlic odor of the breath, noticed in the previous experiment, was again recognized. It remained in evidence throughout the experiment and for some days thereafter. The languor and sleepiness prominent in the first experiment were not especially noticeable in this. There was no sickness; loss of appetite was the only approach to it.

The urine was not quite as dark in color as before. Albumin, bile pigment, sugar, and abnormal sediment were absent from the urine in all cases. None of the samples of urine gave off sufficient methyl telluride to be detected by the sense of smell. The fæces were little altered, although they acquired the characteristic bluish-black appearance during the oxide period, due, as previously stated, to metallic tellurium. They contained no unusual quantity of mucus; only once was the garlic odor perceived. In this experiment also, the cast-off hair had the usual garlic odor, but we were unable to detect any appreciable quantity of tellurium in the hair shed during the oxide period.

The tables given herewith (pages 118 and 119) present the data of this experiment.[1] They show conclusively, we think, that tellurous

[1] Indoxyl was determined with uniform quantities of urine and reagents so as to make colorimetric observations directly comparable. The dry weight of the

SECOND EXPERIMENT.

Date, 1899. Feb.	Body weight. K.	Food. Nitrogen. Grams.	TeO_2. Grams.	Urine.							Fæces.	
				Vol. c.c.	Sp. gr.	Reaction. litmus.	Indoxyl. Coloration.	Nitrogen. Grams.	Total P_2O_5. Grams.	Total SO_3 Grams.	Dry weight. Grams.	Nitrogen. Grams.
Fore Period.												
1	10.6	6.725	..	580	1016	Acid	Strong	7.002	1.283	0.691		
2	10.6	6.725	..	610	1014	Acid	Strong	7.224	1.341	0.732	11.28	
3	10.5	6.725	..	631	1015	Acid	Weak	7.138	1.076	0.704		
4	10.5	6.725	..	570	1013	Acid	Strong	6.007	0.984	0.477		
5	10.6	6.725	..	450	1016	Alkaline	Strong	5.138	0.863	0.502		
6	10.5	6.725	..	586	1015	Alkaline	Strong	7.021	1.071	0.684	15.71	1.539
7	10.6	6.725	..	498	1014	Acid	Weak	5.378	0.793	0.472		
Tellurous Oxide Period.												
8	10.6	6.725	0.1	564	1014	Acid	Weak	5.982	0.993	0.548		
9	10.5	6.725	0.2	599	1013	Acid	Weak	7.081	1.227	0.682		
10	10.4	6.725	0.3	642	1012	Acid	Strong	7.236	1.364	0.754	14.81	
11	10.4	6.725	0.3	598	1013	Acid	Strong	7.024	1.197	0.707		

12	10.5	6.725	0.3	473	1015	Acid	Weak	5.121	0.762	0.445		
13	10.6	6.725	0.2	584	1014	Alkaline	Strong	6.434	0.889	0.578		
14	10.6	6.725	0.1	557	1016	Alkaline	Strong	6.339	0.946	0.629	10.63	1.647
After Period.												
15	10.5	6.725	..	618	1014	Acid	Strong	7.034	1.313	0.664		
16	10.5	6.725	..	550	1016	Alkaline	Weak	6.228	0.974	0.574		
17	10.4	6.725	..	637	1013	Acid	Strong	7.331	1.287	0.761	13.94	
18	10.6	6.725	..	482	1015	Acid	Strong	5.367	0.705	0.438		
19	10.5	6.725	..	598	1014	Acid	Weak	7.097	1.304	0.681		
20	10.4	6.725	..	590	1013	Alkaline	Strong	7.146	1.106	0.637	14.67	1.832
21	10.5	6.725	..	469	1015	Alkaline	Strong	5.234	0.797	0.481		
Daily averages for each of the Three Periods.												
Fore		6.725	..	561	....	....	....	6.415	1.059	0.609	3.85	0.220
Tellurium		6.725	0.21	574	....	....	....	6.460	1.054	0.620	3.64	0.235
After		6.725	..	563	....	....	....	6.491	1.069	0.605	4.09	0.262

oxide in quantities as large as could well be retained had little metabolic influence that could be measured chemically. Body weight was constant; volume, reaction, and specific gravity of the urine showed little variation, total phosphoric and sulphuric acids were unchanged in quantitative elimination; and nitrogenous excretion was only slightly in excess of ingestion in each of the three periods. The distribution of nitrogen in the excreta is stated in the following summary:

Total nitrogen.	Fore period. Grams.	Tellurous oxide period. Grams.	After period. Grams.
Nitrogen of food	47.075	47.075	47.075
Nitrogen of urine	44.908	45.217	45.437
Nitrogen of fæces	1.539 } 47.341	1.647 } 47.972	1.832 } 48.215
Nitrogen of hair	0.894	1.108	0.946
Nitrogen balance	− 0.266	− 0.897	− 1.140
Ratio to nitrogen ingested.	Per cent.	Per cent.	Per cent.
Nitrogen of urine	95.4	96.1	96.5
Nitrogen of fæces	3.3	3.5	3.9
Nitrogen of hair	1.9	2.4	2.0
Nitrogen balance	− 0.6	− 2.0	− 2.4

These results are in accord with those of the previous experiment. The unimportant excess of excreted nitrogen in each period can hardly be given much significance from any standpoint, as each amount is within the ordinary limits of error in work of this kind.

It is worthy of note that no particular influence on normal putrefactive changes in the intestine was manifested, for indoxyl could be detected in every day's urine. The normal fluctuations were quite noticeable. The indoxyl reactions were obtained most distinctly on or

fæces is recorded on the day of elimination. The nitrogen of the fæces was determined in the combined excreta of each period. The average daily weight of shed hair was: fore period, 1.02 gm. (0.128 gm. N); tellurous oxide period, 1.30 gm. (0.158 gm. N); after period, 1.10 gm. (0.135 N).

about the days of defecation, indications that the formation of indigo bodies was greatest when the matter in the intestines was largest in amount. The fæces collected throughout this experiment showed even less variability than was noticed in the previous experiment. Not only were the quantities eliminated in each period approximately equal, but nitrogen content, also, was practically the same. It may be assumed, therefore, that there was little interference with absorption in this experiment.

This animal seemed to bear the tellurium dosage especially well. At the end of the equilibrium experiment 0.75 gm. of the oxide was given with the usual morning meal. It did not cause vomiting, although a few hours thereafter the odor of methyl telluride in the expired air was almost unbearable, and it remained strong for several weeks. Even languor and sleepiness were not particularly noticeable.

Third Experiment; with Sodium Tellurite and Tellurium Tartrate.

In several preliminary experiments both the tellurite of sodium and the tartrate of tellurium seemed to be more distinctly toxic than tellurous oxide, facts which are doubtless dependent on the greater solubility of the former compounds.[1] The dog weighed 9.8 kilos. Equilibrium was established in four days. The daily food was composed of 160 gms. prepared meat (5.856 gms. N), 40 gms. cracker dust (0.604 gm. N), 30 gms. lard, and 400 c.c. water. The total nitrogen was 6.460 gms. The experiment was carried through four periods, each a week in length. Throughout the second period sodium tellurite was given in meat capsules with the food as before; in the fourth, tellurium tartrate. The third or intermediate period gave the animal time to recover from any influence of the tellurite, and, serving as an "after" as well as a "fore" period, enabled us to note any possible cumulative effect of the dosage.

The largest dose of the tellurite was 0.15 gm. with half the daily quota of food, the smallest 0.05 gm. The greatest amount of tellurium tartrate given with any one meal was 0.025 gm., the smallest 0.0125 gm. On the evening of the sixth day of the sodium tellurite period, the dog ate the usual portion of food only after much persuasion. Loss of appetite was very marked. On the next day,

[1] It should not be forgotten, however, that tellurites are transformed into the hydrated oxide by the acid of the gastric juice. The oxide likewise becomes tellurite in the alkaline liquids of the intestines.

assuming that the limit of dosage had been reached, and wishing to prevent vomiting, the dose was decreased to the smallest quantity of the period. No trouble was experienced with the tellurium tartrate. We were, however, afraid to increase the dose over 0.05 gm., as 0.1 gm. had caused vomiting in another dog. Possibly for this one the dose might have been raised somewhat.

Within half an hour after the ingestion of the first dose of tellurite, the garlic odor of the breath was very noticeable. It continued throughout the whole experiment. On the day the tellurium tartrate was first administered, nothing resulted save an unmistakable increase in the odor. With the exception of the loss of appetite on the sixth day of the tellurite period, and the garlic odor of the breath, there was nothing at any time to indicate that the dog was not normal. The urine showed little variation in color and nothing abnormal could be detected in it. Even the fæces were only a little blackened by metallic tellurium; in all other outward appearances they were perfectly normal. No methyl telluride could be detected at any period in the solid excreta even directly after passage.

The accompanying tables, pages 124 and 125, giving detailed analytic data[1] for this experiment, point to the same general conclusions that were drawn from the first and second experiments. These non-toxic doses induced very little alteration in the course of metabolic events. The weight of the animal fluctuated very little; the volume, specific gravity, and reaction of the urine were practically constant throughout; and the quantity of sulphuric acid excreted was the same in each period. The nitrogen showed little deviation from the normal, although slight stimulation, after dosage, was again indicated. On page 123 are the figures for the distribution of nitrogen in the various excreta, which emphasize the conclusions already drawn.

In this experiment we determined quantitatively the amount of combined sulphuric acid in order to measure more definitely than was the case in the previous experiment the effect of tellurium on intestinal putrefaction. It will be noticed that the normal fluctuations

[1] Nitrogen was determined, every two or three days, in combined urines. (See note, bottom of page 111). Total SO_3 of the urine, and the nitrogen and ether-soluble matter of the fæces, were determined in the excreta for the whole period. Combined SO_3 was determined in the urine passed on the days of elimination and also in the combined urines of each period. Dry weight of fæces is recorded on days of defecation. The average daily amount of cast-off hair varied between 0.77 gm. and 0.89 gm.; the content of nitrogen between 0.099 gm. and 0.115 gm. The dosage appeared to have no influence in this connection.

are here again emphasized and that the combined sulphuric acid was greatest in quantity in the urine on or about the days of defecation. In only one case was the amount of combined sulphuric acid in the urine of the day of defecation less than the average daily output of

Total Nitrogen.	Periods.			
	Normal. Grams.	Sod. tellurite. Grams.	Intermediate. Grams.	Tell. tartrate. Grams.
Nitrogen of food	45.220	45.220	45.220	45.220
Nitrogen of urine	41.878	41.452	40.432	41.300
Nitrogen of fæces	3.126 } 45.757	3.896 } 46.042	3.781 } 44.934	3.812 } 45.916
Nitrogen of hair	0.753	0.694	0.721	0.804
Nitrogen balance	− 0.537	− 0.822	+ 0.286	− 0.696
Ratio to nitrogen ingested.	Per cent.	Per cent.	Per cent.	Per cent.
Nitrogen of urine	92.6	91.7	89.4	91.3
Nitrogen of fæces	6.9	8.6	8.4	8.4
Nitrogen of hair	1.7	1.5	1.6	1.8
Nitrogen balance	− 1.2	− 1.8	+ 0.6	− 1.5

the same for the whole period. The ratios of combined to total sulphuric acid are here summarized; from these it is evident that tellurium, in the quantities and forms administered, had no material influence on intestinal putrefaction.

Periods.	Grams.		Ratio.	Per cent of Total.
	Combined SO_3.	Total SO_3.	Combined to Total.	Combined SO_3.
Normal	0.361	4.461	1 : 12.4	8.1
Sodium tellurite	0.411	4.398	1 : 10.7	9.3
Intermediate	0.461	4.537	1 : 9.8	10.1
Tellurium tartrate	0.427	4.316	1 : 10.1	9.9

THIRD EXPERIMENT.

Date. 1899. April.	Body weight. K.	Food. Nitrogen. Grams.	Urine. Vol. c.c.	Urine. Sp. gr.	Urine. Nitrogen. Grams.	Urine. Total SO_3. Grams.	Urine. Comb. SO_3. Grams.	Fæces. Dry weight. Grams.	Fæces. Nitrogen. Grams.	Fæces. Ether-soluble matter. Grams.	Fæces. Ether-soluble matter. %	
Normal Period.												
18	9.8	6.460	461	1018	....	....	(0.054)	10.80				
19	9.8	6.460	360	1016	11.476							
20	9.7	6.460	503	1018								
21	9.7	6.460	430	1014	12.821	....	(0.059)	22.63				
22	9.7	6.460	435	1014								
23	9.8	6.460	392	1019								
24	9.7	6.460	510	1015	17.581	4.461	(0.061)	21.72	3.126	16.201	29.4	
Sodium Tellurite Period.												Na_2TeO_3. Gram.
25	9.7	6.460	450	1017	....	....	....	....	....	....	....	0.3
26	9.8	6.460	373	1019	11.732	....	(0.068)	13.94	....	....	....	0.1
27	9.6	6.460	535	1019	....	....	....	....	....	....	....	0.1
28	9.6	6.460	304	1019	11.981	....	(0.052)	24.62	....	....	....	0.1
29	9.7	6.460	440	1019	....	....	....	....	....	....	....	0.2
30	9.7	6.460	485	1019	....	....	....	....	....	....	....	0.3
May 1	9.6	6.460	446	1015	17.739	4.398	(0.084)	25.11	3.896	22.581	35.4	0.1

2	9.7	6.460	451	1015							
3	9.8	6.460	370	1019	11.531						
4	9.8	6.460	445	1018	....	....	(0.098)	34.61			
5	9.9	6.460	353	1016	11.764						
6	9.8	6.460	480	1016							
7	9.7	6.460	467	1016	....	....	(0.083)	25.21	3.781	19.878	33.2
8	9.8	6.460	430	1016	17.137	4.537					
Tellurium Tartrate Period.											$Te(C_4H_5O_6)_4$. Gram.
9	9.9	6.460	415	1015	....	....	....	....	....	....	....	0.050
10	9.8	6.460	502	1017	12.003	....	....	....	....	....	....	0.050
11	9.7	6.460	410	1019	....	....	(0.089)	22.18	....	....	....	0.025
12	9.6	6.460	485	1016	12.178	....	....	....	....	....	....	0.025
13	9.6	6.460	406	1018	....	....	(0.071)	15.74	....	....	....	0.050
14	9.7	6.460	471	1017	....	....	....	....	....	....	....	0.025
15	9.7	6.460	448	1019	17.119	4.316	(0.092)	24.41	3.812	18.792	30.1	0.025
Daily averages for each of the Four Periods.												
Normal.		6.460	441	....	5.982	0.637	0.052	7.88	0.447	2.314	29.4	Na_2TeO_3
Sod. Tellurite.		6.460	433	....	5.922	0.628	0.059	9.10	0.557	3.226	35.4	0.171
Intermediate.		6.460	428	....	5.776	0.648	0.066	8.55	0.540	2.840	33.2	$Te(C_4H_5O_6)_4$
Tell. Tartrate.		6.460	448	....	5.900	0.617	0.061	8.90	0.545	2.685	30.1	0.036

The increased quantity of ether-soluble matter in the fæces, recorded in the table of the first experiment, is repeated in this experiment after the administration of the tellurium compounds. The ratio of the fat and nitrogen to the whole quantity of the fæces for each period is shown in the summary:

Periods.	Grams.			Per cent.	
	Fæces.	Ether-sol. matter.	Nitrogen.	Ether-sol. matter.	Nitrogen.
Normal	55.15	16.201	3.126	29.4	5.7
Sod. tellurite	63.67	22.581	3.896	35.4	6.3
Intermediate	59.82	19.878	3.781	33.2	6.3
Tell. tartrate	62.33	18.792	3.812	30.1	6.1

There is seen to be a rise in the quantity of both ether-soluble and nitrogenous matter during the dosage periods; this, though very slight, indicates some interference with absorption, and probably an increase in the quantity of mucus and epithelial cells. The action here may be relatively more marked because the soluble substances would naturally have more decided local action than the insoluble oxide. However, these differences are entirely too slight for more than reasonable guesses.

At the close of the experiment 0.1 gm. of tellurium tartrate given with the usual morning meal caused vomiting in little less than an hour. Two days thereafter 0.5 gm. of the tellurite produced the same effect in three hours. The odor of methyl telluride in the breath was especially strong at the time of vomiting.

Fourth Experiment; with Sodium Tellurate.[1]

With the results of the first three experiments before us it appeared altogether unlikely that non-toxic amounts of tellurates would have a more decided action than that already observed. It

[1] The preparation of tellurates in a pure condition is a most difficult problem. After working several months, with the assistance of the late Dr. Herman A. Loos, Professor Lenher succeeded in making for us 9.5 gms. of almost chemically pure sodium compound. This preparation was recrystallized at least twenty times. Its only impurity was a very small proportion of sodium tellurite. It is probable that commercial tellurates are no purer than this preparation and that their effects, when given as drugs, are modified by the small quantities of tellurite which they contain.

seemed desirable, however, to determine experimentally the influence of sodium tellurate on metabolism, because of the therapeutic employment of this particular compound. The dog used in this concluding experiment weighed 15.5 kilos.[1] The diet consisted of 275 gms. of prepared meat (9.675 gms. N), 50 gms. of cracker dust (0.755 gm. N), 30 gms. of lard and 600 c.c. of water; it contained in all 10.430 gms. of nitrogen. This diet was given for eight days, until the weight of the animal remained constant, when the experiment was begun. It was carried through three periods; the first and third were each a week in length; the second, eight days. During the second the tellurate was given daily with the food in the accustomed way. The largest dose of the tellurate, 1 gm., was given on the last day of the second period with the morning meal. With the first food of the tellurate period 0.5 gm. was given, and none for the rest of the day. The amount regularly administered was 0.25 gm. with each portion of food.

During the night of the third day the dog vomited a little greenish mucus. As this indicated cumulative action no tellurate was given on the fourth day. The vomited mucus was mixed with the food given the next morning. There were no manifestations of illness other than vomiting, and no toxic symptoms were exhibited even after the administration of the unusual dose during the morning of the last day of the tellurate period. Sleepiness, however, was very marked at the end of the second and at the beginning of the third periods. Within a very short time after the first ingestion of tellurate the alliaceous odor of the breath was very marked. It seemed to increase steadily, and was, of course, strongest after the administration of the largest dose; for more than two months thereafter it was still very perceptible.

The urine manifested the customary coloration changes — became more coffee colored with tellurium dosage — but no abnormal constituents could be detected in it, except occasionally a garlic odor.[2] Its reaction was acid throughout and indoxyl could be

[1] Six months previous to this experiment a gastric fistula had been made in this dog for experimentation in other connection. At this time the cannula had not been opened for a little more than a month. The fistula was kept closed throughout each of the three periods. The dog remained in perfectly healthy condition to the end of the experiment.

[2] By an unfortunate oversight we failed to look for tellurium in the urine. After the largest dosage it is probable that the urine did contain the substance. See results in this connection on pages 111 and 135.

FOURTH EXPERIMENT.

Date. 1900. April.	Body weight. K.	Food. Nitrogen. Grams.	Urine. Vol. c.c.	Sp. gr.	Reaction. litmus.	Total solids. Grams.	Total solids. %	Nitrogen. Grams.	Urea. Grams.	Urinary Total Solids. Nitrogen. %	Urinary Total Solids. Urea. %	Fæces. Dry weight. Grams.	Na_2TeO_4. Grams.
Fore Period.													
25	15.5	10.43	485	1025	Acid	28.27	5.83	9.61	20.45	33.99	72.34		
26	15.6	10.43	345	1028	Acid	22.50	6.52	6.69	14.24	29.72	63.29		
27	15.5	10.43	720	1021	Acid	35.23	4.89	10.20	21.71	28.96	61.62	26.7	
28	15.5	10.43	455	1028	Acid	29.68	6.52	9.08	19.31	30.58	65.06		
29	15.6	10.43	635	1021	Acid	31.07	4.89	8.91	18.96	28.68	61.02		
30	15.5	10.43	330	1036	Acid	27.68	8.39	9.86	20.97	35.61	75.76		
May. 1	15.6	10.43	405	1025	Acid	23.59	5.83	9.15	19.46	34.78	74.00	32.7	
Sodium Tellurate Period.													
2	15.7	10.43	395	1026	Acid	23.93	6.05	8.62	18.33	36.00	76.60	....	0.5
3	15.6	10.43	610	1024	Acid	34.11	5.59	11.93	25.38	34.97	74.41	18.3	0.5
4	15.5	10.43	540	1025	Acid	31.45	5.83	12.10	25.75	38.48	81.87	....	0.5
5	15.7	10.43	335	1032	Acid	24.98	7.46	9.40	20.00	26.87	80.07		
6	15.6	10.43	645	1024	Acid	36.07	5.59	10.99	23.40	30.49	64.88	28.1	0.5
7	15.8	10.43	270	1034	Acid	21.39	7.92	8.81	18.75	41.20	87.66	....	0.5
8	15.7	10.43	535	1028	Acid	34.90	6.52	12.45	26.50	35.69	75.93	....	0.5
9	15.6	10.43	445	1034	Acid	35.25	7.92	12.52	26.64	35.52	75.57	31.4	1.0

After Period.													
10	15.7	10.43	380	1031	Acid	28.45	7.22	10.53	22.41	37.02	78.77		
11	15.5	10.43	575	1024	Alkaline	32.15	5.59	10.07	21.43	31.32	66.65	28.3	
12	15.6	10.43	460	1029	Acid	31.08	6.76	10.73	22.84	34.53	73.48		
13	15.6	10.43	550	1022	Alkaline	28.19	5.13	9.60	20.44	34.07	72.50	16.4	
14	15.7	10.43	410	1028	Acid	26.75	6.52	8.68	18.47	32.45	69.05		
15	15.6	10.43	290	1042	Acid	28.38	9.79	10.55	22.45	37.18	79.11		
16	15.5	10.43	590	1029	Acid	39.87	6.76	11.26	23.97	28.26	60.13	21.4	

General Summaries.

Periods. I. Totals.	Nitrogen. a. Food. Grams.	Nitrogen. b. Urine. Grams.	Nitrogen. Ratio. a : b.	Nitrogen. Difference. Grams.	Urine. Vol. c.c.	Urine. Total solids. Grams.	Urine. Total solids. %	Urine. Urea. Grams.	Urine. Urea. % of T. S.	Fæces. Dry weight. Grams.	Na_2TeO_4. Grams.
Fore, 7 days	73.01	63.50	1 : 0.87	+ 9.51	3375	198.02	5.87	135.10	68.2	59.4	
Tellurate, 8 days	83.44	86.82	1 : 1.04	− 3.38	3775	242.08	6.41	184.75	76.3	77.8	4.0
After, 7 days	73.01	71.42	1 : 0.98	+ 1.59	3255	214.87	6.60	152.10	70.7	66.1	
II. Averages. Fore	10.43	9.07	1 : 0.87	+ 1.36	482	28.29	5.87	19.30	68.2	8.5	
Tellurate	10.43	10.85	1 : 1.04	− 0.42	472	30.26	6.41	23.09	76.3	9.7	0.5
After	10.43	10.20	1 : 0.98	+ 0.23	465	30.70	6.60	21.71	66.1	9.4	

detected in each sample. The fæces contained a little more than the normal amount of mucus, during the second and part of the third period, and the bluish-black color of deposited tellurium which had been noticed before was again observed; otherwise there was nothing unusual to be noted.

In this experiment nitrogenous metabolism was measured by the output of nitrogen in the urine only. The nitrogen was determined by the hypobromite method. Urea was calculated from the nitrogen. The accompanying tables summarize the data of this experiment (pages 128 and 129).

Here again the results are essentially a repetition. Body weight as well as the volume, reaction and specific gravity of the urine were unaffected. The total solids of the daily urine were practically the same in each period, but nitrogen (urea) was increased enough to indicate, as in the case of all of our previous experiments, that metabolism had been slightly stimulated. With the exception of the vomiting on the third day and the continuous elimination of methyl telluride in the expired air, there were no visible toxic effects of the tellurate. The dog was particularly sleepy for a short time, as already mentioned, but did not suffer from loss of appetite, a symptom observed in each of the preceding experiments. In fact, the tellurate seemed to be especially devoid of toxicity, for even 1.5 gm. given on an empty stomach with a small piece of meat at the close of the experiment, caused vomiting only after seven hours. The quantitative eliminations of the fæces, it will be seen from the tables, were so constant that it may safely be said that no particular effect was produced on intestinal absorption, except, perhaps, a slightly diminished assimilation of fat.

Review.

In reviewing the results of these metabolism experiments it should be mentioned that the occasional vomiting was quite in accord with the original observations of Hansen (2) and the experience of subsequent workers. The alliaceous odor of the breath after the introduction of tellurium has been observed by all investigators except Rabuteau (3) and Combemale and Dubiquet. Reisert (4), inquiring into the cause of the so-called bismuth breath, found that when men took only 0.000,000,5 gm. of tellurous oxide, in solution, the odor of garlic could be noticed in the breath 75 minutes after-

ward, and that it continued for about 30 hours. Before Wöhler and Dean's[1] and Heeren's[2] observations were made this odor had been attributed to ethyl telluride by Wöhler and his pupils.[3] Heeren assumed that the volatile substance exhaled was in reality the telluride of methyl. Hofmeister (12) has lately proved by chemical means that synthesis of methyl telluride occurs in almost all parts of the body after the introduction of tellurium in any form, and Beyer has found that the process does not take place in the absence of oxygen. Hofmeister has also shown that methyl telluride is formed in worms and crustacea, as well as in dogs and rabbits, and Hofmeister and Czapek and Weil observed similar production after administration of tellurium to frogs. Neither Knop (5) nor Bokorny (11), who have found that small quantities of tellurium compounds exert little or no destructive action on plants, observed this synthesis on the part of vegetable cells.

The very evident languor, sleepiness, and loss of appetite in some of these experiments, first noted by Gmelin (1), were reported by Hansen among the results of experiments on himself, and were observed also by Neusser. The color and odor of the urine and fæces, the increase of mucus, and the presence of tellurium metal, in the latter, confirm previous observations by Hansen, Beyer and Reisert. The latter found that the garlic odor, after ingestion of 0.015 gm. of tellurous oxide, could be perceived in the urine 382 hours; in the sweat, 452 hours; in the fæces, 79 days. In the breath it was still present at the end of 237 days.[4]

Tellurium appears to have had no influence at all on intestinal putrefaction. This result, however, harmonizes with the very recent observations of Scheurlen (14) and Klett (15), who found that the development of various forms of bacteria, for example, Staphylococcus

[1] Wöhler und Dean: Annalen der Chemie und Pharmacie, 1855, xciii, p. 233.

[2] Heeren: Chemisches Centralblatt, n. F., 1861, vi, p. 916.

[3] Wöhler: Annalen der Chemie und Pharmacie, 1840, xxxv, p. 111; *Ibid.*, 1852, lxxxiv, p. 69. Also, Mallet: *Ibid.*, 1851, lxxix, p. 223. Also, Wöhler: Journal für praktische Chemie, 1840, xx, p. 371.

[4] We are greatly indebted to Professor John Marshall for calling our attention to Reisert's work. It seems that subsequent foreign investigators of the behavior of tellurium in the animal body were unaware of Reisert's results. It is probable, however, that Kunkel refers to these results when he says, "The odor (of methyl telluride) has been detected in the fæces of man over two months and in the breath more than a half-year, after the last dose of tellurium." Handbuch der Toxicologie, erste Hälfte, 1899, p. 365.

pyogenes aureus and B. mesentericus vulgatus, was not materially hindered by small proportions of tellurite. Klett observed that the virulence of such bacteria as B. anthracis was not perceptibly decreased by the action of small quantities of the same salt.[1] In all of our experiments much of the ingested tellurium was quickly transformed to the passive metallic state. As a consequence, the proportion of active tellurium in the intestinal contents must have been very slight.

Attention has already been called to the fact that Beyer's brief and imperfect experiment on the excretion of urea after intravenous injection of sodium tellurate was the only previous attempt to deter-

Tellurium compound used.	Nitrogen ingested daily. Grams.			Nitrogen excreted daily. Grams.			Total balance of nitrogen for each period. Grams.		
	Fore.	Dosage.	After.	Fore.	Dosage.	After.	Fore.	Dosage.	After.
1. TeO_2	9.854	10.839	9.854	9.827	11.147	9.667	+0.191	−3.079	+1.309
2. TeO_2	6.725	6.725	6.725	6.763	6.853	6.888	−0.266	−0.897	−1.140
3. Na_2TeO_3	6.460	6.460	6.460	6.537	6.577	6.419	−0.537	−0.822	+0.286
$Te(C_4H_5O_5)_4$	6.460	6.460	..	6.419	6.559	..	+0.286	−0.696	....
4. Na_2TeO_4	10.430	10.430	10.430	9.070	10.850	10.200	+9.510	−3.380	+1.590

The figures for excreted nitrogen in Experiment 4 represent only that eliminated in the urine, so that the corresponding figures under "total balance" represent differences between food and *urine* nitrogen.

mine the metabolic influence of tellurium. He found that the normal amount of urea eliminated in the urine of a healthy dog during three preliminary days was 9.45, 10.41 and 7.62 per cent respectively, an average of 9.16 per cent. After injection of 0.75 gm. of sodium tellurate (0.27 gm. tellurium) into the jugular vein the urea in the urine on five successive days was 1.79, 6.06, 8.50, 7.98, 9.00 per cent, an average of 6.67 per cent. This falling-off in the amount of urea was due, undoubtedly, to the refusal of the dog to eat on the first and second days of the tellurium period, and as Beyer does not give any analytic data regarding the food, it is impossible to attach any

[1] Our attention was first called to the work of Scheurlen and Klett by Dr. P. H. Hiss, to whom we are also indebted for valued suggestions.

special importance to his results in this connection. After the injection of tellurium, albumin and bile pigment were eliminated in the urine for several days. On the first day after injection of tellurate, 0.062 gm. of metallic tellurium was eliminated, on the second 0.081 gm., on the third a trace. More than half the amount injected, therefore, was eliminated through the kidneys.

Our own results with respect to nitrogenous catabolism are shown in the above general summary, page 132.

II. Influence on Digestion and on the Gastro-Intestinal Tract in General.

In our metabolism experiments we noted that vomiting occurred in the first and fourth experiments, soon after ingestion of 0.5 gm. of tellurous oxide and several hours after 0.25 gm. of sodium tellurate had been administered. At times there was loss of appetite and in practically all of the experiments the elimination of mucus in the fæces was increased. We saw, also, that tellurium compounds were reduced in the gastro-intestinal tract, that absorption of fat was diminished and that methyl telluride mingled with the fæcal gases. We have attempted to determine by additional experiments some of the other special influences of compounds of tellurium in the alimentary tract.

Experiments on the Normal Dog.[1]

The following abbreviated reviews present the essential points observed in this connection, together with other facts of interest:

1. **With tellurous oxide.** *1899. Jan. 7.* — Dog weighed 14 kilos. Had received no food during previous 24 hours. Was given a total of 3.5 gms. of TeO_2, with 280 gms. fresh meat, in equal portions — 0.5 gm. TeO_2 in pieces of meat weighing 40 gms. — at 1.30, 3.30, 5.00, 8.15, 9.15, 10.15, and 10.45 P.M. Drank 200 c.c. water with first dose. Odor of methyl telluride in room very strong at 2.30 P.M. At 9.00 animal very sleepy and odor sickening. Continued so throughout experiment. At midnight had neither vomited nor passed urine. *Jan. 8.* — Considerable vomit found in morning; full of undigested pieces of meat, with heavy white and greenish black mucus. Contained much undissolved TeO_2. Was acid to litmus; no free acid. Dog very languid.

[1] The dogs of these experiments were kept in the cage used in the metabolism work. Its arrangement favored separation of solid matter in the vomit from fluid, as well as the separation of fæces from urine. Tests for free acid were made with Günzburg's reagent and tropæolin oo.

12.30 P. M., first food offered — 20 gms. meat with 0.5 gm. TeO_2 — eagerly eaten; water refused. 1.45, all vomited, with much greenish black mucus in strings and lumps. Acid to litmus; no free acid. Contained undissolved TeO_2. 11.30 P. M. (no food or water during interval), vomited again. Mostly clear fluid with much mucus. Acid to litmus; no free acid. *Jan. 9.* — 10 A. M., drank 500 c.c. water; no food given. Ten minutes later 100 c.c. vomited: neutral to litmus. 10.30, 175 c.c. urine eliminated. The urine yellowish green, like diluted bile, though no bile pigments were present. No coagulable proteid. 150 gms. meat at 6 P. M. *Jan. 10.* — 150 gms. meat, 200 c.c. water at 9 A. M. 30 gms. meat with 0.5 gm. TeO_2 at 5.15 P. M. At 8.00, fæces — bluish-black, streaks of blood, much mucus. Urine also, 80 c.c., not as dark in color as on 9th. No coagulable proteid. 8.30, 30 gms. meat with 0.5 gm. TeO_2. *Jan. 11.* — No vomiting since last doses. 11 A. M., refused food and water — none for 26 hrs. Nose very warm and dry. Refused food repeatedly all day. Persisted in sleeping. Fever high at midnight. Dog not easily roused from stupor. *Jan. 12.* — 9 A. M., 100 gms. meat, in several pieces, eaten; vomited in 10 minutes. Solid portion eaten; again quickly thrown up. This occurred three times in half hour. Fluid each time acid to litmus; no free acid. Greenish mucus very abundant. 2 P. M., vomited again; acid to litmus; no free acid. *Jan. 13.* — Ate small quantities of meat and drank water, with increasing appetite throughout day. 300 c.c. urine in morning; not particularly dark. *Jan. 14.* — Recovering rapidly. Odor of methyl telluride undiminished. *Jan. 15.* — 10 A. M., 50 gms. meat in one piece with 1.0 gm. TeO_2 and 200 c.c. H_2O. 12 M., 60 gms. piece with 1.0 gm. TeO_2. 4 P. M., 30 gms. piece with 1.0 gm. TeO_2. Up to midnight no action except increased methyl telluride and stupor. *Jan. 16.* — Vomit found at 9 A. M. — 30 and 40 gms. pieces meat unchanged, with contained TeO_2 in place. Greenish fluid, full of greenish and bluish shreds of mucus. Strongly acid to litmus; no free acid. Urine normal in appearance, 250 c.c. at 9.30. At 12.30 P. M., vomited again. 60 gms. piece meat thrown up, undiminished in size; putrid. Strings of blue mucus half foot in length. Some TeO_2 undissolved. Vomit acid to litmus, none free. 5.00, bloody fæces; bluish-black in places. *Jan. 17.* — 9 A. M., unusually lively. 30 gms. meat, 1.0 gm. TeO_2, 200 c.c. water. 3 P. M., fluid vomit; green and blue mucus; acid to litmus, none free. 5.00, tried to vomit, without success. 5.30, vomited 30 gms. piece meat given at 9 A. M. TeO_2 powder in blue mucus. Acid to litmus; none free. Midnight, 115 c.c. very dark urine. Contained coagulable proteid; no bile pigment.

Jan. 18. — *Post-mortem* (chloroform, 9 A. M.). Methyl telluride from abdominal cavity and separate organs. Blood, liver, lungs, brain, spleen, normal in outward appearance. Gall bladder greatly distended. Alimentary tract lined throughout with greenish and bluish-black layer of metallic tellurium in granules. Small intestines much inflamed. Contents of stomach acid to

litmus; no free acid. Pepsin present. Intestinal contents bluish-black; much mucus. Kidneys very dark, cortical layer black. Urine in bladder very dark; no tellurium in suspension. Walls of bladder normal in appearance.

Analytic results. Qualitative analysis of various parts by method outlined on page 109 gave following results for tellurium: *positive*, liver, blood, stomach, intestines, muscle from back, urine, contents of stomach and of large and small intestines, bile, fæces; *negative*, lungs, spleen, pancreas, brain, heart. The amount in the fæces was surprisingly large, 75 gms. of the desiccated material yielding 0.977 gm. of tellurium — 1.3 per cent of dry substance.[1]

2. **With tellurous oxide.** *1899. Mar. 13.* — Bitch weighed 16 kilos. 9.30 A. M., 1.0 gm. TeO_2, 125 gms. meat, 300 c.c. H_2O. Ten minutes later nearly all vomited; all solid portion licked up at once. At 11.00, large quantity thrown up again; all eaten quickly. This repeated at 3.00, 6.30, 8.45 and 11.15 P. M. Vomit less and less each time; proportion of bluish-black mucus correspondingly greater. Samples of each vomit acid to litmus; no free acid. No haemoglobin in any, but bile pigment in some. Increasing number of bacteria. Each gave good precipitate with $AgNO_3$ and HNO_3 after removal of albuminate and proteose. Kelling's and Uffelmann's [2] tests for lactic acid gave negative results. Urine had usual coffee color. Odor of telluride of methyl very strong soon after first dosage.

3. **With sodium tellurite.** *1899. Apr. 7.* — Bitch weighing 6.2 kilos. 9 A. M., full meal meat, bread, water. 3.30 and 4.30 P. M., 15 gms. meat enclosing 0.1 gm. Na_2TeO_3. 5.30 and 6.30, same quantity meat with 0.25 gm. Na_2TeO_3. At 4.00, methyl telluride very noticeable about cage; more and more intense throughout day. 6.35, vomit — fluid and mucus. Acid to litmus, no free acid. 7.35, more vomit — three pieces of meat given during afternoon thrown up little altered, with parts of fourth. Blue mucous strings. Fluid acid to litmus; none free. 9.30, refused food. Sleepiness pronounced. 10.00, 150 c.c. urine, somewhat darker, otherwise normal.

4. **With tellurium tartrate.** *1899. Apr. 8.* — Same dog used Apr. 7th, 8.30 A. M., urine normal in appearance. 9.30, 100 c.c. water, 15 gms. meat in piece enclosing 0.3 gm. $Te(C_4H_5O_6)_4$. 10.45, same quantity water and meat with 0.43 gm. $Te(C_4H_5O_6)_4$. Methyl telluride stronger an hour after first dosage. Vomit at 11.15, 11.30, 11.40, 11.55 A. M., and 12.10 and 12.35 P. M. Unchanged pieces of meat came up at 11.15 and 11.30 A. M. Much fluid and mucus thereafter. Each vomit acid to litmus, with no free acid. Dog very ill during afternoon; recovered rapidly during evening. At first refused food. 10 P. M., ate largely and eagerly; food retained. 11.30, 125 c.c. normal urine.

5. **With sodium tellurate.** *1900. Apr. 16.* — Dog weighed 7.3 kilos. Good meal night before. 12.30 P. M., 0.5 gm. Na_2TeO_4 with 100 gms. meat,

[1] See quantitative results on page 143.

[2] SIMON: A manual of clinical diagnosis, 1897, pp. 156–157.

two pieces. Methyl telluride very strong within an hour. 5 P. M., 0.5 gm. Na_2TeO_4 in 100 gms. meat, three pieces. Sleepiness very marked, odor unusually strong at 7 P. M. No other marked symptoms. *Apr. 17.* — 9 A. M., odor of methyl telluride in room almost unbearable.[1] No food given. Very sleepy. 10.00, vomited — two pieces meat each weighing nearly 30 gms., with considerable quantity grayish-black mucus. Vomit acid to litmus; none free.

Post-mortem (Chloroform, 11 A. M.). Only pathological conditions noted were inflammation of intestines; bluish-black lining of gastro-intestinal tract due to granular tellurium in epithelium; and methyl telluride from abdominal cavity and organs. No tellurium could be separated from the lungs.

Many of the results in the above experiments confirm observations made in our metabolism experiments and in those of previous investigators, especially Hansen, Rabuteau and Beyer; but particularly striking is the fact that there was never any free acid in any of the mixtures thrown from the stomach. It is quite evident from these experiments that irritation of the gastric mucous membrane is usually very marked, although it required at times a surprisingly large quantity of tellurium compound to cause irritation. The intestines were also much inflamed by tellurium. The mucous cells appeared to be greatly stimulated, judging from the large quantities of mucus secreted. Slight intestinal hemorrhage was also produced, as was occasionally shown by the bloody fæces. The results of each of these experiments seem to combine to prove that tellurium exerts an inhibitory action on the secretion of acid in the stomach. Certainly not enough acid is found to furnish free acid, even when only a small amount of proteid is present there to combine with it. This must be one of the causes of the indigestion repeatedly observed throughout these experiments. It does not seem probable that mere transformation of the small quantity of tellurium compounds administered could account for the disappearance of free acid. We have not recorded, above, the individual results regarding the presence of proteolytic enzyme. Pepsin was contained in active quantity in each particular

[1] A dog of 15 kilos weight which had been perfectly healthy during the six months he was in our charge was chained near the animal on which the experiment was being performed. During the night he vomited twice. This seemed to be due entirely to inhaled methyl telluride. The windows and doors of the room had been closed for the night, so that the telluride accumulated. See personal reference on page 147.

vomit. When an equal amount of 0.2 per cent hydrochloric acid was added, giving distinct blue reaction with congo red, fibrin in relatively large quantity was quickly digested in all samples.

Experiments on a Dog with Gastric Fistula.

In order to test the above conclusion regarding interference with secretion of hydrochloric acid, we conducted on a dog with gastric fistula some experiments designed to give even more direct evidence in this connection. The dog weighed 15.5 kilos. The cannula was put in place, toward the pyloric end, on the 9th of November, 1899, five weeks before the experiments were begun. Entire recovery speedily resulted and the dog seemed to digest normally.[1]

1. **Preliminary control experiments.** — *I. 1899. Dec. 15.* — 12.15 P. M., free acid in stomach contents. 12.45, 150 gms. of meat given in four pieces of equal size. 5.50, free acid in contents. Time from feeding to first appearance of free acid, 5 hr. 55 min.[2]

II. 1899. Dec. 17. — 9.50 A. M., free acid in stomach contents. 10.00, 50 gms. of meat given in one piece. 12.00, free acid detected. Time required for appearance of free acid, 2 hr. 0 min.

2. **Experiments with tellurium compounds.** — *I.* **With tellurous oxide.** — *1899. Dec. 18.* — 9 A. M., free acid in contents of stomach. 9.15, fed 150 gms. of meat in four pieces of equal size, each containing 0.1 gm. TeO_2. 2 P. M., some undissolved TeO_2 in contents. 3.00, vomited small amount of thick mucus. Stomach contents scanty. Was given 25 c.c. H_2O. 4.20, drank 150 c.c. H_2O. 7.00, stomach contents faintly alkaline to litmus. 9.30, still no free acid. Contents neutral to litmus. 9.30, time since ingestion of food, with no free acid, 12 hr. 15 min. At 9.30 P. M., 50 gms. of meat given in one piece with 100 c.c. water. 10.30, free acid. The fresh meat seemed to act as a special stimulant, and in the absence of the oxide, which we assume had been mostly removed, was able to call forth abundant secretion of acid.

II. **With sodium tellurite.** — *1899. Dec. 19.* — 10.30 A. M., no free acid in stomach. Given 50 gms. of meat in single piece with 0.1 gm. Na_2TeO_3. 12.45 P. M., trace of free acid. Interval to appearance of free acid, 2 hr. 15 min.

[1] *Methods.* On the day preceding each experiment the dog was well fed and received all the water it desired. On the day of the experiment only the meat mentioned in the above summaries was fed; no water was given except when specially recorded. About 10–15 c.c. of fluid were taken from the stomach at intervals of from 15 minutes to an hour. Acidity to litmus, congo red, Günzburg's reagent and tropæolin oo was determined qualitatively in each sample withdrawn.

[2] See Chittenden, Mendel, and Jackson: This journal, 1898, i, p. 194. The time until free acid appears is here lengthened, probably because no fluid was ingested. Note, however, the result of our last control experiment.

III. **With tellurium tartrate.** — *1899. Dec. 20.* — 10 A. M., no free acid in contents. 10.15, 150 gms. of meat in four pieces, equal in size, with total of 0.3 gm. $Te(C_4H_5O_6)_4$. 10.15 P. M., still no free acid. 10.30, interval of no free acid, 12 hours. At 10.30, 50 gms. of meat given in one piece. 12.15 A. M., no free acid. Experiment discontinued. These results might indicate that tellurium tartrate has even more decided inhibitory action than the oxide.

IV. **With sodium tellurate.** — *1900. May 25.* — 10 A. M., no free acid in contents. 10.15, 50 gms. of meat in single piece with 0.3 gm. of Na_2TeO_4. 2.15 P. M., first appearance of free acid. First appearance of free acid at the end of 4 hours.

V. **With sodium tellurite.** (Direct continuation of Exp. IV.) — 2.45 P. M., abundance of free acid. 3.00, 100 gms. meat in two pieces, with 0.3 gm. Na_2TeO_3. 10.15, first trace of free acid. First trace of free acid after an interval of 7 hr. 15 min.

Note. — The odor of methyl telluride in the exhalations always became more pronounced an hour or two after the ingestion of the meat containing the tellurium compounds. Frequently bile pigment was detected, with Gmelin's test, in the stomach contents after tellurium dosage, but not at any other time. All of the various samples tested contained pepsin which, after the addition of an equal quantity of 0.2 per cent HCl, showed vigorous digestive action on fibrin shreds. Contents almost always acid to litmus.

3. **Final control experiment.** — *1900. June 1.* — 11.15 A. M., no free acid in contents. 11.30, 50 gms. of meat fed; one piece. 11.45, free acid. Same at 12.00, 12.30 and 1 P. M. Time from feeding till free acid was detected, 15 min.

No.	Meat. gms.	Time of feeding.	First trace free acid.	Time interval.	Conditions.	Average interval.
1 (II)	50	10.00 A.M.	12.00 M.	2 hr. 0 min.	Prelim. control	1 hr. 7 min.
3	50	11.30 A.M.	11.45 A.M.	0 hr. 15 min.	Final control	
1 (I)	150	12.45 P.M.	5.50 P.M.	5 hr. 55 min.	Prelim. control	5 hr. 55 min.
2 (II)	50	10.30 A.M.	12.45 P.M.	2 hr. 15 min.	0.1 gm. Na_2TeO_3	3 hr. 7 min.
2(IV)	50	10.15 A.M.	2.15 P.M.	4 hr. 0 min.	0.3 gm. Na_2TeO_4	
2 (V)	100	2.45 P.M.	10.15 P.M.	7 hr. 15 min.	0.3 gm. Na_2TeO_3	7 hr. 15 min.
2 (I)	150	9.15 A.M.	*	12 hr. 15 min.†	0.4 gm. TeO_2	12 hr. 7 min.§
2(III)	150	10.15 A.M.	*	12 hr. 0 min.†	0.3 gm. $Te(C_4H_5O_6)_4$	

* No free acid when experiment was discontinued. † At least. § Minimum.

Direct comparison of the results, in the preliminary and final "control" experiments with those in which the meat fed contained tellurium, clearly brings out the fact that free acid invariably appeared in shorter time when no tellurium was given. The above summary of these experiments, page 138, in which the results for equal portions of meat are grouped together, shows this, and our data indicate, we think, that the secretion of hydrochloric acid in the gastric juice is markedly inhibited by tellurium compounds.

Influence on Zymolysis.

All evidence in our experiments up to this point, bearing on digestive conditions, appeared to favor the view that tellurium compounds, in the quantities given, have no special inhibitory action on pepsin proteolysis in the presence of free hydrochloric acid. The secretion of pepsin did not seem to be materially affected. When it is recalled, however, that traces of pepsin manifest great proteolytic power under favorable conditions, it cannot be safely inferred, from any results we have presented, that its secretion was not interfered with. In the case of the acid, however, its more definite quantitative relationship to proteolysis in the stomach makes deduction regarding its formation in these experiments much more reliable.

With a view of ascertaining roughly the action of percentages of tellurium compounds, equal to and somewhat higher than those in the stomach throughout the previous experiments, we conducted a few test tube experiments with "pepsin — HCl" and fibrin, and then, incidentally, also determined the effects of similar quantities on ptyalin and trypsin under appropriate artificial conditions.[1] We give our results briefly in summary:

1. Pepsin — HCl, 0.2%.
   I. With sodium tellurite. (Alkaline in reaction to litmus. In quantities above 0.6%, is transformed in great part into hydrated TeO_2, which

[1] *Methods.* I. "Pepsin — HCl" was prepared by dissolving 0.5 gm. of pepsin scales (P. D. & Co., 1–2000) in a litre of 0.2 per cent HCl. II. Neutral solution of trypsin was made by Kühne's method. (Given in Studies from the Yale Laboratory of Physiological Chemistry, vol. i, p. 101.) III. Neutralized, filtered saliva was used in the amylolytic experiments. IV. Proteolysis was determined by the disintegration and disappearance of purified fibrin in shreds; amylolysis on starch paste, 0.5 per cent, with iodine and Fehling's solutions as indicators. The volumes of the digestive mixtures were 15–20 c.c. Time: usually 30 minutes to an hour, at 40° C. In all cases control experiments were made to determine the activity of the enzyme solutions.

is precipitated. Reaction of mixture also becomes alkaline). Digestive action quickly obtained with amounts not over 0.625%. In presence of this quantity some acid is uncombined.

II. With tellurium tartrate (acid). Rapid digestion with as much as 1.25%.[1]

III. With sodium tellurate (containing trace of tellurite; slightly alkaline). Digestion with 1.25%.

2. Trypsin (neutral).
   - I. With sodium tellurite. Rapid digestion in presence of 2.50%.[1]
   - II. With tellurium tartrate. Some digestion in presence of 0.85%.
   - III. With sodium tellurate. Rapid digestion in presence of 2.50%.[1]
3. Ptyalin (neutral).
   - I. With sodium tellurite. No digestion with quantities above 0.02%.
   - II. With tellurium tartrate. No digestion with quantities above 0.02%.
   - III. With sodium tellurate. No digestion with quantities above 0.35%.

It seems quite evident, from these results, that pepsin and trypsin are not destroyed by quantities of tellurium compounds under 0.6 per cent and are active with as much as 1.25 per cent and 2.5 per cent, respectively, of some compounds. Ptyalin appears to be the most sensitive to destructive influence, trypsin least so. The reactions of the compounds appear to influence greatly these results, the tellurate (only very faintly alkaline from admixed tellurite) having the least destructive action. It may be reasonably concluded, then, that interference with digestion in the dog, after dosage with comparatively small amounts, has resulted more from disordered secretion than from direct influence on zymolysis itself.

Effect on Absorption and on the Fæces.

From the experimental data here presented we can draw hardly more than very general deductions regarding influence on absorption. The chief evidence of disturbed absorptive function is given in the figures for ether-soluble matter in the fæces of the first and third metabolism experiments, indicating decreased fat assimilation. During the dosage periods the cells of the villi take up metallic tellurium and their absorbing capacity may therefore be much diminished. The variations in nitrogen content of the fæces shown in the tables of the first three metabolism experiments are too slight to warrant the conclusion that food proteid had accumulated in the intestines. Besides, it has been very evident, in almost all our experiments that the secretion of mucus was considerably increased in the presence of tellurium, and the larger quantity of nitrogen in the fæces after dosage

[1] Effects of larger quantities were not determined.

may have been due entirely to that cause. It is perhaps unwise, however, in the absence of direct experimental evidence, to lay any stress on these points, since the digestive and absorptive changes in the intestines are far too complex, and are influenced by too many interdependent relations, for us to ascribe the increase of ether-soluble matter and nitrogen of the fæces to any one general disturbance, or to consider it a result of any specific abnormality.

Since secretion of acid in the stomach is interfered with, it may be reasonably supposed that secretory inhibition results in the intestines also and that perhaps digestion of fat was retarded for that reason. Certain it is, at all events, that loss of appetite, gastric indigestion. irritant action resulting in vomiting and disturbed secretion of gastric juice, result from sufficient dosage of tellurium; that the mucous cells in the membrane lining the gastro-intestinal tract throw out an abnormal quantity of their product; that excessive doses of tellurium may cause intestinal hemorrhage; that the cells of the mucous membrane reduce tellurium compounds to the metallic state; and that the fæces, somewhat more bulky in the dosage periods, carry off, in the form of the metal, much of the ingested tellurium. Intestinal putrefaction does not seem to be especially influenced, and methyl telluride is formed somewhere in the tract and eliminated in part, at least, per rectum.

III. Effects and Distribution after Subcutaneous Injection.

No effort has previously been made to determine quantitatively the distribution of tellurium, although its presence in almost all parts of the body, after intravenous injections, has been shown quite satisfactorily by histological methods. We give here the toxicological data of one experiment in which tellurium tartrate was injected under the skin, together with the results of some analyses of the glands and tissues.

1. **Injection experiment. With tellurium tartrate.** *1899. April 9.* Bitch weighed 6.2 kilos (same animal had previously been used; in experiments 3 and 4, page 135). 10 A. M., full meal given. 3.30 P. M., 0.25 gm. $Te(C_4H_5O_6)_4$ (5 c.c. of 5 per cent sol.) injected on side, posteriorly. Marked local irritant action. 3.50, very restless. 4.00, tremor in limbs. 4.10, garlic odor very strong. 4.20, tongue and jaws moving continually, as if to get rid of ill-tasting matter. 4.30, 0.2 gm. $Te(C_4H_5O_6)_4$ injected, near same place (4 c.c. of 5 per cent sol.). 4.50, breathing more labored. 5.10, muscles

twitching all over body. 5.30, 1.0 gm. $Te(C_4H_5O_6)_4$ injected, opposite side (5 c.c. of 20 per cent sol.). 6.00, very unsteady. 6.20, movements of tongue and jaws less frequent. 8.30, stupor; aroused with difficulty. 8.45, 90 c.c. urine — coffee colored, containing coagulable proteid and bile pigment; no sugar. (Urine, night before, normal.) 9.15, defecated — very watery. 12.00, midnight, hardly able to stand. Refused food. Senses dulled. Nose cold and moist. *April 10*, 8.30 A. M., nose dry and warm. Unable to rise. 8 P. M., remained in any unnatural position, however uncomfortable. 9.00, arose with difficulty to defecate — diarrhœa. Food refused. 10.15, profound stupor. *April 11.* 9 A. M., odor of telluride remarkably strong. Temperature very much lowered — extremities cold. 10.30, convulsive movements. Unable to rise. 12.15 P. M., breathing slow and deep for several hours. Fæces — watery and bluish-black (color doubtless due to tellurium from ingested compounds in previous experiment). 3.15, no control of movements. 5.10, brownish red vomit, with much mucus. Acid to litmus, none free; contained pepsin. 8.00, unable to move, even with mechanical stimulation. 8.30, reddish black urine, 110 c.c., containing coagulable proteid. 9.15, coma. 9.45, convulsions. 9.50, breathing intermittent. 9.55, convulsions; death.

Post-mortem. 10.15 P. M., garlic odor from abdominal cavity. Blood very black. Not laky. No crystalline forms found in blood, such as Rabuteau described. Kidneys very black in cortical layer. Heavy deposit of metallic tellurium about points of injection, and some pus. Intestines very much inflamed. Gastro-intestinal tract lined with metallic tellurium (from previously ingested compounds). Stomach contents deep red, alkaline; contained pepsin. Liver congested. No other lesions observed. Parts removed for analysis.

These results tend to show that subcutaneous injections of tellurium salts are followed essentially by the general effects noted after intravenous injections, especially by Rabuteau and Czapek and Weil, except that with subcutaneous injections the effects are much more gradual. Particularly noticeable in this experiment were general depression, weakening of the reflexes, increasing stupor, paralysis, coma, and convulsions preceding death from asphyxia.

2. **Distribution of tellurium.** — We determined quantitatively[1] the amounts of tellurium distributed to the various organs of the dog into which the tellurium tartrate had been injected,[2] with results agreeing

[1] By the method outlined on page 109.

[2] This same animal had previously ingested 0.7 gm. Na_2TeO_3 and 0.73 gm. $Te(C_4H_5O_6)_4$, in experiments 3 and 4, page 135. Most of this was vomited, however, and much that remained in the tract finally passed out with the fæces, or was held in the intestinal mucous membrane. The total quantity of $Te(C_4H_5O_6)_4$ injected under the skin was 1.45 gm. — containing approximately 0.31 gm. Te.

in the main with the qualitative conclusions drawn by previous observers. The figures given below show relative distribution, and they indicate that tellurium is readily soluble in the tissue fluids and, as Beyer has demonstrated histologically, may be carried to and deposited in almost all parts of the system:

	Te in mgs.
Muscle and skin about points of injection[1] (300 gms.)	38
Liver	12
Kidneys	9
Blood, clots from heart and large vessels (150 gms.)	8
Bile, 11 c.c.	7
Stomach	5
Urine, 110 c.c. (April 11)	4
Brain	4
Bladder	2
Stomach contents	2
Muscle, from shoulders and fore legs (150 gms.)	trace
Lungs, pancreas, spleen	trace

We see from the above results that the liver and kidneys contained a fairly large proportion of tellurium, and it is obvious that these organs have much to do with its separation from the blood and subsequent elimination. The comparatively large quantities in the urine and bile show this conclusively. In spite of the strong odor of the breath, the lungs contain at any one moment only traces of tellurium.

IV. Elimination of Tellurium.

Tellurium compounds appear to be quickly reduced after they enter the body. In all our feeding experiments the fæces contained much of the bluish-black metal, the walls of the gastro-intestinal tract were lined with reduced tellurium and even the material in the vomit — pieces of meat as well as mucus — showed reducing action by holding tellurium in metallic form. Consequently a great part of ingested tellurium is eliminated in metallic form with the intestinal excrementitious matter. When dosage was excessive, or when tellurium was introduced under the skin, appreciable quantities were eliminated in solution in the urine.[2] When the quantities carried into the stomach were small, only traces of tellurium appeared in the urine — frequently none could be detected. After subcutaneous

[1] Discoloration (bluish black) extended far beyond the limits of the excised tissue, so that much more tellurium was deposited near by.

[2] Identical results were obtained by Hansen, Czapek and Weil and Beyer. Also by Kletzinsky: Wiener medicinische Wochenschrift, 1858, viii, p. 355.

injection we have found tellurium in the urine and in the bile — proof of the elimination of that substance from the body by both the liver and the kidneys. The glandular and tissue cells appear to reduce the bulk of soluble tellurium compounds coming in contact with them and to retain the metal, although, as Hofmeister and Beyer have shown, they form methyl telluride also — probably from the metal.

This reduction takes place very readily, in contact with any protoplasmic substance. We ourselves have observed it when tellurium compounds were brought in contact with fresh meat. Scheurlen and also Klett have lately shown that bacteria reduce tellurite to tellurium and that the bacterial cell is colored by the metal under such conditions, thus furnishing a very satisfactory indicator of reducing power on the part of these organisms. Hansen first referred to this process in explanation of the pigmentation of the glands and the contents of the gastro-intestinal tract. Hofmeister noted that the methyl synthesis and the process of reduction are entirely independent of each other, and that the latter may take place all over the body. Beyer, working by histological methods, observed that granular tellurium was deposited only in form-elements — in nerve and glandular cells, leucocytes and striated muscle particularly. Endothelium, unstriated muscle, nerve and connective tissue fibres, on the other hand, were found to have no affinity for tellurium.

The continuous evolution of methyl telluride in the breath (noted by practically all observers under all circumstances, and a symptom in all our experiments), implies transformation of deposited metal into soluble and diffusible form and subsequent transference to the lungs. This elimination, as we have seen, invariably continues so long after the last dosage of tellurium that *gradual transformation of deposited metal* seems to be a necessary deduction.[1] Tellurium in the form of methyl telluride is thrown from the body, not only by the lungs, but also with the epidermal excretions, in the fæces and intestinal gases, and may, as Neusser has pointed out, give special odor to eructations.

[1] Hofmeister has, in fact, proved this. He injected pulverized, chemically pure metallic tellurium, suspended in 0.7% NaCl solution, into the jugular veins of rabbits. At first there were no special symptoms. After 2–3 days, however, the odor of methyl telluride appeared in the expired air and continued to develop. In this way, also, much of the metal deposited under the skin in our subcutaneous injection experiment must have been slowly transformed (page 142).

V. Personal Experiences.

There are no cases of fatal tellurium poisoning on record, so far as we have been able to ascertain, although comparatively small quantities have been destructive of life in the lower animals. Comparatively few facts have been collected regarding the action of tellurium in the human system. Sir J. Simpson records a case [1] in which a student inadvertently swallowed a dose of tellurium, which was followed by the evolution of such a persistent odor that for the remainder of the session he had to sit apart from his fellow-students.

Berzelius [2] found hydrogen telluride more irritant in its action and more poisonous in effect than the corresponding compound of sulphur. Both he and Kölreuter [3] have reported that the oxides of tellurium, as well as a number of salts of telluric and tellurous acids, have a very unpleasant metallic taste resembling that of compounds of antimony,[4] and that some have a nauseating action and are strongly emetic. Wöhler, at the time of his discovery of ethyl telluride,[5] stated that it is very poisonous. At that time and subsequently, while engaged in his chemical researches on ethyl telluride, Wöhler observed that his sweat and breath took on an odor closely resembling that of the substance he was working with.[6] One night while perspiring very freely, the garlic odor in his sweat became so great that he himself could hardly bear it. It persisted in his breath for weeks. During seven successive days Hansen took a total of 0.34 gm. of potassium tellurite. Unusual sleepiness, oppression in the cardiac region, nausea and abundant salivation were the chief symptoms observed. At the end of the dosage period there was complete loss of appetite. The gastric symptoms did not disappear completely until after a lapse of two weeks. The characteristic odor of the breath continued seven weeks. Hansen was unable to separate any tellurium from his urine. An experiment on his friend Von Röder presented essentially the same results. Heeren [7] states that when

[1] Quoted from Blyth: Poisons, their effects and detection, 1885, p. 559.

[2] Th. Husemann und A. Husemann: Handbuch der Toxikologie, 1862, p. 773.

[3] L. Gmelin: Handbook of Chemistry (Watts), 1850, iv, pp. 398, 399, 402, 403. Also, *Ibid.*, 1856, x, p. 309, and Berzelius: Traité de chimie, 1846, ii, pp. 225, 230.

[4] See foot-note, page 148.

[5] Wöhler: Annalen der Chemie und Pharmacie, 1840, xxxv, p. 112.

[6] Quoted from Hansen's paper. Also referred to by Gorup-Besanez: Lehrbuch der physiologische Chemie, 1878, p. 552.

[7] Heeren: Chemisches Centralblatt, n. F., 1861, vi, p. 916.

compounds of ethyl and methyl tellurides are merely touched with the fingers their characteristic odor is carried to all parts of the body, the breath acquiring it, also, in a few days. In addition to the facts, already referred to in the experience of Reisert,[1] metallic taste, after ingestion of 0.015 of tellurous oxide, was observed in an hour and persisted for three days. We have already alluded to the clinical observations of Neusser, Pohorecki and Combemale and Dubiquet.[2]

We are highly favored in being permitted to present the following statement from Professor Victor Lenher in this connection.

Professor Lenher says, " My work with tellurium was largely from a metallurgical standpoint. I frequently had occasion to make large quantities of tellurium. The oxide is volatilized at high temperatures. In the process of fusion of the metal some of it escaped into the air and a considerable quantity was involuntarily inhaled into the lungs. Inhalation of the volatile tellurous oxide was accompanied by a distinctly metallic taste, and the breath and secretions from the skin quickly took on the characteristic garlic odor. In my own personal experience this disagreeable odor remained for months. In one case it persisted for about a year. When particularly large quantities of the oxide were inhaled, great depression and weakness followed. One day, after having fused metallic tellurium in the open air for several hours, I was so overcome by the influence of the volatile oxide that I lay on my bed to sleep for a little while, intending to arise shortly and resume my work; but I slept soundly for eighteen hours without awakening once during that time. Severe constipation followed the inhalation of the oxide and even purgatives, such as compound cathartic pills and Rochelle salt, failed to move the bowels for several days at a time and occasionally for a week. The inhaled oxide did not diminish intestinal putrefaction. The fæcal odors were stronger than normally and, besides, distinctly garlic. As the tellurium disappeared from the system a return to normal conditions was experienced and the odor of the expired air steadily diminished. A few days after my worst experience I analyzed a large quantity of the urine, but could not detect any tellurium in it. The fæces were not closely examined, but they were not blackened by metallic tellurium. After inhalation of fumes of the oxide I have frequently felt nauseated, although I have never vomited."

We ourselves have had no particularly toxic experiences, although

[1] Pages 130 and 131.

[2] Pages 105 and 131.

the following facts observed by Dr. Gies may not be without some interest: At the close of the first metabolism experiment (see footnote, page 111) Dr. Gies had occasion to make a journey of some length. He was very much surprised to learn that a pronounced alliaceous odor was observed not only in his breath but also in the excretions from the skin. This information was offered independently by several friends. It seems probable, therefore, that some of the tellurium, in the methyl compound breathed out by the dog, was inhaled by him and retained in his system and then was gradually eliminated in the same form. Dr. Gies is certain that he did not at any time come in personal contact with the oxide, but while stooping over the dog to hold the dish containing the weighed food — from five to ten minutes at a time twice a day for over two weeks — he breathed the eliminated telluride in relatively large quantities. These brief intervals of special inhalation were usually followed by drowsiness, and sometimes by nausea. Each symptom was, however, of short duration.

VI. Summary of Conclusions.

Non-toxic doses of tellurium (in the forms of oxide, tellurite, tartrate and tellurate) did not materially affect metabolism in dogs brought to a state of nitrogenous equilibrium even when dosage was continued for a week. These substances appeared to stimulate proteid catabolism only slightly. They increased somewhat the weight of dry matter in the fæces and diminished, in small degree, the absorption of fat. The urine was unaffected in volume, specific gravity and reaction, but became dark brown in color during the dosage periods.

Large doses retarded gastric digestion, induced violent vomiting, loss of appetite and somnolence. They caused, besides, inflammation and disintegration of the mucous membrane of the gastro-intestinal tract and also intestinal hemorrhage.

Introduced under the skin, tellurium (tartrate) caused restlessness, tremor, weakening of the reflexes, somnolence, diarrhœa, paralysis, unconsciousness, stoppage of respiration and death, in convulsions, from asphyxia. At the point of injection much of the tellurium was deposited in metallic form, but it was also distributed in large quantity to most of the organs and tissues.

Methyl telluride invariably appeared in the breath a few minutes after introduction into the system of even very small quantities of tellurium. It persisted for months after the last dosage. The odor

of this substance was also detected in the fæces and urine, about the viscera and in the epidermal excretions.

Secretion of mucus in the stomach and intestines was greatly stimulated by tellurium. Regurgitation of bile into the stomach was a frequent result. Tellurium compounds, even in small proportion, markedly arrested the secretion of acid in the gastric juice.

In the gastro-intestinal tract tellurium compounds were quickly reduced and the metal deposited in great part in, and on, the mucous membrane. Intestinal putrefaction did not appear to be influenced in any degree. The intestinal contents were pigmented by reduced tellurium and much of the ingested substance was eliminated in metallic form in the fæces.

The action of trypsin and pepsin outside the body was not very perceptibly diminished by quantities of tellurium compounds under 0.6 per cent. Zymolysis was almost unaffected in the presence of as much as 1.25 per cent of some of the salts. Ptyalin was more easily affected, even by the faintly alkaline tellurate. Trypsin appeared to be least sensitive to destructive influence, acting rapidly in the presence of even 2.5 per cent of tellurite.

Tellurium was eliminated in metallic form in the fæces; as methyl telluride in the breath, urine, fæces and epidermal excretions; in a soluble form, in small quantity, in the urine and in the bile.

The urine was colored brown to yellowish green after heavy dosage with tellurium compounds, but return to normal coloration was rapid after administration had been discontinued. Albumin and bile pigment, besides tellurium, were the abnormal constituents of the urine, found after subcutaneous injections. Toxic quantities given by the mouth caused the appearance of coagulable proteid but neither bile pigment nor sugar in the urine.

In man tellurous oxide taken into the lungs in fairly large quantity caused nausea, metallic taste, somnolence, depression and constipation. Methyl telluride was excreted in the breath, through the skin and with the fæces. Inhalations of methyl telluride induced sleepiness and nausea and the breath and the excretions from the skin under these circumstances acquired, and retained for a long time, the odor of that substance.

In many respects the action of tellurium in the body is like that of selenium, arsenic and antimony.[1]

[1] CZAPEK and WEIL have come to the same conclusion. It is interesting to note, in this connection, that tellurium is believed by some chemists to be in

VII. Bibliography.

1. Chr. Gmelin.
1824. Versuche über die Wirkungen des Baryts, Strontians, u. s. w., auf den thierischen Organismus. Tübingen, p. 43.

2. Hansen.
1853. Annalen der Chemie und Pharmacie, lxxxvi, p. 208.

3. Rabuteau.
1869. Gazette hebdomadaire de médecine et de chirurgie, xvi, pp. 194, 241.

4. Reisert.
1884. American journal of pharmacy, lvi, p. 177.

5. Knop.
1885. Botanisches Centralblatt, xxii, p. 35.

6. Neusser.
1890. Wiener klinische Wochenschrift, iii, p. 437.

7. Pohorecki.
1891. Jahresbericht über die gesammten Medicin, xxvi, I, p. 398.

8. Combemale et Dubiquet.
1891. Semaine médicale, xi, Annexes, p. 24.

9. Combemale.
1891. Bulletin général de thérapeutique, cxx, p. 14.

10. Czapek und Weil.
1893. Archiv für experimentelle Pathologie und Pharmakologie, xxxii, p. 438.

11. Bokorny.
(*a*) 1893. Chemiker Zeitung, xvii, ii, p. 1598.
(*b*) 1894. *Ibid.*, xviii, ii. p. 1739.

12. Hofmeister.
1894. Archiv für experimentelle Pathologie und Pharmakologie, xxxiii, p. 198.

13. Beyer.
1895. Archiv für Physiologie, p. 225.

14. Scheurlen.
1900. Zeitschrift für Hygiene und Infectionskrankheiten, xxxiii, p. 135.

15. Klett.
1900. Zeitschrift für Hygiene und Infectionskrankheiten, xxxiii, p. 137.

References in which only casual mention of effects of tellurium appear are given in the footnotes throughout this paper, pages 105, 131, 143, 145, and 146.

reality a mixture of elements, containing an antimony, arsenic-like body. Brauner calls one of the presumed constituents of the tellurium complex, *austriacum*, which may be the *dwitellurium* predicted by Mendeléeff. See Brauner: Journal of the Chemical Society (London), Trans., 1889, lv, p. 382, and Grünwald: *Ibid.*, Abstracts, 1890, lviii, p. 434; also, Dictionary of applied chemistry, Thorpe, 1893, iii, under "Tellurium." (See footnote, p. 105.)

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CHEMICAL CHANGES IN THE BODY IN WHICH THE METHYL GROUP MAY BE INVOLVED.

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The writer has read, with much interest and profit, the very valuable résumé of leading facts in our knowledge of the "changes of substances in the organism," written by Dr. J. W. Wainwright, and recently published in this journal (this volume, p. 92). The paper referred to presents a timely and systematic review of many important facts which could be brought together so admirably only by one thoroughly versed in the subject.

For the sake of imparting further historical accuracy to Dr. Wainwright's very acceptable review, which no doubt will serve as a guide to many in the future, the writer would call attention to several slight inaccuracies in the discussion of synthetic changes in which the methyl group is involved.

Dr. Wainwright states (at the bottom of page 96) that we "know three cases in which the methyl group has paired in the body. One is the appearance of methyl tellur*ate* after feeding with telluric acid (Hofmeister). The substance was recognized by the odor, *but not by analysis, none being made.* The glandular organs, and especially the testicles, are able to form a large amount of methyl tellur*ate*. Selenic acid similarly yields methyl selen*ate*."

The author is not prepared to say that methyl tellur*ate* and methyl selen*ate* may not be formed after administrations of the acids—nobody knows, he thinks—but it should be pointed out that no one has yet demonstrated their synthesis in the organism. Compounds *analogous to methyl sulphide*, tellur*ide* and also selen*ide* of methyl, however, are formed in abundance after the entrance of tellurium and selenium compounds into the body and, quite contrary to Dr. Wainwright's statement, it has been shown very definitely, in a chemical way, that such syntheses of these volatile, alliaceous bodies can and do occur.

In his early experiments on dogs with tellurous acid, Gmelin, (1824), on post mortem examination, detected a peculiar garlicky odor on opening the abdominal cavity. Hansen (1853) observed this same odor in the breath of dogs to which potassium tellurite and tellurous acid had been given. The odor was assumed, by Wöhler and his pupils at that time, to be due to ethyl telluride. Various investigators, chiefly Rabuteau (1869), Reisert (1884), Neusser (1890), Czapek and Weil (1893) and Hofmeister (1894), have since confirmed the fact that this peculiar odor may be detected in the breath of animals and man after the administration of tellurium and selenium in various forms (including the metallic), the so-called "bismuth breath" being due to tellurium impurities is bismuth products used medically.

Reisert's investigation of the cause of "bismuth breath," following the therapeutic use of various commercial preparations of bismuth, showed that as little as 0. 000,000,5 gram of tellurous oxide, given in solution to men, was followed by the smell of garlic from the expired air in 75 minutes and that it continued for about 30 hours.

The odor has also been found to proceed from

the secretions of the skin, from the urine and feces and from the blood of poisoned animals; also, from the minced fresh glands, etc., after treatment outside of the body with tellurium or selenium compounds.

Experiments with tellurium compounds already published by the author, and additional ones in progress with selenium, confirm all of these facts.

On the discovery of methyl telluride the resemblance of its odor to the garlicky odor of the breath, etc., after administration of tellurium compounds convinced Wöhler that *methyl* not ethyl telluride, as he had previously assumed, was formed in the body and eliminated from it under such circumstances. This view was at once generally accepted. Hofmeister eventually proved, in a chemical way, the fact of methyl telluride synthesis and also showed satisfactorily the formation of the corresponding methyl selenide on administration of selenium compounds.

Hofmeister's method of detection of methyl telluride was as follows: Sodium tellurate, 0.03-0.06 gram, was injected subcutaneously into dogs and cats. As soon as the garlic odor became evident in the expired air, the latter was passed through saturated solution of iodine in potassium iodide for 20 to 48 hours. The solution decomposed the methyl telluride, retaining each radicle. From it, methyl was separated in the form of methyl sulphide by treatment with sodium sulphide. Tellurium, after evaporation of the solution and treatment with nitric and hydrochloric acids, was precipitated in metallic form with sodium sulphite.

The literature on this subject was reviewed sometime ago by the author: See, "The toxicology of tellurium compounds, with some notes on the therapeutic value of tellurates," *Philadelphia Medical Journal*, 1901, Vol. ii, p. 566.

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23

NOTE ON THE GLYCOSURIA FOLLOWING EXPERIMENTAL INJECTIONS OF ADRENALIN.*

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IT is the object of this communication to call attention to the fact that when a considerable quantity of adrenalin (Takamine) is injected into the peritoneal cavity of a normal dog there follows a rapid and usually considerable excretion of dextrose with the urine. This fact was noted in the course of a research upon the functions of the pancreas, which is being carried on by the writers under the auspices of the Rockefeller Institute for Medical Research.

Apparently the first observations on the influence of the suprarenal gland upon carbohydrate metabolism were those of Blum[1] who found that the urine of animals subcutaneously injected with considerable quantities of an extract of fresh suprarenal gland regularly contained sugar, even when the diet of the animals (chiefly dogs) had been free from carbohydrates. The highest percentage of dextrose observed was 3.8 per cent.; usually the concentration of sugar in the urine was much lower.

The discovery of Blum has recently been confirmed by G. Luelzer[2] and, in this country, by Croftan.[3] The latter writer reaches the conclusion that the glycosuria observed by him in rabbits and dogs depends on the action of a diastatic

*The observations referred to in this paper were made partly in the laboratory of Dr. Herter, partly in the Laboratory of Physiological Chemistry, College of Physicians and Surgeons, Columbia University.

[1] Ueber Nebennierendiabetes, Deutsches Archiv für klinische Medicin, Bd. 71, Heft. 2 and 3, p. 146, 1901.

[2] Zur Frage des Nebennierendiabetes, Berliner klinische Wochenschrift, No. 48, s. 1209, 1901.

[3] Concerning Sugar Forming Ferment in Suprarenal Extract. A Preliminary Report on Suprarenal Glycosuria, American Medicine, Jan. 18, 1902.

ferment in the suprarenal gland which converts the glycogen of the liver into sugar.

The observations made by us on the action of adrenalin in producing glycosuria are strongly opposed to the idea that the suprarenal glycosuria is connected with a diastatic ferment contained in this organ. Further reference will be made to this point.

Two very recent publications[4] relating to the chemistry and pharmacology of adrenalin fail to make any reference to the ability of this preparation to induce glycosuria and it seems fair to infer that this action of adrenalin has not heretofore been noted. The omission is the more noticeable as one of these papers is by Takamine (to whose ingenuity we owe adrenalin) whom one may presume to be fully familiar with the investigations that have been made on the properties of the extract.

The following are brief extracts from the protocols of our experiments:

Experiment I.—A small terrier (about 16 lbs.) received 6 c.c. of a 1-1,000 adrenalin solution (Takamine) in the peritoneal cavity. During the following two hours there were repeated vomiting and great excitement which gave way to prostration and unsteady gait. Urine previous to injection contained no reducing substance. Urine passed six hours after injection contained 6 per cent. of sugar by fermentation (6.11 per cent. by Fehling) and yielded a typical glucozazone. No more urine was secreted. The animal died about twelve hours after the injection, after having had bloody diarrhea.

Autopsy.—Peritoneal cavity contained about 30 c.c. of bloody fluid. Entire gastro-enteric tract, from lower end of esophagus to anus, intensely congested. Mucosa deep purple color, surface denuded of epithelium. Congestion most intense in descending colon and rectum. Pancreas unevenly hemorrhagic and congested throughout. Liver and spleen normal. Kidneys slightly congested. Suprarenals appear slightly congested at junction of cortex and medulla. Tissues preserved in Ohlmacher's fluid. Sections through descending colon show mucosa to be entirely denuded of epithelium in places. Submucosa is seat of numerous and extensive hemorrhages. Pancreas shows considerable congestion, most noticeable in

[4] E. M. Houghton, The Pharmacology of the Suprarenal Gland and a Method of Assaying Its Products, Jour. Amer. Med. Assoc., Jan. 18, 1902, and J. Takamine, The Blood-Pressure Raising Principle of the Suprarenal Gland, Jour. Amer. Med. Assoc., Jan. 18, 1902.

the capillaries close to the islands of Langerhans. Many lobules are the seat of hemorrhage and near such hemorrhages the secreting cells are in all stages of disintegration. Numerous focal necroses involving nearly entire lobules. In some parts the acini are well preserved. Between the necrotic areas and the well preserved acini are zones of acini showing various grades of cell degeneration. In places the cells composing the islands of Langerhans show only slight degenerative alterations of the cell-bodies, the nuclei retaining their normal structure and staining properties. In very many places these cells are markedly altered, the protoplasm of the cell-bodies showing a high degree of granular degenerative change. The cell nuclei have in many instances lost their chromatin, and are barely distinguishable. Many of these nuclei are very pale and some show no coloration by hematoxylin. It is noticeable that in many places the acini about these much damaged cells are comparatively well preserved. The acini that are not necrotic are widely separated and the connective tissue is looser than normal, suggesting the presence of edema. The kidney shows only the signs of acute congestion. The suprarenal structures are unaltered.

Experiment II.—Small dog (about 20 lbs.). Animal bled; 30 c.c. for sugar determination. Urine collected at this time reduces Fehling's very slightly. Soon after bleeding received 6 c.c. adrenalin in peritoneal cavity (1-1,000 solution). Urine passed forty-five minutes later contained an abundance of reducing substance (Fehling's); by fermentation 4.20 per cent. glucose. Vomited several times within a few hours of injection; later had bloody diarrhea. Urine passed during night after injection reduced Fehling's strongly. Urine in bladder twenty-four hours after injection contained 0.4 per cent. glucose. Animal bled for sugar determination, then bled to death.

Autopsy.—Congestion of mucous membrane of stomach, lower jejunum and ileum in patches, intense congestion of greater part of rectum, irregular congestion of colon. Pancreas irregular and finely mottled with pink. Appears normal on section. Kidneys and spleen look normal. Suprarenals look congested in cortex. Blood drawn before injection contained 0.163 per cent. sugar. Blood drawn after injection (when urine contained 0.4 per cent.) contained 0.174 per cent. glucose.

Experiment III.—Large mongrel terrier (about 40 lbs.) received 10 c.c. adrenalin solution (1-1,000) intraperitoneally. Urine drawn within five minutes after injection contained 0.25 per cent. glucose. Urine collected next day did not reduce Fehling's. Animal vomited after injection, but showed no prostration and had no diarrhea. Recovered.

Experiment IV.—Large dog (about 30 lbs.), bled for sugar determination. Urine collected after bleeding free from sugar. Injected intraperitoneally 18 c.c. adrenalin solution (1-1,000) which had been boiled for five minutes. In half an hour animal became violently excited and remained so for more than one hour, howling continually. Vomited several times. Refused food six hours after injection, but drank water. Urine collected four hours after injection contained 3 per cent. sugar by fermentation and reduced Fehling's strongly. Recovered.

Experiment V.—Large dog (about 50 lbs.); urine free from sugar. Received 8 c.c. adrenalin solution (1-1,000) in peritoneum. After remaining quiet about half an hour after injection, vomited and became violently excited for more than one hour. Excitement then gradually wore away. Urine collected four hours after injection contained 9.17 per cent. of glucose and 1.84 per cent. nitrogen. The ratio of nitrogen to dextrose was thus 4.98. Six hours after injection, animal refused meat, but drank water. Day following injection animal appeared in good condition and urine was free from glucose. Recovered.

Experiment VI.—Small dog (about 18 lbs.); urine free from sugar. Received 8 c.c. adrenalin solution (1-1,000) in peritoneal cavity. One hour later urine contained 6.9 per cent. glucose. Eighteen hours after injection urine contained 2.0 per cent. glucose. Forty hours after injection no reducing substance was present in the urine passed at this time. Recovery.

Experiment VII.—Small dog (14 lbs.) received 5 c.c. adrenalin solution (1-1,000) intraperitoneally. Soon vomited. No excitement but some prostration and drowsiness. Urine before injection 0.46 per cent. glucose (Pavy's method); after injection (twenty-one hours later) urine contained 5.26 per cent. glucose.

Experiment VIII.—Large dog (about 40 lbs.). On January 14th given 1 gm. phloridzin under skin. Next day urine, previously free from sugar, contained large quantity of glucose. January

15th received 1 gm. phloridzin under skin. January 16th urine continued to show large quantity of glucose. January 18th urine contained about 1 per cent. glucose. Animal fasted from January 14th to 18th. On evening of January 18th received a moderate allowance of lean meat. Animal then fasted until evening of January 23d. On January 22d, urine being entirely free from reducing substance, the dog was given 10 c.c. adrenalin solution (1-1,000) intraperitoneally. Urine collected four hours after injection failed to reduce Fehling's solution in the slightest degree. Urine passed about twenty hours later reduced Fehling's solution slightly (0.1 per cent. glucose). Soon after last collection animal received 1 gm. phloridzin. Next urine passed contained an abundance of glucose (1.28 per cent. glucose).

Experiment IX.—Dog (24 lbs.) received 7.5 c.c. adrenalin solution (1-1,000) under skin. Urine before injection 0.31 per cent. reducing substance (Pavy); twelve hours after injection 0.47 per cent. reducing substance. After this collection animal received good meal. Eight hours later 10 c.c. adrenalin (1-1,000) in peritoneal cavity. Four hours later urine contained 5 per cent. glucose.

Experiment X.—Dog (30 lbs.) received 10 c.c. adrenalin solution (1-1,000) under skin. Urine before injection 0.48 p er cent. reducing substance (Pavy). First collection after injection 0.66 per cent., second collection 0.52 per cent. reducing substance.

Experiment XI.—Dog (50 lbs.) received 15 c.c. adrenalin solution (1-1,000). Urine before injection contained 0.33 per cent. reducing substance (Pavy); second collection, 0.57 per cent.; third collection, 0.42 per cent.; fourth collection, 0.46 per cent.; fifth collection, 0.44 per cent. reducing substance. Second, third, fourth and fifth collections were made *after* injection.

The observations here recorded show that the intraperitoneal injection of adrenalin solution (1-1,000) in doses varying from 6 to 10 c.c. was in each instance followed by the appearance of glucose in the urine.[5] In one instance the percentage reached 9.17 per cent.; in another it was as low

[5] Since the foregoing was written an observation has been made regarding the local application of adrenalin solution to the pancreas. Dog, previously used in Experiment V., after his recovery and the disappearance of glucose from the urine, was etherized and the abdominal cavity opened. Pancreas exposed and 1 c.c. of adrenalin solution (1-1000) plus 1 c.c. of water applied to its surface by means of a soft brush. After a slight immediate blanching of the gland, congestion followed simultaneously with the appearance of glucose in the urine, ten minutes after application of the adrenalin solution.

as 0.25 per cent. In one instance the sugar appeared in the urine in less than five minutes. Two of the dogs died. All vomited and showed more or less violent excitement after the injection. Those which recovered showed some degree of prostration twenty-four hours after the injection. With two exceptions the dogs used had been on a diet of lean meat previous to the injections.

Experiment VIII. appears of special interest, because an effort was made greatly to reduce the store of carbohydrate material by means of injections of phloridzin together with deprivation of food. It is noticeable that urine collected four hours after the injection did not contain any sugar, although glucose subsequently appeared in very small amount.

Another feature of interest is the fact that an abundant excretion of glucose followed the injection of an adrenalin solution which had been boiled for five minutes. We should expect any diastatic ferment contained in the extract to be destroyed by this treatment. Indeed the method described by Takamine[6] for the preparation of adrenalin renders it most unlikely that any diastatic ferment would resist the injurious action of the heat employed in the course of the process for the purpose of getting rid of albuminoid substances. To accomplish this involves an exposure to 90° to 95° C. for a period of one hour.

It may also be pointed out that adrenalin can be added to a solution of glycogen and kept in the incubator for twenty-four hours without any conversion of glycogen into sugar. These different considerations show that there is no reason for attributing the glycosuria from adrenalin to the presence of a diastatic ferment. There is also no satisfactory ground for referring to a diastatic ferment in the suprarenal body the glycosuria caused by any extract of this gland.

Attention is directed to the observation that the dogs which received adrenalin subcutaneously showed only a slight increase in the reducing substance of the urine. With doses of equal size these results contrast sharply with the glycosuria following intraperitoneal injections.

The alterations noted in the intestine and pancreas in Experiment I. are remarkable. They indicate that the suprarenal extract is capable of inducing changes of a highly destructive character in these parts. How these alterations are brought

[6] Loc. cit.

about we shall not undertake to discuss here, as it is our intention to speak of these lesions more fully in another connection.

While we do not at present care to express an opinion as to the relations between the glycosuria caused by adrenalin and the lesions observed in the pancreas, it may be stated that we have already made observations which suggest that this glycosuria is in reality of pancreatic origin. It is not, however, maintained that advanced pancreatic lesions like those to which we have referred are essential to the glycosuria in question.

Among the topics which we reserve for future discussion in connection with adrenalin glycosuria or diabetes are the glycogen content of the liver and muscles and the sugar content of the blood.

We feel justified in emphasizing the following facts:

1. Adrenalin given intraperitoneally is capable of inducing a marked glycosuria, in which the percentage of sugar may reach 9.17 per cent., and the ratio of nitrogen and dextrose 4.98.

2. Adrenalin glycosuria is not dependent on the presence of a diastatic ferment stored or formed by the suprarenal gland.

3. Adrenalin injections are sometimes followed by destructive lesions of the gastro-enteric tract and pancreas.

4. After a fatal dose of adrenalin the cells composing the islands of Langerhans were found to be the seat of granular degeneration, very pronounced in some places. The nuclei of many of these cells showed extensive loss of chromatin substance. In some parts of the pancreas the cells of the islands of Langerhans were much more injured than the surrounding cells of the secreting acini.

6. With equal doses of adrenalin the intraperitoneal injections proved much more efficient in the production of glycosuria than injections under the skin.

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STUDIES ON THE INFLUENCE OF ARTIFICIAL RESPIRATION UPON STRYCHNINE SPASMS AND RESPIRATORY MOVEMENTS.

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I. HISTORICAL.

PREVIOUS to the discovery of the effect of artificial respiration upon strychnine convulsions, the observation was made by Meissner and Richter (1) that artificial respiration in curarized animals will prevent the outbreak of strychnine convulsions even after the paralyzing influence of curare has worn off. These authors did not ascribe this favorable result to the effect of artificial respiration, but assumed that, during the period of rest enforced by curare, the strychnine was partly eliminated from the body and partly neutralized within the body.

Leube (2), however, came to a different conclusion. Under the direction of I. Rosenthal, Leube studied the alleged immunity of the chicken to strychnine. He found that if artificial respiration be instituted during a strychnine tetanus, the tetanus will soon give way. If the dose of strychnine be too large, or the artificial respiration last only a short time, the convulsions may return.

Uspensky (3), also working under the direction of Rosenthal, a year later studied the influence of artificial respiration upon the spasms brought on by other poisons. He found that the convulsions which

followed poisoning with brucin, thebain or caffein may be inhibited by artificial respiration, but that artificial respiration has no influence upon convulsions following poisoning with nicotin or picrotoxin. The poisons of the latter group, though capable of producing spasms, do not increase reflex irritability, while those poisons, the convulsions of which are affected by artificial respiration, have the common characteristic that they do increase reflex irritability. It appears evident, therefore, that artificial respiration inhibits only such spasms as are of reflex origin.

From the results of the preceding experiments, Rosenthal (4) concluded that artificial respiration exerts its influence upon the spasms by means of the increased oxygenation of the blood. He compared this influence with the effects which artificial respiration exerts upon the mechanism of respiration itself, in the production of apnœa. In both cases the oxygen reduces the irritability of the central organs; in respiration it is the natural irritability of the respiratory centre in the medulla oblongata, whereas in strychnine poisoning it is the exaggerated irritability of the spinal cord.[1]

Shortly after the experiments by Rosenthal and his pupils had been published, Schiff (5) obtained essentially the same results. Schiff observed, also, that after prolonged artificial respiration a few animals survived very large doses of strychnine.

During the succeeding interval a few publications have dealt with the facts and views presented by Rosenthal and his pupils. There is one assertion on record which is in contradiction to previous statements of fact. This was made by Rossbach and Jochelsohn (7) in a brief preliminary communication, which was never supplemented by a full publication of their experiments. They claim that artificial respiration has no soothing influence whatsoever upon strychnine spasms. These observers make additional statements in this connection which are not in conformity with the general experience, but which need not be discussed here. All other investigators confirm, unreservedly, the fact that artificial respiration exerts an in-

[1] According to this view the favorable results in the experiments of MEISSNER and RICHTER were due solely to the artificial respiration. We should like to call attention, however, to the experiments of CH. RICHET (6) in this connection. RICHET found that after poisoning with large doses of strychnine, the life of the animal is greatly prolonged, if, in addition to the artificial respiration, curare is also administered. RICHET makes no reference to the work of either MEISSNER and RICHTER, or to that of ROSENTHAL and his pupils.

hibitory influence upon the strychnine convulsions. There is, however, a divergence of opinion regarding the nature of this inhibitory influence.

Ebner (8) and Buchheim (9) stated that they were able to induce the same soothing effect by simple movements of the body and extremities of the animal, and denied, therefore, that oxygenation has anything to do with the favorable action. They believe that the muscular movements are the favorable factors in the relaxation of the spasms. L. Pauschinger (10), however, working under Rosenthal's direction, could easily dismiss this contention by showing that the authors simply employed the now well-known Schultze's method of instituting artificial respiration without opening the trachea.

Brown-Séquard (11), after confirming the fact that the convulsions may be relieved by artificial respiration, denied that the favorable effect is due, as Rosenthal believed, to a greater charging of the blood with oxygen. He was of the opinion that artificial respiration causes a mechanical stimulation of the nerves of the lungs, thorax, and diaphragm, and thus affects an inhibition of the reflex centres.

The statements of Brown-Séquard were contradicted by Filehne (12). Later we shall have occasion to return to the works of both these observers.

Rosenthal's view was supported by Ananoff (13), who, in a brief communication, reported that animals breathing pure oxygen show a greater resistance to the effects of strychnine.

From 1878 to 1900 there is no publication to be found bearing on this subject. In the last-named year this question was studied by Osterwald (14) in the Pharmacological Institute of Göttingen. Osterwald, like Ananoff, put animals under glass bell jars through which a stream of oxygen was conducted. Experiments with mice did not yield striking results, but a few positive results with guinea-pigs led Osterwald to the unreserved support of the opinion that the favorable influence of artificial respiration is due to the greater introduction of oxygen into the blood.

Similar experiments were made last year by Von Czyhlarz (15) with guinea-pigs as well as with rabbits. His experimental results may be more appropriately discussed farther on.

The present status of this subject is, then, as follows: It is now a well-established fact that artificial respiration may prevent the outbreak of convulsions due to strychnine poisoning, or may inhibit

them if already present, provided the dose of strychnine be not too large. The striking feature of its action is the perfect relaxation of the convulsed muscles, the absence of any muscular rigidity or any kind of tremor.[1] Artificial respiration here, apparently, inhibits the artificial increase of reflex-irritability. It is now the consensus of opinion that this inhibition is produced by increased introduction of oxygen into the blood, and that the mechanical effect of the expansion of the lungs, suggested by Brown-Séquard, has no share in the result.

This position, did not appear to us to be entirely satisfactory, for the following reasons: the soothing influence of artificial respiration upon the increased reflex-irritability due to strychnine is apparently identical with its soothing influence upon respiration itself, *i. e.*, with the production of apnœa.[2] We have already stated above that Rosenthal, who may be said to be the discoverer of these phenomena, looked upon both as processes of identical character — the inhibition of the normal reflex-irritability in one and inhibition of the increased reflex-irritability in the other. As regards the causation of apnœa it now seems to be a settled conviction that this state can be brought about by hyperoxygenation as well as by the mechanical distention of the lungs. Why, then, should it be different with the inhibitory effect of artificial respiration upon the strychnine spasms?

Furthermore, the evidence upon which the present prevailing view is based does not appear to us to be entirely conclusive. The chief points in the evidence are: 1. Filehne's work in disproving Brown-Séquard's claims of the disappearance of the effect of artificial respiration after section of the cord or the vagi; 2. Ananoff's,

[1] Some writers, when speaking of the favorable effect of artificial respiration, mean simply that it prolongs life. Life can be prolonged by artificial respiration, however, even if the administered strychnine dose is very large: but then the tonic and clonic convulsions continue even during the most energetic artificial respiration.

[2] The inhibitory effect of artificial respiration upon the complex mechanism of respiration may show itself in several ways: 1. The animal stops its normal abdominal and thoracic respiratory movements. 2. The concomitant respiratory movements of mouth and nose stop during artificial respiration. 3. All respiratory movements remain quiet for some time immediately after discontinuation of the artificial respiration. Usually only the last form of inhibition is termed apnœa. It is obvious, however, that the arrest of the respiratory movements during artificial respiration also belongs to the inhibition phenomena, and ought to be included in the term apnœa.

Osterwald's, and Von Czyhlarz's experiments in producing the same favorable effect by simple normal inhalation of pure oxygen.

Considering the last line of evidence first, we have to exclude at the outset the testimony of Ananoff. In his short communication Ananoff speaks only of artificial respiration prolonging life, and does not mention the absence of spasms during the process which, as remarked above, is the essential point.

Osterwald's successful experiments were made on guinea-pigs (animals which are very resistant to strychnine), and were few in number. These strictures appear the more important when we read them in the light of the results reported by Von Czyhlarz. This last-named observer made nine experiments with guinea-pigs. In each experiment one animal inhaled pure oxygen and the other (control) inhaled air. In four of these experiments both animals had only marked hyperæsthesia. In one both animals had tetanus and survived. In the remaining four experiments the oxygen animals had marked hyperæsthesia, whereas the control animals had non-fatal convulsions. In the majority of the experiments, therefore, there were hardly any differences between the oxygen-breathing animals and the control animals, while the differences observed in the minority of the experiments were only of a minor character.

Von Czyhlarz's experiments on rabbits are still more instructive. Here he records eight experiments. In three experiments both animals had non-fatal tetani. In two both had fatal tetani. In one the oxygen animal had a fatal tetanus, and the control survived. In the remaining two experiments the oxygen animals died, while the controls survived their tetani. We fail to see in any of these experiments with rabbits even the shadow of proof that the inhalation of oxygen can suppress in these animals the increased reflex-irritability due to strychnine poisoning. Now, all the successful experiments ever made with artificial respiration were upon rabbits! If, however, it be admitted that the results of the above experiments with pure oxygen do prove that the oxygenation of the blood can neutralize to some degree the effect of strychnine, they surely do not prove that the mechanical distention of the lungs has no share in the effects produced by the classical method of artificial respiration.

There remains the work of Filehne, which was conducted in contravention of the claims put forward by Brown-Séquard. The latter observer, as stated above, was of the opinion that the arrest of respiratory movements of the animal (apnœa), as well as the arrest

of the spasms in strychnine poisoning, both of which artificial respiration is capable of effecting, are not due to hyperoxygenation of the blood. The arrest in each case was attributed to the mechanical irritation of the branches of the vagus, the phrenic "or other diaphragmatic nerves," caused by the forced insufflation of air into the lungs. In support of his view Brown-Séquard states that transverse section of the spinal cord above the origin of the phrenic nerves or below their origin, or even section of the vagi, removes the arresting influence which artificial respiration exerts upon the respiratory movements.

In contravention of these statements Filehne reports that he tested these claims in a series of experiments and could not confirm them. An analysis of Filehne's experiments reveals the fact, however, that no experiment was made in which he studied the arrest of strychnine spasms by artificial respiration in animals whose spinal cord was cut. His attempts in this line were confined to the demonstration of the presence of apnœa after the severance of the cord. Even in these he succeeded in cutting the cervical cord in only one experiment. In a few other experiments he tried to crush the cord in young animals by forcibly constricting the cervical column with a string. The crudity of such a method hardly inspires confidence in the results attained by it.

Filehne further records a few experiments in which artificial respiration arrested strychnine spasms after cutting the vagi. These experiments, however, as was pointed out by Filehne himself, seem also to demonstrate that the cutting of the vagi visibly impairs the favorable effect of the artificial respiration.

We see, therefore, that the experiments to show the effect of inhalation of pure oxygen are still far from being decisively in favor of the exclusive oxygen theory. We find, further, that Filehne's work cannot be considered a sufficient refutation of Brown-Séquard's mechanical theory. Apparently, much more work must be done before the questions raised here can be satisfactorily answered.

II. Our Own Experiments with Sections of Cord and Vagi.

From the above analysis it is evident that the claims of Brown-Séquard have not yet been properly tested, and that they deserve, therefore, to be investigated anew.

Brown-Séquard believed that the insufflation of air into the lungs

irritates the endings of the vagus as well as of the phrenic and "other diaphragmatic nerves," whatever the latter may be. He might as well have said, also, that the sensory nerves of the thorax wall and the pleura might be stimulated by the rhythmical pressure of the artificial respiration. A more suggestive conjecture would be that the rhythmical pressure upon the contents below the diaphragm irritates the splanchnic nerves. We now know that stimulation of the central ends of the splanchnic nerves causes inhibition of inspiration (16). Experiments which exclude only one set of nerves, while the other paths of innervation remain intact, afford inconclusive evidence that the mechanical irritation of the nerves has no share in the inhibiting effect of artificial respiration. Our experiments were therefore directed in the first place toward the study of the action of artificial respiration on animals in which the vagi were cut, and, at the same time, the spinal cord severed at one place or another.

General method. — The experiments were made on rabbits, which were kept stretched on a holder, and were under ether anæsthesia during the operations. The artificial respiration was administered by bellows through a tracheal tube. The bellows were fastened to the table on which we operated, and were manipulated by hand. An average of thirty uniform strokes per minute was maintained, which caused a pressure rarely exceeding 36 mm. Hg. Each stroke with the bellows caused a distinct jar of the table upon which the animal was resting, a fact of importance in our experiments. We employed strychnine nitrate. An extensive experience has taught us that white rabbits are more sensitive to strychnine than colored ones. We have found that 0.45 mgm. of strychnine nitrate per kilo is a surely toxic dose for a white rabbit, and 0.5 mgm. for a gray one. Although this knowledge might have sufficed, we employed controls in almost every experiment. While our main object was the study of the influence of artificial respiration upon the spasms of strychnine, we also made note of the relation of artificial respiration to apnœa under these conditions.

Abbreviated protocols of our various experiments are given below:

Experiment I. — Gray and white male rabbit, 1920 grams. Tracheotomy.

5.30 P. M. Cord cut between third and fourth vertebra.

5.34. Strychnine injected, 0.6 mgm. per kilo.

5.35. Artificial respiration started, 30 to 35 mm. pressure.

Experiment I — (*continued*).

5.37. Both vagi cut.

6.07. Artificial respiration discontinued. Animal was watched for ten minutes longer, and then was removed from board. During the forty-seven minutes after the injection of the fatal dose of strychnine, the animal did not show even any hyperæsthesia due to strychnine, although the table was jarred, the rabbit untied and its paws squeezed in testing for reflexes of the paralyzed hind limbs, and the animal *even removed from the table.*

With the dose administered in Experiment I a normal animal would have succumbed to a fatal tetanus in less than thirty minutes! The inhibitory effect of the artificial respiration was distinctly manifest, although the nervous paths of the vagi and the splanchnici were cut off. However, during the entire period of artificial respiration, there was in this experiment no full suppression of the animal's own breathing. Further, the concomitant respiratory movements of mouth and nose continued, and became very pronounced after the vagi were cut. There was no sign of apnœa after stopping the artificial respiration. In short, artificial respiration produced no apnœa. Possibly the artificial respiration with only 30 to 35 mm. pressure was not strong enough to cause apnœa in this large animal. But it remains a noteworthy fact that in an animal in which the paths through the vagi and splanchnici were blocked, a certain degree of artificial respiration was sufficient to influence the strychnine spasms, but not to cause apnœa!

Later the same animal was given another injection of strychnine — 0.5 mgm. per kilo. It had distinct convulsions after sixteen minutes. The noteworthy fact was observed that the convulsions did not appear simultaneously in the anterior and the posterior parts of the animal, but occurred in the part above the section of the cord usually before the part below. Subsequently, when the animal recovered from these convulsions, it was killed by asphyxia. It again had convulsions, which appeared in the hind part later than in the front, both sets of convulsions apparently continuing independently of one another.

Experiment II. — Gray rabbit, 1030 grams. Tracheotomy.

5.37 P. M. Cord cut opposite third dorsal vertebra.

5.42. Injected strychnine, 0.67 mgm. per kilo.

5.44. Artificial respiration started.

5.46. Artificial respiration slackened, signs of convulsive movements appeared. Artificial respiration immediately increased, perfect rest again.

Experiment II — (*continued*).

6.00. Both vagi cut, no independent respirations, but concomitant breathing appears and remains throughout artificial respiration.

6.08. Artificial respiration stopped, soon vibration in upper part, and gasps.

6.09. Artificial respiration resumed, followed by rest again.

6.14. Artificial respiration discontinued, apnœa for a few seconds.

6.15. Convulsions in front parts, not in hind parts.

6.16. Convulsion in hind legs, none in front; soon, however, opisthotonus and death.

In this experiment, with a still larger dose of strychnine, the artificial respiration could not abolish the convulsions permanently, but while it was continued, arrested them for thirty-three minutes, the animal being perfectly relaxed, and even without hyperæsthesia during the entire period. In this smaller animal artificial respiration suppressed the independent respirations of the animal, and even caused a very brief period of apnœa, but it had no effect upon the concomitant respiratory movements of mouth and nose after the vagi were cut.

Experiment III. — White rabbit, 1400 grams. Tracheotomy.

5.03 P. M. Cervical cord cut opposite fifth vertebra, paralysis of hind and fore legs; no voluntary breathing. Artificial respiration begun.

5.11. Strychnine injected 0.6 mgm. per kilo.

5.31. While handled, slight and brief spasms (?).

5.33. Both vagi cut, gasping and other concomitant respiratory movements cannot be suppressed; pinching of any part brings out a tetanic convulsion confined to that part and lasting only as long as the part is handled; reflexes.

5.45. Artificial respiration discontinued, brief apnœa, then attempts to breathe; convulsions in upper part alone, later in lower part alone.

5.46. Rabbit dead.

In white rabbits 0.6 mgm. strychnine per kilo is a rapidly fatal dose. For thirty-five minutes, while the artificial respiration lasted, there were no real convulsions, but throughout the entire experiment there was a marked reflex hyperæsthesia, upon which the artificial respiration had apparently only a moderate inhibiting influence. After the vagi were cut the artificial respiration could not longer arrest the strong concomitant respiratory movements.

Experiment IV a. — Gray rabbit, 1840 grams. Tracheotomy.

5.17 P. M. Cord cut opposite fifth cervical vertebra. Rabbit collapsed, no voluntary respiration, and only faint heart-beat. Artificial respiration, elevation of rear end of rabbit holder, and compression of abdomen.

5.46. Animal fully recovered.

6.01. Strychnine injected (0.06 mgm. per kilo strychnine sulphate + 0.06 per kilo strychnine nitrate).

6.33. Both vagi cut. Extremities squeezed or pulled, board hit, table thumped, but no convulsions, and even no hyperæsthesia. Independent voluntary respiration continually present.

6.41. Artificial respiration discontinued, no apnœa, breathes well.

6.46 to 6.49. Short tetanic convulsions either in front extremities alone, with legs upward, or in the four extremities with front legs downward.

6.50. Tetanus, opisthotonus, and death.

Experiment IV b. — Control, gray rabbit, 1760 grams.

4.44 P. M. Cord cut between third and fourth dorsal vertebræ.

6.03. Injected strychnine (0.06 mgm. per kilo strychnine nitrate + 0.06 per kilo strychnine sulphate).

6.30. On striking table tetanus in all parts at once, opisthotonus and death.

Although the animal in Experiment IV a received a fatal dose of strychnine and was subjected to all sorts of irritations, it manifested neither convulsions nor hyperæsthesia during the entire time it received artificial respiration. The control animal, Experiment IV b, on the other hand, had a fatal tetanus when the table was struck, twenty-seven minutes after receiving the strychnine. Six minutes after discontinuance of artificial respiration the strychnine poisoning became manifest also in Experiment IV a. The artificial respiration apparently only inhibited an increase of reflex-irritability but did not destroy the poison in the body; neither was the strychnine sufficiently eliminated from the body during the period of artificial respiration to prevent tetanus.

In this experiment artificial respiration did not suppress the voluntary breathing, nor did it produce any apnœa after its discontinuance.

Experiment V a. — Gray and white rabbit, 1240 grams. Tracheotomy. Artificial respiration for three minutes, voluntary and concomitant breathing suppressed. Artificial respiration stopped, apnœa only two seconds. Artificial respiration resumed, voluntary and concomitant breathing suppressed in one minute. Artificial respiration stopped after two minutes, apnœa eight seconds.

Experiment V a — (*continued*).

5.45 P. M. Cervical cord cut between fifth and sixth vertebræ, animal in good condition. Artificial respiration resumed, voluntary and concomitant respiration suppressed only after seven minutes. Artificial respiration stopped, apnœa eight seconds. Artificial respiration immediately begun again, no voluntary and concomitant movements.

5.57. Strychnine nitrate injected, 0.65 mgm. per kilo.

6.07. Vagi cut; voluntary and labored concomitant breathing set in, each suppressed after seven minutes.

6.34. Artificial respiration stopped, apnœa eight seconds. Animal observed until 6.52. Although extensively handled during the fifty-five minutes since strychnine was injected, no sign of hyperæsthesia.

Later on asphyxia was caused by inhalation of hydrogen, and again by clamping of the trachea. Tetanic convulsions appeared only in the anterior part; the legs were directed towards the head.

Experiment V b. — Control, gray and white rabbit, 1250 grams.

6.03 P. M. Injected strychnine nitrate 0.63 mgm. per kilo.

6.27. Animal stiff.

6.29. Tetanic convulsion.

6.33. When lifted there was a convulsion which the animal survived.

In this experiment the effect of artificial respiration upon strychnine spasms was very plain. They were entirely suppressed during the fifty-five minutes of observation, although the control animal began to show a distinct strychnine effect even twenty-four minutes after injection. The inhibitory effect upon the respiration was retarded by section of the cord as well as by section of the vagi, but finally a distinct apnœa was attained.

In the last three experiments the vagi and splanchnici, as well as the sensory fibres of the pleura and thoracic wall, at least most of them, were separated from the respiratory centre, etc. The roots of the brachial plexus were apparently divided in two parts, for when convulsive movements occurred in the upper part alone, the front legs were directed toward the head.

Experiment VI a. — Gray and white male rabbit, 1550 grams. Tracheotomy. Artificial respiration (25–30 mms. Hg) for three minutes. Voluntary and concomitant breathing soon suppressed. Artificial respiration stopped, apnœa three seconds.

4.43 P. M. Cord cut "between fourth and fifth cervical vertebræ," breathing stopped. Artificial respiration begun. Heart, lid reflex, etc., all right. Concomitant breathing soon suppressed. Artificial respiration stopped,

Experiment VI a — (*continued*).

apnœa fifteen seconds, soon "head breathing." Artificial respiration resumed again, head breathing soon suppressed.

4.57. Injected strychnine nitrate 0.7 mgm. per kilo.

5.16. Both vagi tied off, concomitant breathing appeared, but disappeared again after four minutes.

5.32. Artificial respiration stopped, apnœa fifteen seconds, then "head dyspnœa"; no hyperæsthesia otherwise. Artificial respiration again.

5.40. Artificial respiration stopped, apnœa fifteen seconds, then gradual development of head dyspnœa and asphyxia.

5.43. Heart stopped. No convulsions.

Experiment VI b. — Control, gray and white rabbit, 1050 grams.

5.01 P. M. Injected strychnine nitrate 0.7 mgm. per kilo.

5.07. Convulsions, did not survive.

In Experiment VI a when artificial respiration was stopped there appeared now and then a very faint indication of thoracic movement. Possibly it was produced passively by the dyspnœic contraction of the cervical muscles. The autopsy showed that the cord was severed at the lower border of the fourth cervical vertebra, but the cut was diagonal and possibly a few fibres of the phrenic escaped. At all events this experiment is a strong demonstration of the efficiency of artificial respiration in suppressing strychnine spasms, and in producing apnœa, even after the vagi, splanchnici, brachial plexus, and almost all of the phrenic nerves are excluded. Although the control animal had a convulsion six minutes after injection (0.7 mgm. per kilo), the animal in Experiment VI a manifested no sign of strychnine spasms either during the forty-three minutes of artificial respiration or during final asphxyia. Furthermore, there was no concomitant breathing during the artificial respiration, and an apnœic pause was present after each interruption.

Experiment VII a. — Gray female rabbit, 1120 grams. Tracheotomy.

5.20 P. M. Cord cut near upper border of fifth cervical vertebra, animal breathes normally. Artificial respiration for a few minutes, voluntary respiration persistent. Artificial respiration stopped, no distinct apnœa. Artificial respiration begun again.

5.30. Both vagi cut, labored, concomitant breathing; voluntary and concomitant breathing subsiding slowly.

5.35. Injected strychnine nitrate, 0.7 mgm. per kilo.

6.05. Artificial respiration discontinued, no apnœa. Animal observed five hours longer. Had no sign of strychnine poisoning. When then

Experiment VII a — (*continued*).

given a comparatively large dose of strychnine, it had a number of short convulsions in either of the two parts, independently of one another.

Experiment VII b. — Control, gray female rabbit, 1050 grams. For better comparison had ether anæsthesia for a few minutes.

5.40. Injected strychnine nitrate 0.7 mgm. per kilo.

5.59. Convulsions, succumbed in six minutes.

This experiment again is a classical demonstration of the inhibitory effect of artificial respiration upon the strychnine spasms even after section of cord and vagi. The effect upon respiration was less pronounced.

Experiment VIII a. — Gray female rabbit, 1750 grams. Tracheotomy.

3.59 P. M. Artificial respiration begun, only very slight concomitant respiratory movements.

4.01. Cord cut between second and third cervical vertebræ, concomitant breathing greatly increased, after a few minutes decreased again.

4.09. Artificial respiration discontinued, apnœa for a few seconds, then dyspnœa. Artificial respiration again.

4.15. Animal recovered from anæsthesia. Injected strychnine nitrate, 0.7 mgm. per kilo. Heart-beat, lid reflex, etc., normal until 4.30, when heart-beats became slower and labored concomitant respiratory movements reappeared.

4.35. The respiratory movements rapidly diminished ; no lid reflex.

4.37. Heart-beats faint.

4.39. Death.

During the fifteen minutes after injection the animal was perfectly normal, but there was no sign of hyperæsthesia.

Experiment VIII b. — Control, gray rabbit, 1700 grams.

4.18 P. M. Etherized (for comparison) and kept under ether until 4.23.

4.19. Injected strychnine nitrate 0.7 mgm. per kilo.

4.33. Tetanic dance.

4.36. Convulsion terminating fatally at once. Although this animal was under the influence of ether while strychnine was injected, fourteen minutes after injection it manifested the unmistakable effects of this drug.

In Experiment VIII a the vagi, splanchnici, and all thoracic nerves, including the phrenici, were excluded. Although the animal died early, it lived long enough, and was normal long enough, to demonstrate that the strychnine had no effect so long as the artificial respiration was continued. There was once also a distinct apnœic pause.

Experiment IX a. — Gray female rabbit, 1750 grams. Tracheotomy.

4.00 P. M. Artificial respiration begun.

4.02. Injected strychnine nitrate, 0.7 mgm. per kilo. The voluntary respirations were completely suppressed. Six minutes of artificial respiration, concomitant breathing not completely suppressed.

4.07. Cord cut at third cervical vertebra. Heart-beat, lid reflex, etc., normal, concomitant respiratory movements increased; remained unsuppressed throughout entire experiment.

4.12. Vagi cut. Animal normal throughout the remainder of the experiment. There was apparently a hyperæsthesia in the lower part, pressing or pulling legs was followed by contraction or tremor in legs, but these continued only as long as pull or pressure lasted. Blowing on animal, hitting table, no effect. Tremor in abdominal muscles; they even seem to contract synchronously with artificial respiration, simulating superficial independent voluntary breathing.

4.42. Artificial respiration stopped, all contraction and tremor disappear immediately (are due apparently to the local stimulus of the artificial respiration); head dyspnœa appears. Artificial respiration resumed again. Extremities and tail repeatedly pinched, pulled, etc., response with local, short reflexes, either during stimulation or immediately after. Pinching ear or other parts of head produce no reflex, but voluntary motion, moving away.

5.03. Artificial respiration discontinued; head dyspnœa, but no other movement of body.

5.04. Slight movement, and later, vibration only in front legs.

5.05. Sudden tetanus in all four extremities, followed by clonic convulsions.

5.06. Artificial respiration resumed. Lid reflex and heart-beat soon normal again, no more convulsions.

5.10. Artificial respiration discontinued again.

5.12. Sudden tetanus. Artificial respiration at once, and tetanus stopped suddenly. This procedure was repeated several times with same result, but sometimes tetanus stopped even while there was no artificial respiration.

Experiment IX b. — Control, gray female rabbit, 1600 grams.

4.22. Strychnine nitrate, 0.7 mgm. per kilo.

4.35. Tetanic convulsions.

4.45. Blown on, immediately violent convulsion, succumbs.

In Experiment IX a the section of the cord was above the phrenici, and the influence of the vagi and all other nerves concerned was positively excluded. The animal had a dose of strychnine which proved

fatal to the control rabbit in less than half an hour. Although the animal in Experiment IX a was continually handled, and the reflexes, etc., tested, for an hour, while artificial respiration lasted, there was no reaction which could be ascribed to the effect of strychnine. Pounding the table or blowing on the animal had no effect at all. Pinching or pulling a leg brought out a local reflex which was apparently due only to the increased reflex-irritability caused by the section of the cord. Pressing one hind leg, for instance, would bring out a short flexion or extension of the opposite, or of a front leg. The artificial respiration caused short contractions of the abdominal muscles. But pinching any part of the head caused no reflex-response. The strychnine, however, was not destroyed within the body, nor sufficiently eliminated from it. Soon after stopping the artificial respiration there appeared convulsions and tetani, which by their entire character were apparently due essentially to the strychnine and not to asphyxia, or at least not to asphyxia alone. But these convulsions also could be stopped instantly by artificial respiration.

The influence of artificial respiration upon apnœa was not carefully noted in this experiment, but the concomitant respiratory movements continued during the hour while the artificial respiration lasted, although their intensity gradually decreased.

Our first series of experiments brought out one positive result. The claim of Brown-Séquard, that section of the cord or of the vagi abolished the arresting influence which artificial respiration exerts upon strychnine spasms, is entirely unfounded. Not only does section of the vagi alone, or of the cord alone, fail to impair this influence, but even cutting the vagi, combined with such section of the cord as excludes all influences of the splanchnic, diaphragmatic, and thoracic nerves, apparently does not interfere with the inhibitory influence of artificial respiration upon strychnine spasms. There were no convulsions in any of our experiments as long as sufficiently strong artificial respiration was administered. In many experiments no convulsions appeared even after the artificial respiration was stopped, although in all cases doses of strychnine were employed which by control experiments were proved to be surely toxic and mostly fatal. In some experiments artificial respirations arrested instantly the tetanic convulsions which were permitted to break out.

The doses of strychnine which we employed were, however, not

much above the toxic or fatal minimum. Possibly section of the cord or vagi does interfere somewhat with the degree of favorable influence which artificial respiration might exert under such conditions. Filehne, who admits some impairment due to the section of the vagi, does not state the weight of his animals. Possibly, however, the doses which he employed were a trifle too large. Overdosage might also explain the claims put forward by Brown-Séquard. But the description of his experiments is too brief to permit any very definite interpretation. In fact it is not even evident that Brown-Séquard's conclusions regarding the relations of the sections of cord, or vagi, to the arresting influence of artificial respiration upon strychnine spasms were derived from actual experiments, and that they were not mere inferences from the experiments he made on the production of apnœa.

Regarding the latter, our own experiments have indeed demonstrated that section of the cord and the vagi impairs more or less the production of apnœa by artificial respiration. In some cases after section of the cord, and especially after additional section of the vagi, neither the voluntary respirations nor the concomitant respiratory movements could be suppressed. This was observed in some of the larger animals. Possibly the degree of ventilation employed in our experiments was not sufficient to accomplish this end in an animal with a comparatively large thorax. However, in all the experiments, section of the cord, or of the vagi, even during artificial respiration, immediately brought out again the voluntary breathing of the animal and especially the concomitant respiratory movements. It invariably took a much longer time to suppress the latter after section than before it.

Our experiments also showed that while artificial respiration completely suppresses the increased reflex-irritability due to strychnine-poison, it does not interfere, at least not strikingly, with the increased reflex-irritability induced by section of the cord. In all cases we were able, with little or no difficulty, to produce distinct reflexive movements by pinching a leg, touching an eye, etc., the posterior extremities responding more readily than the anterior ones. In one case, with section above the phrenici, each blow of the bellows brought out a contraction of the abdominal muscles simulating spontaneous breathing, which ceased on stopping the artificial respiration.

We noticed also, in the cases in which mild convulsions appeared after artificial respiration was stopped, that the parts lying above the

line of section of the cord and those lying below it had their convulsions independently of one another. They were mostly insynchronous. In the experiments in which section of the cord was near the fifth cervical vertebra, the interesting observation was made that when the convulsions occurred in the anterior part, the anterior legs took part in it by moving toward the head, and that when the posterior part was convulsed the anterior legs moved toward the tail, pressing against the body. When, however, a violent tetanus broke out, the spasm convulsed all parts nearly simultaneously.

Thus it is evident that our experiments have established the fact contended for, but not proved by Filehne, namely, that section of the cord and vagi does not interfere with the inhibitory influence which artificial respiration exerts on strychnine spasms. But does this fact prove that the inhibitory influence of artificial respiration is due to the chemical influence of the oxygenation of the blood and to this alone? Does this fact indicate that the mechanical act of rhythmical insufflation has no share in the inhibitory influence?

The persistence of the favorable influence observed after section of cord and vagi could only then serve as an irrefutable proof if the claim for the mechanical share had been restricted to a hypothesis that the inhibition acts either through the agency of the respiratory centre or through the inhibitory mechanisms of the brain. If this is what Brown-Séquard meant, his theory is surely disproved by our experiments. The favorable influence of artificial respiration against the increased irritability of the spinal cord continues even after the cord has been severed from the controlling parts above it. But why restrict our hypothesis? We know that any reflex may be inhibited within the spinal cord by any mechanical stimulation of any part of the body. We have also seen in our experiments that, in an animal with a severed cord, artificial respiration caused rhythmical contraction of the abdominal muscles. This fact shows that the insufflations into the lungs, and the consequent abrupt increase of pressure upon the organs within the thoracic cavity, result in stimulating also the dorsal nerves imbedded in the abdominal section of the body. Furthermore we know that this insufflation causes an inhibition of centres lying within the medulla (respiratory, vaso-motor, cardio-inhibitory centres). Why then should it not be assumed that the rhythmical insufflations into the lungs stimulate all nerves within the thoracic and abdominal regions and thus inhibit increased reflex-irritability in all parts of the cord?

The hypothesis formulated by Brown-Séquard is certainly untenable. That the arrest of the spasms can be due to the mechanical stimulation of the endings of the vagi, the phrenic and "other diaphragmatic nerves" alone, our experiments with section of the vagi and the cord have proven conclusively. But no cutting of the cord is capable of disproving the hypothesis that rhythmical insufflation is a mechanical stimulus for all the nerves within the trunk, by means of which an inhibition is caused in every section of the spinal cord above a cut as well as below it.

The question, therefore, is still open: Does the mechanical element involved in artificial respiration have a share in the arrest of the strychnine spasms, just as it is now generally assumed that it has a share in the production of apnœa?

III. Artificial Respiration with Hydrogen.

For the solution of this question a method presents itself which at first sight appears to be quite simple. Previous investigators who desired to prove that it is the chemical factor which causes the arrest of the spasms have tried to introduce the oxygen without the complication of the mechanical element. Desiring to test the efficiency of the mechanical factor, we sought to determine the effect of artificial respiration with its chemical factor removed; *i.e.*, artificial respiration with an indifferent gas. It was partly by this method, indeed, that the value of the mechanical element in the production of apnœa was ascertained. We have, therefore, endeavored to study the effect of artificial respiration with pure hydrogen upon the strychnine spasms.

General method. — The method we employed was comparatively simple. Bellows were connected on one side with a gasometer containing pure hydrogen, and on the other side with the trachea of the animal. The tube connecting the bellows with the gasometer contained a valve which permitted the entrance of the gas into the bellows, but prevented it from going back to the gasometer. The tube connecting the bellows with the trachea contained a valve permitting the escape of the gas in the direction of the trachea, but preventing its return to the bellows. The expiratory air escaped through a lateral tube submerged under water (Müller's water valve), by which arrangement air was prevented from entering into the trachea through the expiratory aperture during a voluntary inspiration. All the con-

nections were carefully made air tight. Each suction of the bellows brought hydrogen into it, and each compression drove the hydrogen into the lungs. The pressure was regulated by means of a stop-cock carried by the expiratory tube, and it was registered by a manometer connected by a T tube with the bellows-trachea tube.

We had, of course, no expectation of being able to continue the exclusive inhalation of hydrogen long enough to prevent the development of the strychnine poisoning, in the same manner as we succeeded in preventing it by the artificial respiration of air. We had observed that when once a tetanus broke out in our experiments it could be suppressed instantaneously by artificial respiration. In fact this instantaneous effect appeared to us to be in favor of the theory of a mechanical effect, since an effect due to a sufficient increase of oxygen in the blood could hardly develop so promptly after the first few strokes with the bellows. We therefore had reasonable expectations of witnessing the same instantaneous effect when pure hydrogen would be insufflated, or at least of observing it, long before the unavoidable asphyxia would finally compel the discontinuation of this gas. However, the first preliminary experiment, to determine the effect of insufflation of pure hydrogen upon the production of apnœa, brought us a surprise.

Experiment X. — White rabbit, 1700 grams. Tracheotomy, connected with bellows and gasometer, expiratory tube submerged. Insufflation of hydrogen for a brief period, apnœa for a few seconds. Repeated a few times with same result. Encouraged by the absence of asphyxia, the insufflation was continued consecutively for eighteen minutes, during which time there was no voluntary breathing, no concomitant respiratory movements, and no perceptible cyanosis of visible mucous membranes. After discontinuing the insufflation of hydrogen an apnœa of fifteen seconds appeared, but this was followed immediately by rapid superficial breathing and very rapid, faint heart-beats. Artificial respiration with air improved this condition, but the animal soon died through an accident.

Eighteen minutes' insufflation of pure hydrogen without asphyxia! That was surely an unexpected result. Before discussing it, however, we should quote a few of these hydrogen experiments in which also toxic doses of strychnine were injected.

Experiment XI a. — White rabbit, 1240 grams. Tracheotomy.

4.30 P. M. Injected strychnine nitrate, 0.6 mgm. per kilo.

4.33. Trachea connected with bellows, etc. Continued insufflation without incident till 4.58, when tetanic convulsions set in. Continued

Experiment XI a — (*continued*).

insufflation until 5.01 without favorable effect. Insufflation stopped, animal thoroughly asphyxiated.

5.05. Artificial respiration with air.

5.07. Discontinued, no apnœa, immediately rapid breathing, a minute later convulsions, which continued for a few minutes. Animal killed.

Experiment XI b. — Control, gray and white rabbit.

5.17. Injected strychnine nitrate, 0.5 mgm. per kilo.

5.30. Convulsions broke out.

The animal in Experiment XI a was a white rabbit which, as mentioned above, was more susceptible to strychnine than the gray and white one. It received a larger dose than the gray control animal. Nevertheless, the convulsions did not break out until twenty-eight minutes after the injection, while the control had convulsions thirteen minutes after the injection. In this experiment the insufflation, however, could not put off asphyxia longer than twenty-five minutes, and with the onset of asphyxia the convulsions broke out.

Experiment XII a. — White rabbit, 2600 grams. Tracheotomy.

4.51 P.M. Injected strychnine nitrate, 0.53 mgm. per kilo.

4.55. Trachea connected with bellows, etc.

5.11. Some spasmodic twitching (beginning dyspnœa?). Increased the number and energy of the ventilations, animal quiet again.

5.15. Both vagi cut, " head dyspnœa " sets in.

5.25. Voluntary breathing of the animal appears and gradually increases.

5.28. Insufflation of hydrogen stopped, no apnœa, very labored dyspnœic breathing.

5.42. Trachea clamped, death. No strychnine effect at any time.

Experiment XII b. — Control, white rabbit, 1970 grams.

4.29. Injected strychnine nitrate, 0.45 mgm. per kilo.

5.01. Convulsions, died in two minutes.

In Experiment XII a, the animal received more strychnine than the control, which succumbed thirty-four minutes after injection, but had no convulsions for forty-seven minutes; *i.e.*, during the time it was under observation. The slight twitchings which appeared sixteen minutes after injection were promptly suppressed by the increase in ventilation with hydrogen. The inhibitory effect upon respiration, however, was greatly diminished by the section of the vagi. The

concomitant breathing set in immediately, and the voluntary breathing appeared soon also, and apparently would have terminated in asphyxia, if the hydrogen insufflation had not been discontinued.

Experiment XIII a. — White rabbit, 1660 grams. Tracheotomy.

4.48 P. M. Injected strychnine nitrate, 0.54 mgm. per kilo.

4.50. Trachea connected with bellows, etc. At no time voluntary or concomitant breathing, no sign of hyperæsthesia.

5.21. Insufflation of hydrogen stopped, brief apnœa, then normal breathing. Observed till 5.42, no convulsions.

Experiment XIII b. — Control, white rabbit, 1420 grams.

5.27. Injected strychnine nitrate, 0.49 mgm. per kilo.

5.53. Had convulsions, and died in about two minutes.

The control had a fatal tetanus in twenty-six minutes, while animal XIII a, with a larger dose, showed no strychnine effect for the fifty-five minutes it was kept under observation. The insufflation lasted for thirty-one minutes and exerted a distinct inhibitory effect upon the respiration.

The results we obtained in these experiments were extraordinary indeed. Not only could the effects of fatal doses of strychnine be completely prevented by insufflation of pure hydrogen, but the animal could be kept by such an uninterrupted insufflation, as was seen in Experiment XIII a, for thirty-one minutes without manifesting any signs of asphyxia, dyspnœa, or cyanosis.

We all know very well that spontaneous inhalation of hydrogen alone results in asphyxia almost immediately. This is an old, well-established fact, and we have tested it ourselves by the following direct experiments. When the trachea of an animal was connected directly with the gasometer, without the intervention of the bellows, the animal thus surely inhaling, spontaneously, pure hydrogen, asphyxia set in after thirty to forty-five seconds, and rarely as late as after sixty seconds. Apparently, then, it was the action of the bellows which deferred asphyxia so long.

The first thought which occurs is that the bellows were, after all, not perfectly air tight. We have tested them by letting the animal spontaneously inhale the hydrogen from the gasometer through the expanded bellows without ventilating them. The asphyxia was then, indeed, deferred a little longer than when the inhalation occurred without the intervening bellows. However, the gain was at the utmost a minute or two, and therefore the amount of air which could have found access to the bellows must have been at most

very small. But even granting that during the sudden and forcible expansion of the bellows more air was sucked into them than during the voluntary breathing, the amount of air which was able to penetrate the pores must under all circumstances necessarily have been very small in proportion to the quantity of hydrogen which, under constant pressure, had free access through the open lumen of a wide tube. It must also be remembered that the animal not only had no asphyxia under these conditions, but also that it was constantly in a state of apnœa, — a state which occurs only, it is assumed, when the animal receives more air than normally.

We may add, also, that, according to Osterwald (17), a diminution of oxygen favors the outbreak of strychnine spasms. In our experiments, with surely diminished oxygen there was no sign of convulsions even with fatal doses of strychnine.

These experiments brought us more than we looked for. It was now no longer a simple question whether the mechanical factor of artificial respiration has a share in the inhibition of strychnine spasms. The question which confronted us was whether one of the fundamental and apparently definitely settled principles in the theory of respiration did not require revision.

Searching through earlier literature on the subject of respiration we discovered that we had touched upon a long-forgotten chapter in the discussion whether the absence of oxygen or the presence of carbon dioxide is the cause of inspiration.

In 1862 L. Traube (18) made experiments with insufflation of hydrogen on dogs, in the same manner as we have made them on rabbits, and found, as we did, that artificial respiration with pure hydrogen may be carried on for a long period (forty-six minutes in one experiment), the animal remaining all the while in a state of apnœa. On the other hand the addition of carbon dioxide to the air rapidly caused dyspnœa. Traube, in consequence of these observations, gave up his original idea, that the absence of oxygen is the stimulus for inspiration, and accepted the view that the real cause of respiration is the presence of carbon dioxide. Heidenhain and Krause (19) soon contradicted Traube's statement, and explained his conclusion by assuming that his bellows were not air tight.

Traube (20) repeated his experiments, oiled his bellows, and took all precautions, as he states, to prevent the entrance of air, and insisted on the correctness of his former results, attributing the failure of Heidenhain and Krause to some fault in their technique.

Traube was contradicted also by Thiry (21),[1] and finally by I. Rosenthal (23). Rosenthal did not repeat Traube's experiments, but connected the trachea of the animal with a gasometer of special construction containing pure hydrogen, and found that the animals became rapidly asphyxiated. By special calculations Rosenthal arrived at the conclusion that air which contains only 1 per cent of oxygen is sufficient for the maintenance of the animal, an amount which presumably found its way into the bellows in Traube's experiment. That was the last word, at least the last we found recorded in this discussion.

We may add that Traube's technique suffers from still another objection. In his experiments the opening for expiration had no valve. The animal, therefore, could obtain, during inspiration, sufficient air through this opening, even if it were made very small. As long as it was large enough for expiration it was also sufficient for inspiration. We have established this fact by experiment. The trachea was connected directly with the gasometer while the expiratory tube was submerged: asphyxia in forty-five seconds. The expiratory tube was left free in the air, and the stop-cock turned so as to make the lumen permissibly narrow: the animal went on breathing without noteworthy impediment for some time.

Rosenthal's paper appeared in 1864, and at that time there had not yet arisen the question whether the mechanical distention of the lungs can cause inhibition of inspiration. The only question in the minds of the earlier investigators was whether absence of oxygen or presence of carbon dioxide is the stimulus of respiration. And as the simple inhalation of hydrogen caused asphyxia, this appeared to prove that it is the absence of oxygen which causes respiration. Traube's experiments, therefore, seemed to have no further object. The value of the mechanical element which distinguishes artificial respiration from spontaneous breathing had not yet been recognized. We now know, from the studies of Hering and Breuer, Head, Gad, Meltzer, and others, that the mechanical effect of the distention of the lungs has a distinct inhibitory influence upon respiration.

It is now, furthermore, the general consensus of opinion that both

[1] That, at least, is what THIRY states in his paper in the Zeitschrift für rationelle Medizin (iii), xxi, p. 25. It is not stated on what grounds the opinion is based. MIESCHER-RÜSCH (22), however, quotes THIRY from a French paper as saying that artificial respiration with air and hydrogen causes apnœa. This paper was not accessible to us.

the presence of carbon dioxide, as well as the absence of oxygen, act as stimuli to the respiratory mechanism. But it is surely not the actual immediate need of oxygen for metabolic purposes which in the latter case is the stimulus. The blood and lymph and tissues are provided with a surplus of oxygen for actual oxidative necessities. It is the first intimation of a deficit in this sinking fund which acts as a warning signal,—as a stimulus for increased provision of oxygen, increased inspiration. Is it, then, inadmissible to assume that this warning, this stimulus resulting from diminution in the body's income of oxygen, could be overcome for some time by the inhibitory influence of the rhythmical mechanical effect of distention of the lungs, if sufficient provision were made for the full escape of the carbon dioxide? Our experiments do not, of course, warrant such a positive conclusion. The bellows permitted the entrance of air to some degree, but the amount of air which entered was surely comparatively small. If, therefore, our experiments, as well as those of Traube, do not yet permit positive conclusions in this regard, they are at least suggestive enough to urge the necessity of a reinvestigation of this particular question with more favorable methods. In this relation the necessity of avoiding suction apparatus in the execution of artificial respiration with indifferent gases seems important.

In this connection, also, we wish to call attention to the statement of Head (24) that he caused apnœa by insufflation of hydrogen. His conclusion was that the apnœa was due to mechanical effects. He used bellows, and does not mention any precaution taken to guard against the entrance of air into the bellows. Could not the contention be made against his conclusions also, as it was raised against Traube's, that it was the air which entered through the pores of the leather into the bellows that brought about the observed result?

Regarding the arrest of the strychnine spasm, which we observed, with hydrogen insufflations, it appears very probable that it is due largely to the mechanical effect of the insufflation, and that it is not essentially a result of the admixture of small amounts of air. Here also additional experiments, and by other methods, will have to be made before the question can be definitely settled.

Bibliography.

1. Meissner und Richter: Göttinger Gelehrten Anzeiger, 1862, p. 165; Zeitschrift für rationelle Medizin (3), xviii, p. 76.
2. Leube: Archiv für Anatomie und Physiologie, 1867, p. 629.

3. Uspensky: Archiv für Anatomie und Physiologie, 1868, p. 522.
4. Rosenthal: Comptes rendus, 1867, lxiv, p. 1142.
5. Schiff: Beiträge zur Physiologie, 1896, iii, p. 211; reprinted from l'Imparziale, 1867.
6. Richet: Comptes rendus, 1880, xci, p. 131.
7. Rossbach und Jochelsohn: Centralblatt für die medicinische Wissenschaften, 1873, No. 24, p. 369.
8. Ebner: Inaugural dissertation, Giessen, 1870.
9. Buchheim: Archiv für experimentelle Pathologie, 1875, iv, p. 137.
10. Pauschinger: Archiv für Physiologie, 1878, p. 401.
11. Brown-Séquard: Archives de physiologie, 1872.
12. Filehne: Archiv für Physiologie, 1873, p. 361.
13. Ananoff: Centralblatt für die medicinische Wissenschaften, 1874, No. 27, p. 417.
14. Osterwald: Archiv für experimentelle Pathologie, 1899, xliv, p. 451.
15. V. Czyhlarz: Zeitschrift für Heilkunde, 1901, p. 160.
16. Graham: Archiv für die gesammte Physiologie, 1881, xxv.
17. Osterwald: *Loc. cit.*
18. Traube: Allgemeine medicinische Central-Zeitzung, 1862, p. 296.
19. Krause: Studien aus dem physiologischem Laboratorium zu Breslau, 1863, ii, p. 31.
20. Traube: Allgemeine medicinische Central-Zeitung, 1863, p. 776.
21. Thiry: Zeitschrift für rationelle Medizin (3), xxi, p. 25.
22. Miescher-Rüsch: Archiv für Physiologie, 1885, p. 355.
23. Rosenthal: Archiv für Physiologie, 1864, p. 456.
24. Head: Journal of physiology, 1889, x, p. 40.

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AN EXPERIMENTAL STUDY OF THE SUGAR CONTENT AND EXTRAVASCULAR COAGULATION OF THE BLOOD AFTER ADMINISTRATION OF ADRENALIN.

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Introduction.

EARLY in 1902 the discovery was made by Herter and Richards[2] that the injection of solutions of adrenalin chloride into the peritoneal cavity of dogs was followed by an intense though transient glycosuria. It was also found that the application of adrenalin solution directly to the surface of the pancreas produced a similar effect. As a result of a number of experiments in this direction, the suggestion was offered that this form of glycosuria was in reality of pancreatic origin.

In extending these observations, Herter and Wakeman[3] have found that the power of adrenalin to produce glycosuria, when applied to the pancreas, is not specific but is shared with various substances. The number of such substances is comparatively large, and apparently the only quality common to the series is a reducing activity. A seeming exception to this rule was found in potassium cyanide. When solutions of this substance were applied to the pancreas in amounts far too small to produce general toxic symptoms, glycosuria resulted. This substance, like hydrocyanic acid, while it has no reducing power, exerts a specific action on the animal cells in preventing them from absorbing oxygen.[4] It is natural to suppose that, in the absence of the normal amount of oxygen in the cell, an excess

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[2] Herter and Richards: The medical news, 1902, lxxx, p. 201.

[3] Herter and Wakeman: Virchow's Archiv für pathologische Anatomie und Physiologie und für klinische Medicin, 1902, clxix, p. 479; Herter: The medical news, 1902, lxxx, p. 867.

[4] Geppert: Zeitschrift für klinische Medicin, 1889, xv, p. 208; *Ibid.*, p. 307.

of reducing substances may be formed. It is possible that these substances may act in a manner comparable to those of the above-mentioned series in bringing about the excretion of sugar. From the facts brought out by their observations in this regard, Herter and Wakeman are inclined to attribute the production of glycosuria upon the application of adrenalin and other substances to the pancreas to a toxic action on the cells of that gland which is closely connected with the power of reduction.

If this view of the matter is correct, an important relationship suggests itself between this form of experimental glycosuria and conditions in the human organism which may give rise to an excretion of sugar. The fact that many organs of the body may form reducing substances capable of easy oxidation which may reach the pancreas in the blood stream, carries with it the possibility that, if the normal balance between the amount of these substances and the oxidizing power of the pancreas be disturbed, the production of glycosuria may occur.

Concerning the mechanism through which adrenalin brings about the excretion of sugar no positive statements can as yet be made. The work of Minkowski [1] and his followers, which has furnished the basis of the belief in the existence of an internal secretion of the pancreas exercising a controlling influence on carbohydrate metabolism, justifies an assumption that the sugar elimination is the result of an alteration in the nature, activity, or amount of this secretion. The glycosuria-producing effect of injury of certain parts of the central nervous system,[2] and the increase in sugar formation in the liver which follows stimulation of the cœliac plexus [3] or of the vagus nerve,[4] may lead to the supposition that adrenalin glycosuria results from the action of a nervous mechanism. Finally, it is known that under the abnormal conditions which follow the injection of phlorhizin [5] or chromic acid,[6] glycosuria may occur, owing to an increase in the permeability of the kidney cells. The possibility that adrenalin

[1] Minkowski: Untersuchungen über den Diabetes Mellitus nach Extirpation des Pancreas, Leipzig, 1893.

[2] Cl. Bernard: Leçons sur la physiologie et la pathologie du system nerveux, Paris, 1858, i, p. 401.

[3] A. and E. Cavazzani: Centralblatt für Physiologie, 1894, viii, p. 33.

[4] Levene: Centralblatt für Physiologie, 1894, viii, p. 337.

[5] v. Mering: Zeitschrift für klinische Medicin, 1889, xvi, p. 431.

[6] Kossa: Archiv für die gesammte Physiologie, 1901–1902, lxxxviii, p. 627.

glycosuria is the immediate result of changes in the kidney has not yet been excluded.

Whatever may be the manner by which the effects of adrenalin are brought about, it is probable that the mechanism involved is one which is active, though in a different degree, under normal conditions. A determination of the identity of the mechanism is therefore of importance, not only in explaining the phenomenon in question, but also from the fact that it may throw light on some of the processes connected with the normal metabolism of carbohydrate within the organism.

Before such a determination can be made, however, a more accurate knowledge of the internal conditions antecedent to the excretion of sugar is necessary. With this end in view we have made a somewhat detailed study of the sugar in the blood, after intraperitoneal injection of adrenalin, as well as after application of that substance to the pancreas.

Sugar Content and Coagulation of Arterial Blood after Treatment with Adrenalin.

It has long been known that the glycosuria produced by extirpation of the pancreas,[1] puncture of the floor of the fourth ventricle,[2] and poisoning with certain substances, such as carbon monoxide,[3] is the immediate result of an increased accumulation of sugar in the blood. On the other hand, injections of phlorhizin[4] are followed by the excretion of sugar due to the effect on the kidney. In the latter case the percentage of sugar in the blood never rises above normal, and may even fall below that amount. To determine in which class adrenalin glycosuria belongs, we have made a number of determinations of the sugar content of the blood of dogs which had been subjected to treatment with adrenalin. In this series also we have attempted to ascertain the rapidity with which this substance acts, and the course and duration of its influence.

Method of collection and analysis of blood. — Healthy, well-nourished dogs were anæsthetized with pure ether, a cannula introduced into a femoral artery, and a portion of blood taken. The solution of adrena-

[1] Minkowski: *Loc. cit.*

[2] Cl. Bernard: *Loc. cit.*

[3] Senff: Ueber den Diabetes nach der Kohlenoxydathmung, Inaugural dissertion, Dorpat, 1869.

[4] v. Mering: *Loc. cit.*

lin chloride[1] was then injected by a hypodermic syringe into the peritoneal cavity or, after an incision through the abdominal wall, was painted on the surface of the pancreas with a soft brush. Portions of blood were then drawn from the femoral artery at various intervals.

Having in mind the possible production of glycosuria by means of anæsthetics,[2] as well as by asphyxia,[3] care was taken to keep the anæsthesia as light and as constant as possible. Moreover, we believe that this factor may be left out of account in these experiments, since the control portion of blood, taken before adrenalin treatment, was collected under the same conditions of anæsthesia as the subsequent portions which are compared with it.

The portions of blood were analyzed according to the following procedure. The blood was drawn directly into a beaker containing a solution of phosphotungstic acid in dilute hydrochloric acid.[4] The beaker was counterpoised on a balance and the blood weighed immediately after its withdrawal from the artery. On boiling this mixture the blood proteids are precipitated in a granular form leaving a water-clear fluid free from proteid. The precipitate was washed thoroughly with hot water, a process which is rendered easy by its porosity and its friable character. The combined filtrate and washings were nearly neutralized with sodium hydroxide and evaporated to small bulk on the water bath. The evaporated residue was made up to known volume (50–100 c.c.) with water, and was filtered. The reducing power of this solution was determined by the Allihn method. The results were calculated in terms of dextrose from the weight of the metallic copper. The figures given represent the averages of closely agreeing duplicates.

Method of determining coagulation. — In one of our early experiments we noticed that a portion of blood drawn for the purpose of rinsing the cannula clotted very rapidly. As a result of this observation, in a number of later experiments we have taken separate por-

[1] In all the experiments outlined in this paper, the adrenalin chloride solution ($\frac{1}{1000}$) prepared by Parke, Davis, & Co., by the method of Takamine, was used.

[2] CUSHNY: Pharmacology and Therapeutics or the action of drugs, 1899, p. 160.

[3] DASTRE: Comptes rendus des séances de l'academie des sciences, 1879, lxxxix, p. 669.

[4] This solution contained 70 gms. of phosphotungstic acid and 20 c.c. of hydrochloric acid, sp. gr. 1.20, in a litre. About 5 c.c. are sufficient to completely precipitate the proteids in 1 gm. of blood.

tions of blood to be tested regarding this point. The amount drawn in each case was 2 c.c., collected in a graduated cylinder of 5 c.c. capacity. The time which elapsed between the collection of the blood and the time at which the cylinder could be inverted without loss of its contents, was noted as the time of the coagulation of the blood.

The results of our determinations are given in Table I, pages 40, 41.

These experiments show unmistakably that the administration of adrenalin chloride either by intraperitoneal injection or by painting it upon the pancreas is followed by a marked increase of sugar in the blood. The increase is very noticeable within the first five minutes after the application and reaches its maximum within three hours. A very gradual fall then ensues, which may continue until the percentage of sugar becomes subnormal (Exp. 2). In a dog recently fed (Exp. 1), the blood sugar may be double the normal quantity fourteen hours after the injection. A marked rise occurred in the case of a dog (Exp. 7) which had been starved for six days. In Experiment 8 a fatal dose of adrenalin was given. A slight increase in the sugar content of the blood occurred shortly after. One minute before death ensued, twenty-four hours after injection, the percentage of sugar was approximately normal.

Simultaneously with the production of hyperglycæmia, an effect on the coagulability of the blood is observed. In every case, without exception, the time of coagulation is lessened after adrenalin is given. This diminution is equal in some cases to four-fifths of the coagulation time of the control.

Arthus[1] has shown that the time of coagulation decreases if the blood is allowed to come in contact with blood already clotted or with an exposed tissue surface. Special care has been taken therefore in these experiments to remove the clot from the cannula before each collection. Furthermore the portion for the coagulation test was collected just after that for sugar analysis, a circumstance which insures the rinsing of the cannula.

The recent observation[2] by the same author that the mere withdrawal of large amounts of blood from the body hastens the coagulation of subsequent portions, raises the question whether the results which we have observed may have been due to loss of blood alone. To test this point, a control experiment was made in which the

[1] ARTHUS: Journal de physiologie et de la pathologie générale, 1902, iv, p. 283.

[2] ARTHUS: *Ibid.*, p. 273.

TABLE I.

SUGAR CONTENT AND COAGULATION OF ARTERIAL BLOOD AFTER TREATMENT WITH ADRENALIN.

No. of experiment.	Weight of dog. Kilo.	Time since last fed.	Amount of blood withdrawn. Grams.	Time when taken.	Sugar. Per cent.	Time of coagulation.	Remarks.
1	8.5	12 hrs.	29.0	9.30 P. M.: normal	0.16		Etherized at 9.15 P. M.; 8 c.c. adrenalin chloride solution ($\frac{1}{1000}$) injected intraperitoneally at 9.35 P. M.; anæsthesia continued till 12.30 A. M.; again etherized on following morn. at 11.30.
			30.0	10.35 P. M.: 1 hr. after injection	0.42		
			25.0	11.30 P. M.: 2 hrs. " "	0.56		
			24.0	12.30 A. M.: 3 " " "	0.52		
			33.75	11.45 A. M.: 14 " " "	0.37		
						min. sec.	
2	7.6	32 hrs.	29.0	5.44 P. M.: normal	0.258	4 00	Etherized at 5.30 P. M.; 8 c.c. of adrenalin chloride solution ($\frac{1}{1000}$) injected intraperitoneally at 5.47 P. M.; anæsthesia continued till 7.00 P. M.; again etherized at 10.30 P. M., also at 10.30 A. M. next morning.
			15.0	5.52 P. M.: 5 min. after injection	0.357	2 15	
			11.5	5.57 P. M.: 10 " " "	0.400	2 00	
			10.7	6.20 P. M.: 33 " " "	0.437	2 20	
			11.8	6.47 P. M.: 1 hr. " "	0.430	4 10	
			10.8	10.35 P. M.: 5 hrs. " "	0.307	3 05	
			27.3	10.38 A. M.: 17 " " "	0.099	3 00	
3	12.0	48 hrs.	22.4	3.00 P. M.: normal	0.112		Etherization continued throughout experiment; 3 c.c. of adrenalin chloride solution ($\frac{1}{1000}$) applied to pancreas with a brush at 3.08 P. M.
			21.5	3.15 P. M.: 7 min. after painting	0.182		
			22.5	3.23 P. M.: 15 " " "	0.178		
			19.7	3.38 P. M.: 30 " " "	0.188		
			20.0	4.08 P. M.: 1 hr. " "	0.204		
			21.4	5.06 P. M.: 2 hrs. " "	0.214		
			20.8	6.00 P. M.: 3 " " "	0.165		
4	7.1	48 hrs.	21.9	7.55 P. M.: normal	0.173		Etherized from 7.45 till end of experiment; 4 c.c. adrenalin chloride solution ($\frac{1}{1000}$) painted on surface of pancreas at 8.12 P. M.
			27.3	8.17 P. M.: 5 min. after painting	....		
			21.1	8.27 P. M.: 15 " " "	0.277		
			20.4	8.42 P. M.: 30 " " "	0.237		
			21.8	9.12 P. M.: 1 hr. after painting	0.291		
			21.3	10.12 P. M.: 2 hrs. " "	0.256		

						min.	sec.	
5	13.2	24 hrs.	19.9	10.45 A.M.: normal	0.239	5	20	Ether given throughout the experiment; 3 c.c. of adrenalin chloride ($\frac{1}{1000}$) applied to pancreas at 10.55 A.M.
			23.8	11.00 A.M.: 5 min. after painting	0.291	3	00	
			25.8	11.10 A.M.: 15 " " "	0.354	1	57	
			19.4	11.25 A.M.: 30 " " "	0.388	2	07	
			20.3	11.54 A.M.: 1 hr. " "	0.433	1	12	
			21.2	12.54 P.M.: 2 hrs. " "	0.477	1	35	
			19.3	1.54 P.M.: 3 " " "	0.519	1	15	
			20.0	2.55 P.M.: 4 " " "	0.465	0	50	
6	7.0	24 hrs.	17.9	4.52 P.M.: normal	0.131	6	50	Ether given throughout experiment; 2 c.c. of adrenalin chloride solution ($\frac{1}{1000}$) applied to pancreas at 5.00 P.M.
			17.0	5.05 P.M.: 5 min. after painting	0.205	3	05	
			20.5	5.15 P.M.: 15 " " "	0.217	1	40	
			19.0	5.30 P.M.: 30 " " "	0.264	1	20	
			18.3	6.00 P.M.: 1 hr. " "	0.315	1	47	
			17.0	7.00 P.M.: 2 hrs. " "	0.371	1	40	
7	7.4	6 days	17.0	3.34 P.M.: normal	0.154	2	00	Ether given throughout experiment; 3 c.c. of adrenalin solution applied to pancreas at 3.43 P.M.
			17.6	3.49 P.M.: 6 min. after painting	0.192	2	25	
			18.2	4.01 P.M.: 18 " " "	0.173	2	40	
			17.0	4.22 P.M.: 29 " " "	0.191	2	05	
			16.5	4.58 P.M.: 1 hr. 15 min. aft. painting	0.206	2	15	
			17.7	5.43 P.M.: 2 hrs. after painting	0.143	1	55	
			18.5	6.37 P.M.: 3 " " "	0.169	1	16	
8	13.7	5 days	14.9	9.23 P.M.: normal	0.138	4	30	Anæsthetized with mixt. of chloroform and ether; 12 c.c. of adrenalin chloride sol. ($\frac{1}{1000}$) injected intraperitoneally at 9.33.30 P.M.; kept under ether till 11.15 P.M.; etherized at 10.30 following morning; animal very much prostrated; no ether given at night; characteristic bloody mucous passed in fæces; died 1 min. after last portion of blood was collected.
			15.6	9.35 P.M.: 1 min. 30 sec. aft. injection	0.104	1	20	
			11.3	10.18 P.M.: 45 min. after injection	0.095	2	20	
			10.4	11.03 P.M.: 1 hr. 30 min. aft. injection	0.199	3	15	
			19.0	10.31 A.M.: 13 hrs. after injection	0.058	2	40	
			26.5	9.38 P.M.: 24 " " "	0.102	5	30	

amount of blood taken was very small. The details of the experiment are as follows:

A small dog, 5.3 kilos in weight, which had received no food for twenty-eight hours previous to the experiment, was etherized and a cannula introduced into the femoral artery. 2 c.c. of blood collected at 2.34 P. M., coagulated in 5 min. 50 sec. An incision was made through the abdominal wall and 2 c.c. of adrenalin solution applied to the pancreas with a soft brush at 2.43 P. M. Subsequent samples of blood (2 c.c. each) coagulated as follows:

5 min.	after	adrenalin	application	(2.48 P. M.)	coagulated	in	1 min.	45	secs.
16 "	"	"	"	(2.59 P. M.)	"		2 "	0	"
33 "	"	"	"	(3.16 P. M.)	"		2 "	32	"
1 hr.	"	"	"	(3.45 P. M.)	"		3 "	10	"

Other control experiments in which large amounts of blood were removed, and no treatment with adrenalin given, show no such marked changes as are seen in the observations in Table I.

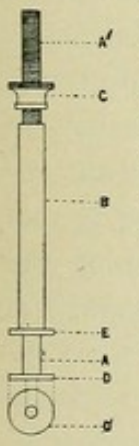
The Source of the Excess of Sugar in the Blood in Adrenalin Glycosuria, as Indicated by Comparative Analysis of Blood Collected Simultaneously from the Portal and Hepatic Veins and the Femoral Artery.

The results just detailed show clearly that the phenomenon of adrenalin glycosuria is due to an increase of sugar in the blood. The source of this excess of sugar is of great importance in determining the mechanism by which this effect is brought about. We have endeavored to trace the source of the sugar by means of analysis of blood taken simultaneously from the portal and hepatic veins and from the femoral artery. In these experiments it was necessary to collect successive portions of blood at various intervals from the same blood-vessel without interfering with the normal circulation in that vessel. The methods which have hitherto been devised for the collection of portal and hepatic blood appeared to be inadequate for our purpose, as well as somewhat difficult and uncertain of application.[1] We have therefore made use of an original method, the description of which follows. A cannula of special design[2] (see accompanying diagram) is the essential feature of the method.

[1] For description of older methods, see Seegen: Die Zuckerbildung im Thierkörper, 2 Auflage, Berlin, 1900, p. 66.

[2] The special cannulas used in these experiments were made for us very skilfully by Mr. John T. Hoyt of the Department of Physiology in this institution.

General method. — The method of fixing the cannula into a vein is as follows: The vessel is carefully exposed, and the outer connective tissue sheath dissected away. Loose ligatures are passed about the vessel at each end of the cleared portion, which should be about 2 cm. in length. Before introducing the cannula, its parts should be so arranged that the flange of the outer tube is separated by a space of about 0.5 cm. from that of the inner tube. The brass rod should be in position, totally filling up the bore of the inner tube. The ligatures about the vessel are then tightened momentarily by an assistant, and a longitudinal slit made in the wall of the vessel between them. This slit should be a trifle shorter than the diameter of the flange. The flange of the inner tube is then introduced into the interior of the vessel through the slit, the outer tube pressed down till the wall of the vessel is held tightly between the two flanges, and the nut screwed down so that the hold is retained. The ligatures are then loosened and the normal circulation is resumed. With a little practice the operation can be accomplished with no loss of blood and an interruption of the circulation of only thirty to forty seconds. On connecting a rubber tube with the inner tube of the cannula and removing the brass rod, blood can be withdrawn at pleasure.



Explanation of Diagram. — The cannula consists of three parts, viz.: an inner tube (*A*), an outer tube (*B*), and a nut (*C*). The inner tube is about 6.5 cm. in length. Its outer diameter is 4 mm., its inner diameter, 2 mm. On it a screw thread (*A'*) is cut, extending for 3.5 cm. from one end. The other end is provided with a flange (*D*) 9 mm. in diameter. The end view of this flange is shown at *D'*. The outer tube (*B*), 3.5 cm. long, fitting closely over the inner tube, terminates in a flange (*E*) similar to the one on the latter. By means of the nut the two tubes may be held in such a position that their flanges are in close contact. All parts of the instrument are made of brass. The cannula is also provided with a brass rod (not shown in the diagram) about 28 cm. long, of such a thickness that it fits closely into the bore of the inner tube. A small shoulder brazed on the rod at the proper point insures the complete occlusion of the cannula when desired.

In our experiments to determine the sugar content of the blood flowing to and from the liver, we have introduced cannulas of this type into the portal vein at its juncture with the pancreatico-duodenalis, and into one of the larger hepatic veins at a point between the liver and the diaphragm.

In order to expose the vessels, a transverse cut was made through the abdominal wall, following the curvature of the free border of the ribs and extending for about three inches on each side of a point on the median line just below the xyphoid cartilage. Bleeding was prevented by ligaturing the vessels which it was necessary to cut. The abdominal organs were protected from exposure by cloths moistened with warm saline solution. The time necessary for the operation and the introduction of the cannulas into both veins usually amounted to about one hour.

In drawing blood from the hepatic vein it was necessary to use a suction pump to overcome the negative pressure, which is very manifest in the venous circulation at this point. For this purpose, the beaker containing the phosphotungstic mixture, previously weighed, was placed under a small bell-jar which was connected by its lower opening with a suction pump. A small glass tube inserted through the rubber stopper which closed the upper opening of the bell-jar, and terminating at a point directly over the beaker, served to conduct the blood into the precipitating fluid. On connecting the cannula in the hepatic vein with the glass tube, and applying suction, the estimated amount of blood is easily obtained.

For collecting arterial blood, a glass cannula of the ordinary type was introduced into the femoral artery.

The order of procedure in obtaining the portions of blood simultaneously was as follows: The beakers which were to hold the femoral and portal blood were counterpoised on balances placed at the side of the operating table. Small glass tubes were clamped in a position to lead the blood into them. The beaker for collection of hepatic blood was placed under the bell-jar arranged as described above. The brass rods were removed from the cannulas in the portal and hepatic veins, and connection with the proper glass tubes made, passage of blood being prevented by clamping the rubber tubes. The connection between the cannula in the femoral and the third glass tube was made. At a given signal the clamps were removed and the blood collected. The brass rods were immediately replaced in the cannulas, and the portions of blood weighed. The time necessary for collection of all three portions of blood has seldom amounted to more than ten or fifteen seconds.

The analysis of the blood was carried out in a manner exactly similar to that previously described.

By means of these methods we have succeeded in obtaining blood simultaneously from the three vessels mentioned, both before and after the application of adrenalin chloride to the surface of the pancreas. The results of sugar determination are given in the table on page 45.

The results of Experiments 1, 2, 3, and 6 present, we believe, a true picture of the events in this connection succeeding the application of adrenalin to the pancreas. The samples of blood taken before treatment with adrenalin agree fairly closely in their sugar content. On the ground of these figures it cannot be said that the amount of sugar in the blood issuing from the liver is greater than that of the femoral artery or portal vein. After treatment with adrenalin, however, the relations are changed. In Experiment 3, four minutes after

TABLE II. — Sugar Content of Blood collected simultaneously from the Hepatic and Portal Veins and Femoral Artery before and after Treatment with Adrenalin.

No. of exp.	Weight. Kilo.	Time since last fed. Hrs.	Amount of blood from			Time of collection. P. M.	Time of application of adrenalin. P. M.	Time of collection compared with time of application.	Sugar in blood from			Remarks.
			Femoral artery. Gms.	Portal vein. Gms.	Hepatic vein. Gms.				Femoral artery. Per cent.	Portal vein. Per ct.	Hepatic vein. Per ct.	
1	9.0	72	17.5	31.4	31.2	9.31	9.37	6 min. before	0.10	0.10	0.08	Chest opened[1] and artificial respiration employed.
			14.4	11.4	30.0	9.57		20 min. after	0.15	0.08	0.25	
2	9.2	24	12.7	14.9	16.8	4.23	4.39	16 min. before	0.160	0.165	0.164	At time of first collection of blood, pancreas and intestines normal in appearance. At second collection, pancreas somewhat congested, intestines somewhat cyanotic. At third drawing of blood, the pancreas was very much congested and small intestine very blue.
			13.4	14.4	16.2	4.49		10 min. after	0.247	0.197	0 284	
			13.8	8.4	12.3	5.20		41 " "	0.322	0.245	0.346	
3	7.8	20	12.9	13.7	21.0	4.21	4.40	19 min. before	0.159	0.169	0.136	At time of first and second collections of blood, the pancreas and small intestines were normal in appearance. When the third and fourth series were taken, these organs were much congested.
			12.6	17.6	13.2	4.44		4 min. after	0.187	0 167	0.201	
			12.4	14.5	13.3	5.06		26 " "	0.215	0.187	0.252	
			13.4	13.6	19.1	5.46		66 " "	0.208	0.207	0.209	
4	15.65	24	15.6	15.5	13.85	5.29	5.42	13 min. before	0.311	0.282	0.316	At first drawing of blood, the pancreas and intestines were normal. At the second, the small intestine was congested and cyanotic.
			13.6	20.1	15.45	6.09		27 min. after	0.385	0.314	0.348	
5	5.35	26	15.3	16.5	13.8	3.15	3.25	10 min. before	0.221	0.227	0.210	Pancreas and intestines normal at time of first collections. Pancreas congested and small intestine congested and cyanotic at time of second.
			13.4	11.7	12.0	3.46		21 min. after	0.242	0.158	0.240	
6	13.2	25	14.7	15.15	11.5	5.26	5.36	10 min. before	0.201	0.181	0.208	Pancreas and intestines normal in appearance at first collections. Pancreas congested and intestines congested and cyanotic at time of second.
			16.0	12.4	19.1	6.01		25 min. after	0.208	0.223	0.227	

[1] In this experiment only was it found necessary to open the chest in order to insert the cannula into the hepatic vein.

the application of the substance to the gland the sugar content of the arterial blood rises 0.028 per cent, that of the portal blood remains practically the same, while the increase in reducing power of the blood emerging from the liver amounts to 0.065 per cent. The same relation, though on a higher plane, is apparent twenty-six minutes later. Sixty-six minutes after, the sugar percentage from all the vessels is approximately the same. Precisely similar results are obtained in Experiments 1 and 2, and in a lesser degree in Experiment 6. Judging from the results of these analyses then, a formation of sugar in the liver must be the cause, in part at least, of the increase of sugar in the blood.

Experiments 4 and 5 apparently form exceptions to this conclusion. It will be noticed, however, that the percentage of sugar in the samples taken before adrenalin treatment are abnormally high, especially in Experiment 4. It is possible that the mechanism which takes part in the production of adrenalin glycæmia has already been affected by the operative disturbance. It is not an unfair assumption that the additional impulse given by the application of adrenalin is on that account less effective and its result more transient. Consequently at the time of the collection of the second portions of blood the secretion of sugar is lessening. The same reasoning may hold good with regard to Experiment 5.

Comparison of the sugar content of the portal blood with that from the femoral artery and hepatic vein in Experiments 1, 2, 3, and 5 shows that the increase of sugar following treatment of the pancreas with adrenalin is least in the portal vein. While in the control samples the sugar percentage of the portal is as high or higher than that of the femoral or hepatic blood, after treatment with adrenalin, it is lower in every case. In this connection we would call attention to certain changes in the appearance of the organs of the abdomen. At the time of collection of the first samples of blood the appearance of the intestines and pancreas was normal. As the experiment proceeded, however, the pancreas became congested and the intestines cyanotic. The latter symptom is due, probably, to a partial obstruction of the circulation by the formation of a clot at the flange of the cannula. The effect of this partial obstruction is a partial asphyxia of the tissues. That the *relative* decrease in the sugar of the portal blood is dependent upon increased utilization within the tissues through which it passes there can be no doubt. Whether this consists in a mere increased oxidation of sugar, owing to the increased

TABLE III.

SUGAR CONTENT OF BLOOD TAKEN SIMULTANEOUSLY FROM THE HEPATIC AND PORTAL VEINS AND THE FEMORAL ARTERY, TREATMENT WITH ADRENALIN BEING OMITTED.

No. of experiment.	Weight. Kilo.	Time since fed. Hours.	Amount of blood from			Time of collection.	Sugar in blood from			Remarks.
			Femoral artery. Grams.	Portal vein. Grams.	Hepatic vein. Grams.		Femoral artery. Per cent.	Portal vein. Per cent.	Hepatic vein. Per cent.	
1	13.6	22	14.5	13.1	16.4	4 45 P. M.	0.236	0.213	0.210	Appearance of the pancreas and intestines normal throughout experiment.
			12.9	12.9	11.6	5.05 P. M.	0.220	0.193	0.242	
			13.6	11.7	13.7	5.30 P. M.	0.190	0.198	0.205	
			13.5	15.1	13.7	6.05 P. M.	0.217	0.185	0.217	
2	7.7	29	12.0	14.2	13.8	4.24 P. M.	0.174	0.160	0.166	Intestines became somewhat blue at the time of second and third collections of blood. Pancreas normal.
			14.3	13.5	16.7	4.46 P. M.	0.183	0.179	0.188	
			15.8	11.9	13.2	5.16 P. M.	0.194	0.173	0.174	
3	17.15	48	13.5	15.1	13.3	12.10 P. M.	0.215	0.174	0.216	Intestines somewhat blue at time of second blood collection. Pancreas normal.
			14.5	25.8	14.4	12.42 P. M.	0.264	0.218	0.260	
4	5.7	24	13.5	13.7	18.15	11.50 A. M.	0.188	0.176	0.194	Intestines somewhat blue at second collection. Pancreas normal.
			13.9	13.7	20.7	12.30 P. M.	0.182	0.198	0.190	
5	11.4	24	14.7	14.1	14.9	2.59 P. M.	0.206	0.204	0.202	Pancreas and intestines normal.
			13.4	12.7	17.0	3.34 P. M.	0.175	0.149	0.185	

supply of that substance, or whether there is a decomposition of another character in increased amount, owing to lack of oxygen in the tissues, our experiments do not decide.

In referring to Experiments 4 and 5, the idea has been expressed that the high sugar content found in the control samples was possibly due to the effects of operative disturbance. The question might naturally be raised as to whether the effects noted in our other experiments might not be due to that cause rather than to the influence of adrenalin. To settle this point we have made a series of five control experiments in which the blood was collected in a manner similar to that described, the treatment with adrenalin being omitted. The results are given in Table III, page 47.

These figures show an essential difference from those given in Table II. In only one case does the blood of the hepatic vein contain considerably more sugar than that of the femoral artery. In only one experiment (3) is there an essential rise in the sugar of the femoral blood. The results indicate, therefore, that while in a small percentage of experiments carried out according to this method, the operation may give rise to a hyperglycæmia similar to that produced by adrenalin, in the majority of cases we are justified in attributing the results to the action of adrenalin.

Sugar in the Blood of the Pancreatico-duodenal Vein after Treatment of the Pancreas with Adrenalin.

It has been shown in the experiments of Series II, page 45, that in the hyperglycæmia which follows the application of adrenalin to the pancreas, the increase of sugar is least in the blood of the portal vein. We have attributed this circumstance to an increased decomposition of sugar in the intestinal tissues, and have suggested that it may be connected with a partial obstruction in the circulation of the blood through those tissues. To ascertain whether the congestion of the pancreas which is regularly observed after treatment of that gland with adrenalin takes part in this phenomenon, we have tested the blood from the pancreatico-duodenal vein. Though an increased decomposition of sugar in the pancreas would be at variance with our ideas regarding the events taking place there, its possibility has not been positively excluded.

The method of collecting blood was as follows: A cannula of the design previously described was introduced into the portal vein at a

TABLE IV.

SUGAR CONTENT of BLOOD FROM THE PANCREATIC VEIN BEFORE AND AFTER APPLICATION OF ADRENALIN TO THE PANCREAS.

No. of experiment.	Wgt. Kilo.	Time since fed. Hours.	Amt. of blood from		Time of collection. P. M.	Time of application of adrenalin. P. M.	Time of collection compared with time of application.	Sugar in blood from		Appearance of pancreas.
			Femoral artery. Grams.	Pancreatic vein. Grams.				Femoral artery. Per cent.	Pancreatic vein. Per cent.	
1	13.4	22		9.5	4.15	4.27-4.28.05	13 min. before.		0.202	Normal.
				14.7	4.31.05		3 min. after.		0.275	Pale.
				10.4	4.37		8 min. 55 sec. after.		0.307	Very slightly congested.
2	10.4	24		12.4	3.57	4.04 30–4.06.05	9 min. before.		0.209	Normal.
				10.6	4.06.30		25 sec. after.		0.277	Very pale.
				10.1	4.11.30		5 min. 25 sec. after.		0.287	Somewhat congested.
3	18.6	30		12.8	4.02	4.08–4.09.20	7 min. 20 sec. bef.		0.180	Normal.
				13.2	4.10.05		45 sec. after.		0.212	Very pale.
				9.4	4.17.40		8 min. 20 sec. after.		0.215	Somewhat congested.
				15.1	4.25.35		16 min. 15 sec. after.		0.236	Very much congested.
			16.9		4.27		17 min. 40 sec. after.	0.226		
				16.9	4.35.35		26 min. 10 sec. after.		0.218	Very much congested.
			16.3		4.38		28 min. 40 sec. after.	0.210		

point just opposite the entrance of the pancreatico-duodenalis. Loose ligatures were placed about the portal vein, one on either side of the cannula. The cannula was opened at the same time that the ligatures were tightened. Blood is thus obtained from the desired vein, free from admixture with portal blood. The portal circulation is interrupted for a few seconds, but the pancreatic not at all.

We have collected blood in this manner both before and immediately after painting the pancreas with adrenalin, and analyzed it for sugar. The results are given in Table IV.

The results of these experiments are very uniform. In Experiment 1, the percentage of sugar in the pancreatic blood rose 0.073 per cent in the first three minutes after adrenalin was applied. In Experiment 2, an increase of 0.068 per cent occurs within twenty-five seconds. In Experiment 3, the rise in the first forty-five seconds amounts to 0.032 per cent. In the last experiment, we have continued the collection of blood when the gland was very much congested, and have compared these samples with portions taken at the same time from the femoral artery. The analyses show a continued rise in the sugar percentage, and only a slight difference in the blood from the two sources. We are forced to conclude, therefore, that there is not an increase in the decomposition of sugar in the pancreas antecedent to the rise of sugar in the general circulation, and that the difference observed in the second series, between the reducing power of the blood of the portal vein and that of the femoral artery, is not dependent on processes of this nature in that gland.

Summary of Conclusions.

1. The intraperitoneal injection of adrenalin chloride, as well as the application of that substance to the pancreas, gives rise to a marked increase of sugar in the blood. This hyperglycæmia makes its appearance immediately after the administration, reaches its maximum in from one to three hours, and may continue for over fourteen hours.

2. Simultaneously with hyperglycæmia occurs a decided diminution in the time of extravascular coagulation of the blood. This phenomenon appears to be due also to the application of adrenalin to the pancreas.

3. The cause of this form of hyperglycæmia, as indicated by comparative analysis of the blood flowing to and from the liver, is to be

attributed, in great part at least, to an increased sugar formation in that organ.

We are indebted to Dr. C. A. Herter for the suggestion of the subject of this work, and for valuable counsel during its progress. We also wish to express our obligation to Mr. William D. Cutter for assistance in a number of the operations.

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ON THE IRRITABILITY OF THE BRAIN DURING ANÆMIA.

By WILLIAM J. GIES.

[*From the Physiological Institute of Bern University.*]

I. Introduction.

DURING the summer of 1899 I had the pleasure of assisting Professor Kronecker in a study of the irritability of the brain during anæmia.[1] Our research could not be concluded during my stay in Bern that summer, but we both looked forward to completing it together in the following year. Unfortunately for me, return to the Physiological Institute has been impossible thus far, and the work which has been delayed on that account has lately been resumed by Professor Kronecker and Dr. Stumme. At the suggestion of Professor Kronecker, the results of our investigation are presented here in some detail though briefly.

In the preparation of these notes I have received numerous suggestions from Professor Kronecker, who has also revised the statements relating directly to our experiments. Throughout practically all of our research, Professor Kronecker not only directed the work, but did a very large share of it. His well-known generosity to his pupils is again shown by his desire that this investigation, which was chiefly his, shall seem to be wholly mine.

II. Description of Experiments.

In this research we sought especially to determine the order of cessation, as well as the period of continuance, of certain reflexes during anæmia of the brain.

Acute anæmia was brought about by perfusion with the solutions indicated on the next page.

The animals employed were toads, frogs, rabbits, and dogs.

[1] Gies: Report of the British Association for the Advancement of Science, 1899 (Dover), p. 897.

The solutions used were various strengths of pure sodium chloride, Ringer's solution, and modifications of it, Schücking's solution (both of sodium and calcium saccharates), rabbit and horse serum, and 0.7 per cent sodium-chloride solution containing paraxanthin or chloralbacid.

Experiments on toads and frogs. — Perfusion in the cold-blooded animals was conducted with the least possible pressure through the abdominal vein. In this series of experiments we used all of the various solutions already enumerated, except serum.

Seventeen experiments were made, seven with toads and ten with frogs, each of which was continued for a period of from one to nine hours. The total amount of perfused fluid varied from 25 c.c. to 1590 c.c. In most cases perfusion was continued until the heart ceased to beat.

The table on page 133 gives a summary of the more important results obtained in this connection. The terms "skin," "lid," and "nose," in the table, refer to the reflex movements caused by pressure on those parts.

During the period of perfusion, the following functions gradually weakened, and then usually disappeared in this order: (1) respiration, (2) skin reflex, (3) lid reflex, (4) nose reflex, (5) heart beat.

The relative time of cessation of these reflexes varied considerably, not only with the character of the solutions, but also with the rapidity of their perfusion and the amounts used.

Convulsive extension of the limbs occurred in all the experiments in the earlier stages, but toward the close of each experiment and before the reflexive movements of the eyelids ceased, no such manifestations were observed, nor could they be induced by mechanical stimulation.

Perfusion of physiological saline solution containing 0.03 per cent of paraxanthin induced hyperæsthesia at first, but the reflex responses quickly came to an end, as the perfusion continued. Cumulative muscular rigor was the most pronounced feature of the experiment. At the end of the experiment the body was perfectly stiff. With a solution containing 0.015 per cent paraxanthin, moderate hyperæsthesia was observed at first, as in the case of the 0.03 per cent solution, but the rigor of the former experiment was absent in this.

During perfusions with physiological salt solution containing 1 per cent chloralbacid, repeated spasmodic extension of the extremities was the main feature. With the solution containing 0.33 per cent

TABLE I.

Number.	Animal.	Solution used.	Total time of perfusion.	Cessation of reflexes. Time after beginning the perfusion.					Amount of solution perfused.	Red corpuscles at the end of perfusion in fluid from		
				Resp.	Skin.	Lid.	Nose.	Heart beat.		Cannula.	Heart.	Brain.
			h. m.	h. m.	h. m.	h. m.	h. m.	h. m.	c.c.			
1	Toad	NaCl — 0.6%	6 15	4 15	5 25	5 30	6 00	6 15	475	+	+	+
2	"	" "	8 10	6 15	7 40	7 45	7 55	8 10	780	—	+	+
3	"	" — 0.8%	3 45	2 25	3 10	3 25	3 25	3 45	290	+	+	+
4	"	Ringer's [1]	8 30	5 25	7 25	7 45	8 05	8 30	740	—	+	+
5	"	"	9 10	6 00	6 15	6 15	6 30	9 10	1590	—	+	+
6	"	Ringer's [2]	4 45	2 00	3 15	3 30	3 45	4 45	625	+	+	+
7	"	"	3 15	2 15	2 30	2 35	2 35	3 15	575	+	+	+
8	Frog	"	3 00	1 00	2 30	2 20	2 25	3 00	600	+	+	+
9	"	NaCl — 0.6%	3 30	2 15	2 40	2 50	2 55	3 30	275	..[3]	..[3]	..[3]
10	"	" "	3 30	2 00	2 20	2 50	2 50	3 30	275	..[3]	..[3]	..[3]
11	"	" "	1 20	0 30	1 00	1 15	1 15	1 20	180	..[3]	..[3]	..[3]
12	"	NaCl — 0.7% Calcium saccharate — 0.03%	5 50	1 10	2 45	2 50	2 55	....[4]	650	—	+	+
13	"	" "	8 30	2 00	3 20	3 30	3 25	....[4]	730	—	+	+
14	"	NaCl — 0.7% Paraxanthin — 0.03%	1 25	0 35	1 00	1 10	1 05	1 25	95	+	+	+
15	"	NaCl — 0.7% Paraxanthin — 0.015%	2 05	0 30	1 50	1 55	1 55	2 05	145	+	+	+
16	"	NaCl — 0.7% Chloralbacid — 1% . .	0 50	0 24	0 35	0 35	0 33	0 50	25	+	+	+
17	"	NaCl — 0.7% Chloralbacid — 0.33%	1 05	0 29	0 36	0 50	0 52	1 05	120	+	+	+

[1] White's modification: 0.6% NaCl, 0.01% $NaHCO_3$, 0.01% $CaCl_2$, 0.0075% KCl.
[2] Howell's modification: 0.7% NaCl, 0.026% $CaCl_2$, 0.03% KCl.
[3] Not ascertained.
[4] Heart continued to beat long after the conclusion of the experiment.

of chloralbacid, spasmodic twitching in the limbs was the most noticeable incident.

At the end of the experiments with the solutions containing paraxanthin and chloralbacid, after the heart had ceased to beat, solution of calcium saccharate was perfused. In each case this solution caused the heart to begin beating, and rapidly induced the normal stroke and rhythm.

Before passing to the next series, it should be stated that in each of the preceding experiments the animal became œdematous. Even those animals in which perfusion took place at the lowest possible pressures, and for the shortest intervals, showed unmistakable signs of œdema.

It was impossible to remove entirely the blood-corpuscles from the capillaries in the heart and brain, even when the perfusion was continued uninterruptedly for eight hours, and as much as 1590 c.c. of fluid had slowly passed through the body. In all cases the fluid expressed from the heart and brain contained an appreciable number of red and white corpuscles.

In most of the experiments, when the heart had come to a standstill after continuous irrigation with physiological saline solution, also Ringer's solution, rhythmical contractions could be promptly induced by perfusing Schücking's solution. This result was obtained even when mechanical and electrical stimulation had failed to restore the normal beating.

Experiments on rabbits. — We report the results of thirteen experiments on rabbits. In this series we used all of the so-called "indifferent" solutions already mentioned.

Considerable difficulty was experienced in our efforts to devise a method which would prevent almost instant death of the animals, and yet which would speedily result in pronounced anæmia.

Ligaturing the arteries to the brain, before or simultaneously with the beginning of the perfusion, brought on convulsions immediately. This was the case whether the ligatures were placed about the arteries in the neck or in the chest. Even when the perfusion had been begun shortly before the arterial blood was completely shut off, it still remained impossible to prevent convulsions and quickly ensuing death.

In Experiments 1–5 (see the table on page 135), the blood-vessels in the neck were quickly tied as perfusion was begun. In Experiments 6–10, they were tied just above the heart as perfusion was

instituted. Experiments 11–13 were carried out by the following method.

Instead of closing the arteries to the brain, the abdominal aorta, vena cava, and vena porta were ligated, and the heart's action utilized to pump the perfusion fluid through the brain. The warm solution was directed into the heart by way of one jugular, and passed from the brain by way of the other. With this method, anæmia was gradually though quickly induced, convulsions were entirely prevented, and life was considerably prolonged.

In all cases, microscopic examination of the fluid pressed from the brain showed the presence of red corpuscles.

TABLE II.

Number.	Weight of Animal.	Solution used.	Total time of perfusion.	Cessation of reflexes. Time after beginning the perfusions.				Amt. of solution perfused.	Pressure of perfused fluid.	Lung œdema.	Blood drawn at beginning of experiment.	Hæmoglobin in exit fluid at end.
				Resp.	Lid.	Nose.	Heart beat.					
	gms.		min.	min.	min.	min.	min.	c.c.	mm. Hg.		c.c.	per cent.
1	1300	Rabbit serum	17	16	14	14	20	110	90–120	+	..	....
2	1100	NaCl — 1%	7	(?)	(?)	(?)	(?)	30	130–150	+	..	....
3	1500	" "	15	(?)	12	7	18	200	90	..	15	....
4	1800	" "	9	8	7	6	9	250	90–110		23	....
5	1600	" "	7	(?)	2	2	(?)	35	90	..	17	....
6	1800	NaCl — 1% / Calc. sach. — 0.035%	9	4	2	3	6	230	75–85	..	..	10–12
7	2800	" " "	27	(?)	2½	4	25	450	110–140	..	9	8–11
8	1600	Rabbit serum	8	3	3	5	8	110	110–150	..	..	....
9	1400	NaCl — 1% / Calc. sach. — 0.035%	2	(?)	(?)	(?)	(?)	15	100	+	..	....
10	1500	" " "	8	(?)	(?)	(?)	(?)	40	100	+	..	....
11	1800	" " "	20	10	14	16	20	350	....	+	..	....
12	2000	NaCl — 1%	25	20	13	22	26	260	70–110	..	..	6
13	1900	Rabbit serum	14	11	9½	12	14	150	80–120	..	..	....

The disappearance of functions in these experiments was not at all regular in the first ten. The events of each experiment transpired so quickly that it was extremely difficult to note accurately the time of cessation of each reflex. In the last three experiments respiration ceased first in one, second in two; the "lid reflex" disappeared first in two, second in one. In each of the last three experiments, the "nose reflex" was the third to disappear. Heart beat was always fourth in the order of cessation.

Experiments on dogs. — Only two experiments were performed on dogs. The first was by a method similar to that in the tenth experiment with rabbits. The weight of the dog was 12 kilos. The pressure of perfusion was 140–150 mm. Hg. The amount of blood drawn at the beginning of the experiment was 47 grams. The perfusion fluid was a 0.7 per cent solution of sodium chloride containing 0.03 per cent calcium saccharate. Perfusion was continued for forty-two minutes. The volume of fluid perfused was 1125 c.c. The amount of hæmoglobin present in the fluid leaving the jugular vein at the end of the experiment was 30 per cent of the normal content in blood.

Reflex responses failed in the following order: (1) lid and nose reflexes in twenty-six minutes; (2) respiration in forty minutes; (3) heart beat in forty-two minutes.

There were no convulsions at any stage of the experiment.

In the second experiment, with a small dog weighing only 5 kilos, 200 c.c. of blood was taken, and an equal quantity of horse serum immediately afterwards was transfused to take its place. This process was repeated three times at intervals of half an hour. After the fourth blood-letting, the dog ceased to breathe, and did not recover when the new portion of serum was transfused. Aside from variations in heart action and respiration, no special functional changes were observed until the end, when respiration suddenly ceased, and the other functions came to an end about the same time. Death was neither preceded nor accompanied by convulsions.

III. Summary of Conclusions.

The more important conclusions of these preliminary experiments are that when the brain is subjected to anæmia by the process of perfusing solutions, such as Ringer's, Schücking's, serum, etc., its functions soon cease. When the anæmia is induced rapidly, convulsions

ensue. When it is brought about gradually, anæmia may be made acute without causing the appearance of convulsions.

When anæmia of the brain is produced gradually by the methods used in these experiments, the functions here referred to cease usually in the following order:

(A) In cold-blooded animals: (1) respiration, (2) skin reflex, (3) lid reflex, (4) nose reflex, (5) heart beat.

(B) In warm-blooded animals: (1) lid reflex, (2) respiration, (3) nose reflex, (4) heart beat.

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AN EXTREME CASE OF SIMPLE ANÆMIA.

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AND

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NEW YORK.

History.—X——, female, nineteen years old, single, mulatto, waitress, was admitted to Dr. Delafield's service at Roosevelt Hospital, November 13, 1899. Her mother was dead of consumption. She had had no previous illness which could bear on the case. Her health had always been good. In April, 1899, she had been delivered of her first child, at term, in an institution. A normal puerperium followed. She was set to work again as soon as possible. In August she began to feel weak and to suffer from headache and vertigo. About one month later she began to notice dyspnœa on exertion with marked cardiac palpitation, and slight cardiac pain at times. In October a persistent diarrhœa began, of six to eight large, fluid, fecal movements a day, containing no blood or mucus and not associated with tenesmus or colic. Marked anorexia but no vomiting accompanied the diarrhœa. She had to give up work. In November she fainted once and her ankles became moderately œdematous. She continued to nurse the infant till the first week in November. She had not menstruated since her pregnancy.

On admission to the hospital she had almost no red color in her skin or mucous membranes. The moderate pigmentation natural to her race gave the surface of her body a leaden hue, but there was no tendency to a yellow cachectic color. She was poorly nourished but not markedly emaciated. There were no abnormal physical signs over the lungs and no dys-

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pnœa while she was quiet. The heart was normal in size and position, its action weak, rapid, and slightly irregular. There was a hæmic systolic murmur at the apex and one over the second left space. The pulse corresponded to the heart action. The arteries were neither thickened nor contracted. There were no abnormal signs over the abdomen, which was thin and retracted. There was slight œdema of the ankles. The voice was weak, and the general weakness and apathy were very marked indeed. Temperature, 101° F.; pulse, 140; respiration, 36. The urine was acid, pale, clear; s. g. 1.011; it contained no albumin and no sugar. Microscopical examination was negative. Blood: HB., 12 per cent.; R. C., 750,000; W. C., 3,300.

The patient was put to bed and given milk and one solid meal a day, which was increased to three meals two weeks later. She was started on gr. xxiv. of sulphate of iron and gr. $\frac{1}{10}$ of arsenious acid in twenty-four hours, and two weeks later the iron was increased to gr. xxxix. A little codeine was given to control the diarrhœa.

During the first week she was in the hospital, her temperature ran between 101° and 103.8°, averaging 102°; during the second week between 98° and 101°, averaging over 100°, and showing a tendency to reach its maximum about noon and its minimum about midnight; during the third week about the same as in the second the breaks being more pronounced and sustained; during the fourth week between 98° and 100.6°, averaging 99.4°, with the same tendency to rise about noon; during the fifth week it never reached 100°, and after that time it ran a normal course.

The œdema disappeared in a day or two. After ten days' treatment her general strength began to improve. After two weeks her appetite commenced to come back, her voice and pulse grew stronger. Pulse, 90; respiration, 20. At this time, also, the diarrhœa began to yield. During the third week her color began to return and she was able to leave her bed. The changes for the better, once instituted, proceeded with surprising rapidity and she left the hospital, two months after entering it, practically well.

The symptoms, then, which the change in the composition of the blood caused in this case, besides the pallor, were marked general weakness, some headache, syncope, dyspnœa on exertion, cardiac palpitation, disturbed heart action, anorexia, diarrhœa, and œdema of the ankles. The absence of menstruation cannot certainly be attributed to the change in the blood, because of the nursing. It is noteworthy that there was neither bleeding, nor vomiting, nor cachectic color of the skin.

After leaving the hospital she continued to take gr. xv. of sulphate of iron a day for two weeks, and since then she has taken practically no medicine. One month after leaving the hospital she began to menstruate and has been regular ever since. She has been steadily employed for over one year now, and, except for being somewhat prostrated by the extreme heat last summer, has enjoyed perfect health. She was last seen February 5th of this year.

Blood.—The blood was examined once a week during the patient's stay in the hospital, and, after her discharge, at first every two weeks, then every month or two, and latterly at intervals of three or four months. The HB. examinations were made by Fleischl's method. The outlines of the corpuscles were traced with a camera from smeared preparations (A), but, in order to obtain accurate figures of the small and deformed cells, at each of the first eight examinations a few of these were traced from a specimen of fresh blood before crenation had set in (B). Following are the records of each examination:

On admission: HB., 12 per cent.; R. C., 750,000; W. C., 3,300. The red cells varied considerably, but not extremely, both in size and shape. A number of red cells were larger than normal (macrocytes), measuring 10.5 μ in diameter, a number were small and deformed (microcytes). The HB. was moderately unevenly distributed. "Ringing" and extreme pallor of the cells were not present, except in the small deformed cells, at this or any subsequent examination. A very few nucleated red cells (normoblasts) were found. Changes in the coloring matter of the red cells were not observed. The white cells for the most

part were normal. A considerable number of degenerating white-cell nuclei were to be seen and an abnormally large percentage of small mononuclear leucocytes (over thirty per cent.) were present, a feature which obtained in the first seven examinations, *i.e.*, as long as the differential counts were continued. There were no excess of eosinophiles and no myelocytes at that time or later (Fig. 1).

Second week: HB., 18 per cent.; R. C., 645,300;



A B

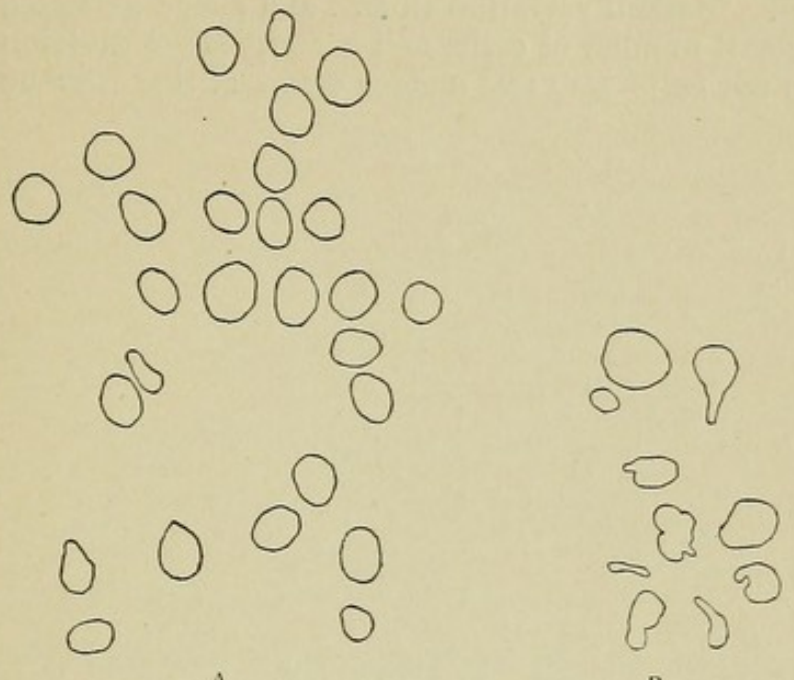
FIG. 1

W. C., 4,200. The appearances were identical with those above described.

Third week: HB., 25 per cent.; R. C., 1,016,000; W. C., 5,600. A distinct improvement in the appearance of the blood was evident. The marked variations in size and shape were less frequent, the moderate variations were still universal, and there were still a number of macrocytes. The HB. was more evenly distributed. One nucleated red cell (normoblast) was found. The white cells were practically normal (Fig. 2).

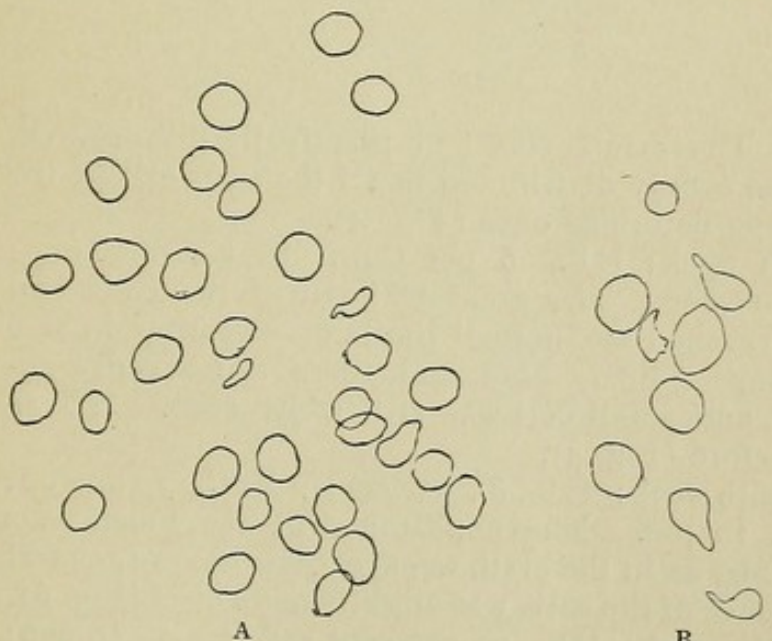
Fourth week: HB., 45 per cent.; R. C., 1,568,000; W. C., 5,600. The universal variation in size and shape was less marked, but the considerable variation

of a certain number of cells persisted, dividing the red cells more or less into two classes. The HB. was pretty evenly distributed except in the small and deformed cells in which it was irregular and often defi-



A B

FIG. 2.



A B

FIG. 3.

cient. The tendency to form macrocytes was less pronounced (Fig. 3).

Fifth week: HB., 58 per cent.; R. C., 2,556,000; W. C., 9,200. The universal change in shape and size was distinctly less than at the preceding examination. Marked variation in size and shape persisted in a small number of cells, and a pretty strict division of the red cells into two classes could at this time be

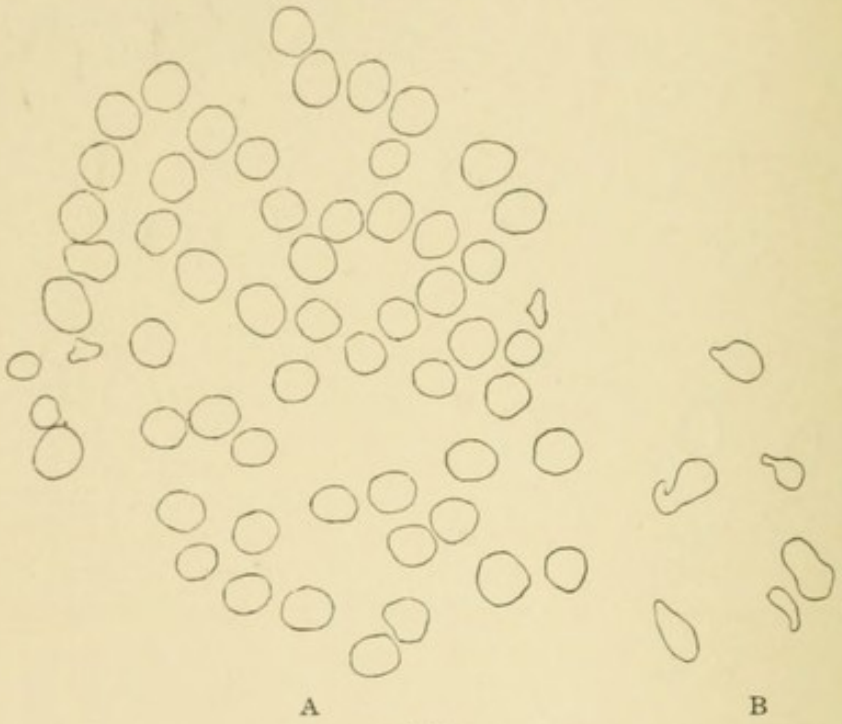


FIG. 4.

made. The macrocytes had practically disappeared. HB. was evenly distributed in all the cells except the small and deformed ones (Fig. 4).

Sixth week: HB., 66 per cent.; R. C., 2,900,000; W. C., 11,800. The great majority of the cells had nearly reached the normal limits of variation in size and shape. Every here and there a markedly deformed and small cell was still to be seen. HB. as week before (Fig. 5).

Seventh week: HB., 85 per cent.; R. C., 3,520,000; W. C., 12,400. The appearance of the blood was practically as in the sixth week, except that the general appearance of the cells was slightly more even (Fig. 6).

Eighth week: HB., 90 per cent.; R. C., 3,556,000;

W. C., 11,400. The appearance of the blood was now normal except for the small and deformed cells usually deficient in HB., which could be found in every second or third field (Fig. 7).

Ninth week: HB., 97 per cent.; R. C., 3,796,000;

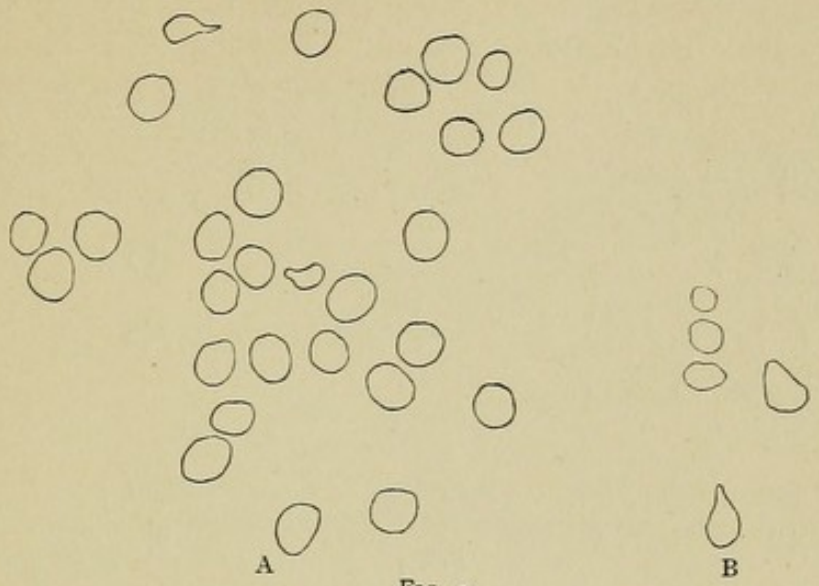


FIG. 5.

W. C., 7,000. The appearance was the same as at the eighth week, but the variation in size and shape of the small cells was not so marked. Just after this examination the patient left the hospital.

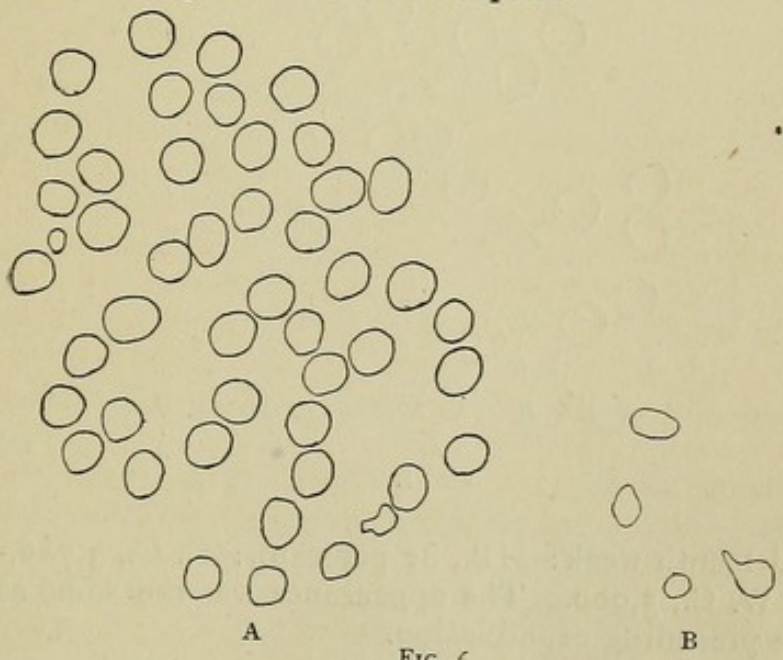
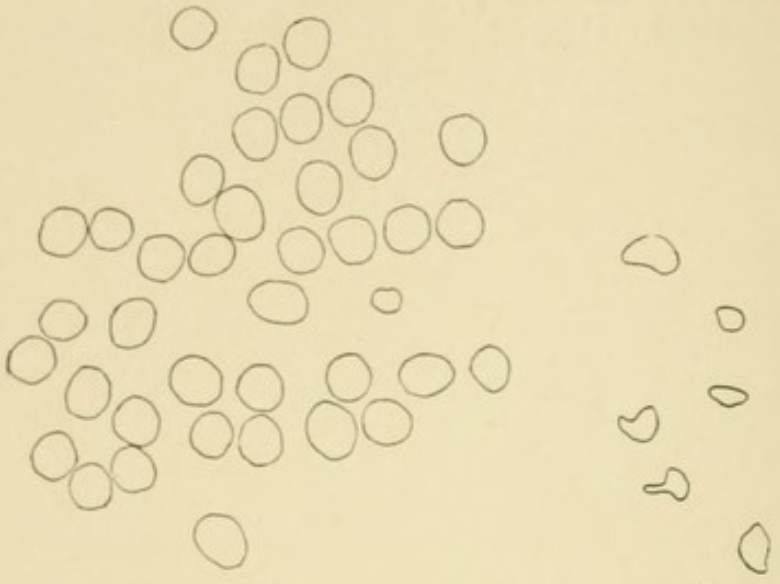


FIG. 6.

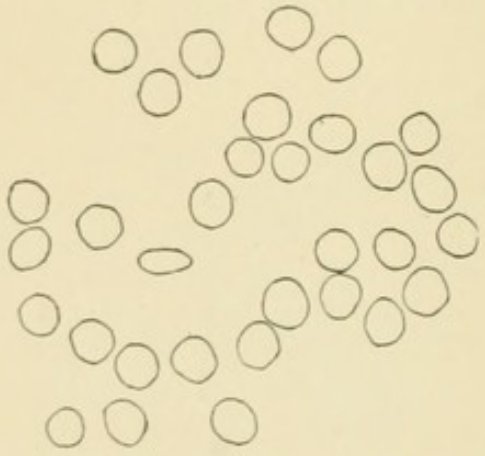
Eleventh week: HB., 78 per cent.; R. C., 3,500,-000; W. C., 10,200. The general appearance of the blood still fell within the normal limits of size and



A B

FIG. 7.

shape variation, but there was slightly more variation than two weeks before. The small and deformed cells were seen in every third or fourth field (Fig. 8).



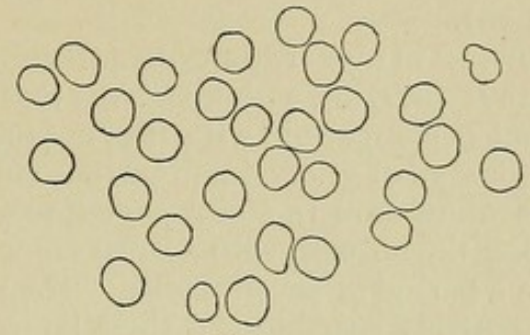
A

FIG. 8.

Thirteenth week: HB., 82 per cent.; R. C., 3,786,-000; W. C., 5,000. The appearance was the same as at the preceding examination.

Fourth month: HB., 76 per cent.; R. C., 4,592,000; W. C., 7,200. The appearance was the same as at the last preceding examination. Small and deformed cells were still present.

Sixth month: HB., 87 per cent.; R. C., 4,496,000;

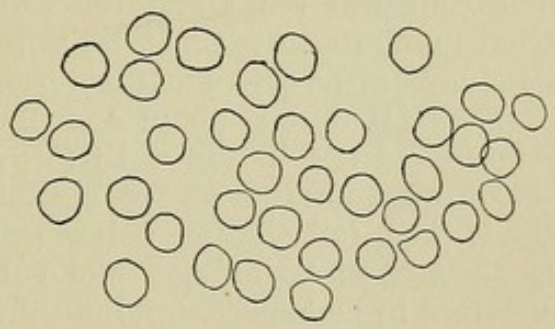


A

FIG. 9.

W. C., 8,200. The appearance was the same as above, but the small and deformed cells were not so frequent.

Seventh month: HB., 77 per cent.; R. C., 4,712,000; W. C., 9,000. The variation in size was still within but close to the normal limits; the shape was regular.



A

FIG. 10.

Small and deformed cells were growing still less frequent.

Eighth month: HB., 80 per cent.; R. C., 4,800,000. The cells had grown a little more regular in shape. The small and deformed cells had practically disappeared—one or two were found (Fig. 9).

Eleventh month: HB., 73 per cent.; R. C., 4,196,-

000; W. C., 5,800. The variation in shape was again within but close to the normal limit. One small and deformed cell was found after considerable search.

Fifteenth month: HB., 70 per cent.; R. C., 4,712,000; W. C., 6,800. The variation in size had again become less. No small and deformed cells were found (Fig. 10).

In the above set of observations the following features are noteworthy: Starting with about the most depleted condition that is compatible with life, the blood, under a maximum dosage of gr. xxxix. of ferrous sulphate and gr. $\frac{1}{10}$ of arsenious acid in a day, passed to a condition approximating the normal, with a subsidence of all the symptoms in a period of seven weeks. Then, the treatment being entirely suspended a little later, the blood continued in about the same condition for one year and one month after convalescence had been established. The variation in size and shape of the cells, at first very marked though not extreme, steadily diminished until the normal limits were reached at the eighth week, never again to be transgressed. An inconsiderable and diminishing number of the cells, however, continued to be small and deformed as late as the eighth month. Nucleated red cells (normoblasts) were found with difficulty at the first three examinations and not at all thereafter. Macrocytes were found in considerable numbers at first but disappeared at the fifth week. The HB. index, about 1 at the first examination, increased till it was a little over 1 and remained so throughout the active period of convalescence. It then fell slightly below 1 and stayed there. The absence of "ringing" and pallor of the cells was therefore to be expected. Both the HB. and the number of red cells reached the full normal limit but at different times. The white cells, reduced in number at first, rose distinctly above the normal count at the seventh week, then fell again to normal and remained there. No myelocytes were seen. The accompanying chart will make some of these points more clear.

The case is classed as simple anæmia because of the rapidity and degree of the recovery. The age, the medication—almost exclusively ferrous sulphate—the absence of cachexia, the continued absence of all

symptoms without treatment, are corroborative points of interest. It is not unlikely that the patient is now tending toward a relapse of moderate severity as is frequent in simple anæmia.

Urine and Fæces.—Our chemical examination of the urine and fæces gave us the following data[1]:

Urine: The urine varied in color from a very pale

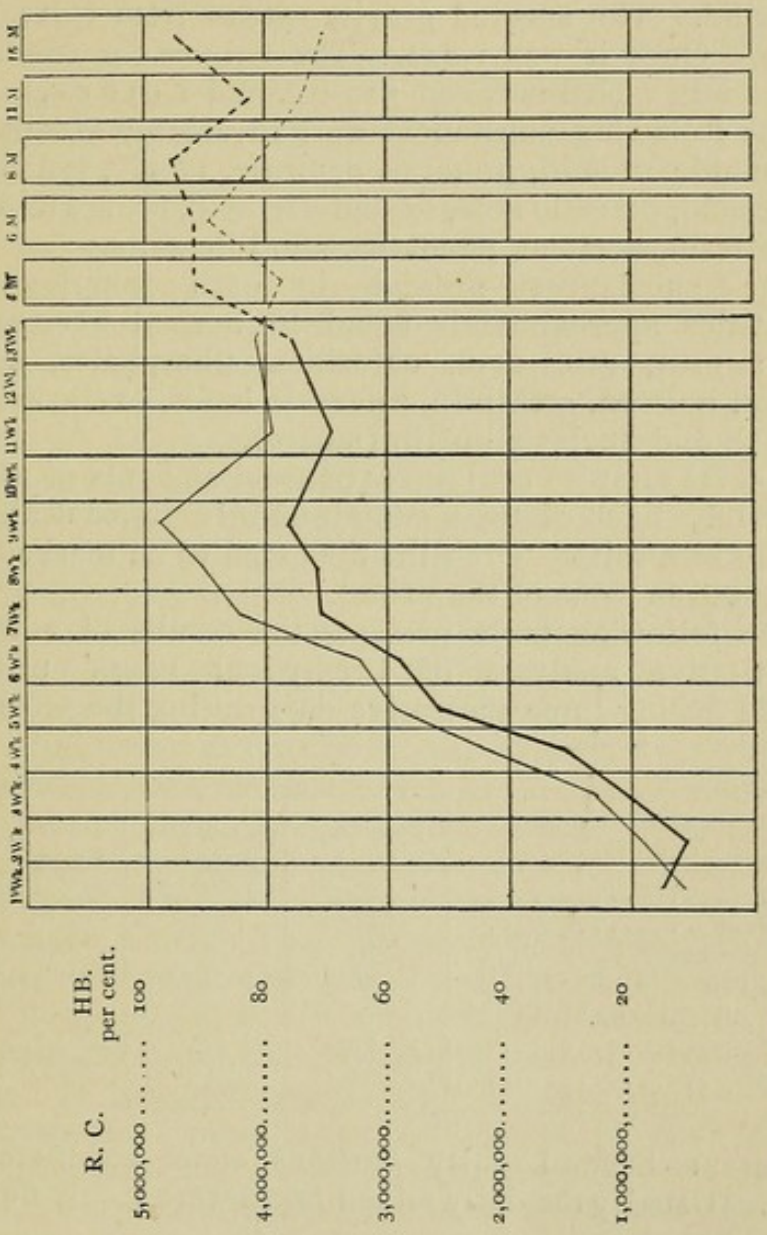


FIG. 11.—Chart to Show the Weekly Changes in the Blood and its Condition at Longer Intervals after the Thirteenth Week. Light line, hæmoglobin; heavy line, red cells.

[1] Analyses of urine and fæces were made daily during the first four weeks, in the department of physiological chemistry of Columbia University, at the College of Physicians and Surgeons.

to a deep golden yellow. Several samples were more highly colored than normally. The reaction was usually alkaline; once or twice amphoteric (litmus). A slight sediment settled out in each twenty-four hours' urine. This usually contained epithelial cells, triple phosphate, and calcium phosphate; now and then pus cells, ammonium urate, and calcium oxalate were found. The sediment never contained casts of any kind. The specific gravity ranged from 1.011 to 1.019. Once it was 1.022. The volume for twenty-four hours varied between 515 c.c. and 1,011 c.c.

The following substances were invariably absent[1]: coagulable proteid, proteose, dextrose, leucin, tyrosin, lactic acid, diacetic acid, oxybutyric acid, hæmoglobin, cholesterin, acetone, ptomains, and bile salts.

The substances present in each sample, in quantities approximately equal to normal amounts, were: urea, uric acid, chlorides, phosphates, sulphates, indican, creatinin, alloxuric bases, oxalic acid, phenol, and nucleo-albumin (mucin).

Several samples contained unusual amounts of uroerythrin. Each of these contained bile pigment also, but no bile salts. Urobilin appeared to be markedly increased in some of the urines.

The following table presents the results of a few quantitative analyses of twenty-four hours' urine passed during three successive days during the second week:

	First Day.	Second Day.	Third Day.
Urea	18.500 gm.	23.600 gm.	19.800 gm.
Uric acid	.481 "	.527 "	.613 "
Alloxuric bases	.066 "	.093 "	.080 "
Ratio, uric acid to urea	1 : 38.5	1 : 44.8	1 : 32.3
Ratio, alloxuric bases to uric acid	1 : 7.2	1 : 5.6	1 : 7.7
Volume	680 c.c.	884 c.c.	862 c.c.

Fæces: Several daily portions were completely fluid. Usually the daily mixed fæces were partly fluid

[1] In all of our chemical tests, on both urine and fæces, we employed the methods our own experience and the work of others have shown are the most satisfactory.

and partly solid. The odor was always very strong, those of aromatic bodies and fatty acids predominating. A few samples were yellowish in color; usually they were greenish-gray to greenish-black. The more solid portions were never homogeneous, varying much in color and composition. Small mucous clots were contained from time to time. The reaction was always alkaline.

The solid matter, examined under the microscope, contained starch granules, muscle fibres, connective-tissue fibres, fat droplets, pigmented particles (yellow), epithelial cells, triple phosphate, soap crystals, calcium phosphate, and myriads of bacteria.

We were unable at any time to find albumin, peptone, proteose, urea, ptomains, blood corpuscles, Charcot-Leyden crystals, hæmatoidin crystals, or cholesterin in crystalline form.

The following substances, in dissolved form, were readily identified, however, by the usual methods, some only occasionally: cholesterin, bile salts, fatty acids, indol, skatol, phenol, tyrosin, alloxuric bases, stercobilin, hydrobilirubin, nucleo-albumin (mucin), and bile pigment.

A review of the chemical data of this case brings out the fact clearly that little was found which may be attributed to any single line of metabolic disturbance —practically nothing that may be said to be peculiar to the anæmic condition.

The specific gravity of the urine was low, its color usually pale, and the volume somewhat less than the average, yet the fluctuations were mostly within the normal limits. The sediment contained nothing of special significance. The amount of urea was low, that of uric acid and alloxuric bases at, or somewhat above, normal; but the ratios of urea to uric acid, and of uric acid to alloxuric bases were within the customary fluctuations. Chlorides, phosphates, and sulphates appeared to be relatively as great in amount as urea. Deductions from our quantitative results cannot be very accurate, however, since it was impossible in this instance to regulate satisfactorily the quantity and character of the patient's food.

Albuminuria of hæmic origin was suspected in view

of the fact that albumin has been eliminated in the urine during anæmia, but coagulable proteid could not be detected at any time. The uroerythrin we found several times, along with a little bile pigment, and the increased urobilin in some of the urines, rather suggest that the normal pigment metabolism was somewhat disordered, but as the first two substances were present only a few times, little importance can be attached to their occurrence. Although ptomains, putrescin particularly, have been found in the urine during pernicious anæmia, they were entirely absent in this case. The absence of lactic acid is also noteworthy, several observers having assumed it to be a constant constituent of the urine in pernicious anæmia.

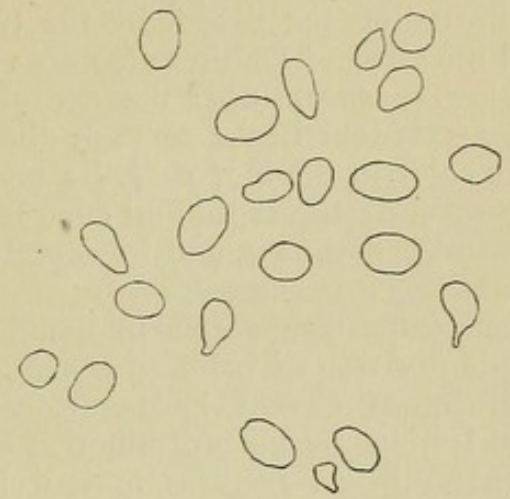
There appeared to be little of special significance in the composition of the fæces.

These results are valuable, we think, chiefly because of their negative character, and we are inclined to believe that, up to the present, few if any special chemical qualities of the excreta have been definitely established as pathognomonic of the various anæmic conditions. Unfortunately, practically all of the work of the past in this direction has been of a very fragmentary character; the results differ widely, and most of them have been recorded with little regard for such influences modifying the usual course of metabolism as the quantity, character, and composition of the food; various secondary pathological conditions; to say nothing of other incidental factors of importance.[1]

A Compared Case of Pernicious Anæmia.—In connection with this case we wish briefly to call attention to another. A woman, sixty-four years old, had been gradually losing strength but not flesh, and getting pale and sallow for one year. She had grown much more rapidly worse for two months. She presented marked anorexia, no vomiting, constipation, no

[1] We are indebted to Dr. William A. Taltavall for the following facts: He examined the urine and fæces in three cases of pernicious anæmia; putrescin and cadaverin were invariably absent; urobilin was not increased; uric acid and nuclein bases appeared to be diminished somewhat. The latter consisted mostly of hypoxanthin.

pulmonary or cardiac symptoms, no evidence of bleeding anywhere. When admitted to the hospital she was well nourished, but very pale and sallow. Her lungs were normal. Her heart was normal in size and position; its sounds were very weak, its action was slightly irregular, the pulse corresponding. Artery was normal. No abnormal signs were elicited over the abdomen. Slight œdema of the ankles was present. The voice was very weak. Extreme general prostration



A

FIG. 12.

was evident. Temperature, 99.4° F.; pulse, 76; respiration, 28. The urine was acid, s. g. 1.012, with a considerable trace of albumin and a few hyaline casts.

Blood: HB., 22 per cent.; R. C., 756,000; W. C., 4,800. The red cells varied considerably, but not extremely, both in size and shape. There was some tendency to the formation of macrocytes. A moderate number of small deformed cells were present. The HB. was fairly even in most of the cells, but often very deficient in the small ones. One normoblast was found after prolonged search, but no megaloblast. The HB. index was somewhat over 1. There was nothing abnormal about the white cells. No myelocytes were found (Fig. 12).

The patient was put on fluid diet and given gr. xx. of ferrous sulphate, gr. $\frac{1}{4}$ of arsenious acid, and ʒ ii. of carnogen every day. Anorexia and vomiting be-

gan very soon. Attacks of syncope occurred. She grew steadily weaker and became restless. The temperature rose over 100° F. only on the last two days of her illness. Death occurred at the end of one week from asthenia.

The blood on the day before death was HB., 16 per cent.; R. C., 576,000. The appearance of the blood was in no way changed from that of the week before. Although no autopsy was held, the case was considered clinically typical pernicious anæmia.

The similarity of the blood in the two cases is striking and the clinical features also are very similar, with the following exceptions: the age—nineteen in the first case, sixty-four in the second; the absence of the cachectic color in the first case, its marked occurrence in the second; the rapid and complete response to treatment in the first case, and its complete absence in the second.

The above comparison seems to demonstrate that even the crude division of primary anæmias into simple and pernicious cannot be made, especially in severe blood lesions, by the examination of the blood alone, but that clinical features must weigh equally with it in establishing a diagnosis. We believe that to obtain a satisfactory classification of anæmias it will be necessary, besides counting and studying the peripheral blood, (1) to understand the life history of the blood cells, which can be accomplished only through study of the physiology and pathology of the blood-making and blood-destroying organs; (2) to investigate more thoroughly the interrelations between the normal and diseased processes occurring in the blood and those occurring in the other body tissues; (3) carefully to correlate the results of such studies with those obtained by clinical experience.

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A CASE OF PANCREATIC FISTULA OF THREE YEARS' DURATION, WITH A CHEMIC STUDY OF THE FLUID ELIMINATED.

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In about 80% of the operated cases of pancreatic cyst on record, Gussenbauer's method of incision and drainage was employed, and the mortality due directly to the operation was less than 6%. While this method is attended with such favorable results and is safer than extirpation, which is limited in application, there are certain disadvantages attending it in that the healing process is slow, at times most tedious, and there is also the danger of a permanent fistula. The duration of the healing process varies from one to several months, and in a case recorded by Körte, 2½ years elapsed before the fistula closed.

As to the number of cases resulting in permanent fistula we possess no definite information, since in many instances details are wanting as to the ultimate result of the fistula still existing when the case was reported. It is probable, however, that in a small percentage of the cases the fistula was permanent, as not a few were cystadenoma. In such cases the conditions favor permanency of the fistula, and extirpation of the entire cyst offers the only means of cure. Since duration of healing after Gussenbauer's method varies from one month to 2½ years (as in Körte's case), one should wait a long time before deciding in a given case that the fistula is a permanent one. If, however, at the time of operation we had to do with a cystadenoma, which did not allow of extirpation, then the question of permanency of the resulting fistula would be more easily decided.

In the case we report the fistula has existed over three years, has not diminished to any extent, in depth, and

its secretion, while, as a rule, of small amount, steadily continues. The clinical history of the case was reported at the meeting of the New York Clinical Society in December, 1900, and was published, with two other cases of pancreatic disease, in *American Medicine*, January 25, of the present year. Since that time a very thorough chemic study has been made of the fluid eliminated.

The significance of the results of this chemic examination can be appreciated only in the light of the full clinical history of the case, which is, therefore, again given in brief detail:

CLINICAL HISTORY.

Before Admission.—M. D., female, 19 years old, admitted May 8, 1899, to St. Luke's Hospital. Family history: Father

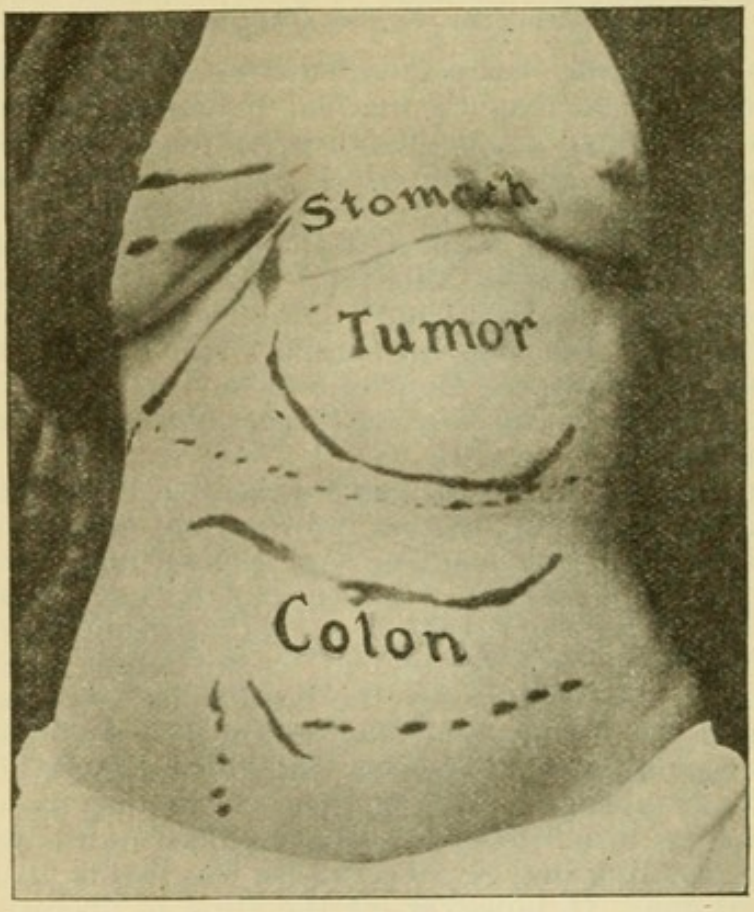


Fig. 1.

died of kidney trouble, mother of heart disease. Personal history: Patient had good health until seven years ago, since then has suffered from attacks of gastritis, lasting three weeks at a time. The attacks were attended with vomiting of foul and greenish material, or coffee-grounds matter; no blood. During

the attacks there was epigastric tenderness and sharp shooting-pains in the stomach and radiating to the back, pain worse after eating. No history of clay-colored or fatty stools, no jaundice, very constipated. Has had no appetite and has lost 10 pounds in the past month. Six weeks ago her physician discovered a tumor the size of an egg in the epigastric region; since then the tumor has steadily increased in size. The tumor appeared just after an attack of gastritis, but there is no history of sharp pain or collapse. Since then the patient has been free from pain or stomach symptoms. Last menstruation in March; had previously been regular.

On Admission.—Patient well nourished, skin dirty yellow, mucous membranes pale, slight acne about face. Tongue coated, teeth poor. Lungs normal. Heart negative, excepting a systolic apex murmur transmitted to left; no accentuation of

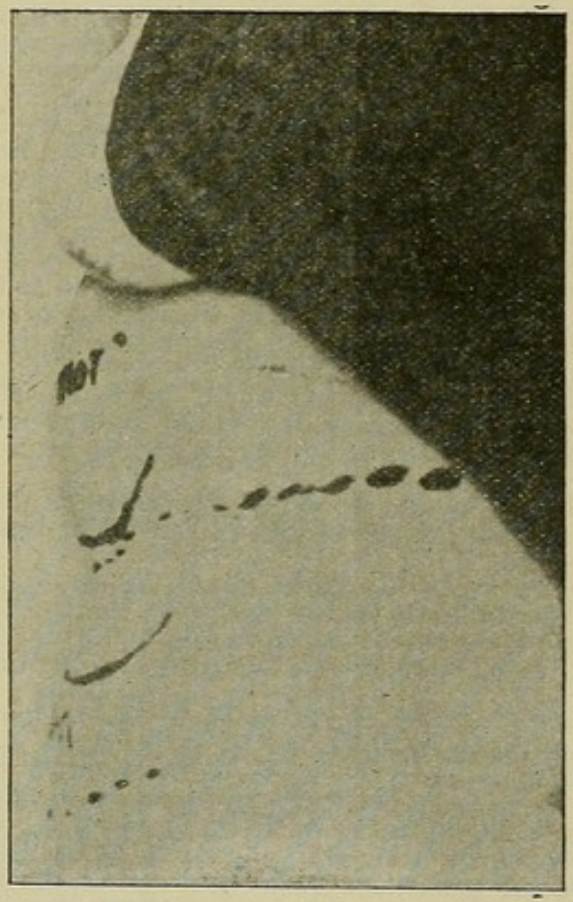


Fig. 2.

second pulmonic. Liver: dulness begins at fourth right interspace and extends to free border, edge not felt. Stomach resonance a little higher than normal. There is a smooth, hard, tense, semifluctuating tumor, about the size of a large cocoanut, which is a little to the left of the median line in the epigastric and umbilical regions, and extends into the left hypochondriac region. Its area of flatness begins just below the stomach and extends to the level of the umbilicus, where it meets the reso-

nance of the transverse colon. The flatness runs to the left and backward to the spine, its upper border behind being 1½ inches below the angle of the scapula. About two inches below the most prominent portion of the tumor in front, a free, sharp, smooth edge can be felt running through the umbilicus to the left in a curved direction. Right kidney palpable, freely movable. Temperature 100°, pulse 90, respirations 24. Urine light yellow, acid, sp. gr. 1,010, no sugar nor albumin, contains a few vesical epithelia.

Diagnosis, pancreatic cyst.

Operation, May 13, 1899.—Ether anesthesia. Through an incision in median line beginning just below the ensiform and continuing downward for four inches, the peritoneal cavity was opened. No adhesions found, stomach displaced upward, and colon downward.

Pearl-colored cyst, seen presenting behind the gastrocolic omentum, which was adherent to anterior wall of cyst. Peritoneal cavity was walled off by gauze packing, large aspirating needle thrust into cyst, and 30 oz. of clear straw-colored limpid fluid withdrawn. As the wall of the cyst collapsed, it was drawn up into the abdominal wound and its cavity exposed through a three-inch incision, and several ounces of similar fluid removed by sponges. Cyst was thin-walled and lined with a smooth membrane, and ran upward behind stomach. Careful probing failed to reveal any communication between cavity of cyst and adjacent organs. Incision in cyst wall partially sutured with catgut, upper half of abdominal incision closed with silk sutures, cyst wall sutured with silk to parietal peritoneum in lower half of abdominal incision. Large rubber drainage tube inserted into cavity of cyst, edges of wound protected with rubber tissue and iodoform gauze; sterile gauze dressing over all. Operation lasted about an hour, patient sent to ward in good condition. Slight reaction followed operation, and convalescence was soon established.

The discharge from the cyst was profuse for about 10 days, and then it gradually decreased, requiring a change of dressing several times daily.

Chemic Qualities of the Cystic Fluid.—Pathologist reported that the fluid removed from the cyst was alkaline, sp. gr. 1,018, opalescent, and contained free fat, cholesterin and leukocytes. It emulsified fats, changed starch into glucose and digested albumin.

Convalescence.—The convalescence was uneventful, the wound gradually became smaller, the discharge lessened, and on June 21 the patient was referred to the out-patient department with a narrow fistula discharging a small amount of thin yellowish fluid. The fistula was about five inches in depth, and passed downward into the left hypochondrium. Since discharge from the hospital the patient has been kept under observation, and while the fistula has never healed, there has been a great improvement. She is strong and able to work, has no more attacks of indigestion, bowels are regular, and there has been a great increase in weight.

For a year after the operation a small rubber drainage tube was worn, but owing to the steady contraction of the wound in the abdominal wall, it was necessary to substitute a straight silver tube three inches in length and of 32 F. caliber. This prevents any retention of secretion and is worn with no discomfort, and at present one small dressing of gauze suffices for 24 hours. Should, however, the patient become excited or

nervous, the secretion of pancreatic fluid is greatly increased, and at such times frequent dressings are needed.

Remarks.—This was a case of pancreatic cyst in a young adult, and as far as could be ascertained at the operation the cyst arose from the distal portion of the gland. The etiology is obscure; there was no history of traumatism, and the attacks of severe epigastric pain and vomiting occurring at intervals during the seven years probably bore an etiologic relation to the formation of the cyst. It should be observed that the cyst was first discovered after one of these attacks, and since then there has been complete cessation of pain and vomiting. This fact may suggest pancreatic calculus as the cause, but as no stone was ever seen in the feces, and as none was found at operation, although sought for by the finger and probe, there is no substantial foundation for considering calculus as the etiologic factor.

The cyst was of large size, had thin walls and was lined with a smooth glistening membrane, and presented none of the appearances of cystadenoma. Incision and drainage was deemed safer owing to the firm adhesions to the neighboring organs. The diagnosis was made before operation from the location of the cyst, and its relations to the stomach and colon when distended with air, by the history of rapid growth, and finally by the absence of cystic disease elsewhere in the abdomen. Aspiration was not resorted to, as it is attended with danger, and it should be discarded in favor of exploratory incision.

Sugar was never present in the urine, and fatty stools were never seen. Efforts to heal the fistula by local treatment have been made steadily and at frequent intervals. Injections of iodin, silver nitrate, carbolic acid, nitric acid, curetting and packing of the fistula, have been tried, but so far in vain. The fistula is still about five inches in depth, and its apparent capacity less than an ounce, but on two occasions when the drainage tube was left out, fluid to the amount of six ounces accumulated in the cavity. The distention of the cavity by the retained secretion caused nausea and some epigastric pain, which was quickly relieved by the introduction of the drainage tube.

The general condition of the patient at present is excellent, she has gained in weight and strength, has no trouble with digestion, is able to work and suffers no inconvenience from the fistula. Local treatment of the fistula is still continued, and should it be considered

necessary for any reason to excise the remaining portion of the cyst, it will be approached by a counter incision through the lumbar region.

In Fig. 1 may be seen the size of the cyst, and its relations to the stomach and colon as determined by percussion, the stomach and colon being distended with air. The dotted line below the tumor represents the position and course of the free, sharp edge felt upon examination at admission.

Fig. 2 shows the tumor in profile as it presents below the ribs and between the colon and stomach.

RESULTS OF RECENT CHEMIC STUDY.

As has already been indicated, the chemic examination of the cystic fluid at the time of the operation, and of that discharged somewhat later, showed that pancreatic constituents characterized it. It seemed desirable at this late stage of elimination, also, to ascertain by chemic means whether the continued discharge is from a permanent pancreatic fistula or whether the fluid has other than a pancreatic origin.

The daily flow of fluid has been considerable. Usually the liquid has been thin, watery, turbid, almost colorless. Occasionally it is tinged with hemoglobin or hemoglobin derivative, and is somewhat mucigenous.

Our analyses were made of fluids collected at wide intervals and under varying conditions, with a purpose of securing a representative average of results. In the examinations referred to below the fluid was collected either with a syringe or a catheter, the patient lying on her back or side during the process. Coughing favored the discharge of the fluid, and was resorted to occasionally, by direction, during the first two periods of collection, in order to facilitate withdrawal. The methods of analysis were those commonly in use. Chemic examination was made immediately or within a few hours after the fluid had been collected.

A. January 21, 1902.—After the silver tube had been removed from the fistula, a small amount of amber colored fluid could be withdrawn at frequent intervals with a syringe. The flow gradually increased, and, in the course of a half hour, 45 cc. of fluid was collected. This is designated below as the "first portion." With the aid of a catheter an additional quantity of the fluid, 31 cc., which flowed somewhat more rapidly, was withdrawn in 15 minutes. This was almost colorless, though slightly turbid, and is referred to below as the "second portion."

a. First Portion, 45 cc.—This was amber-colored, opalescent; contained minute flocks and possessed a slight, though distinct

odor, suggestive of volatile fatty acids. A trace of hemoglobin was present, the blood having come from a very slight wound of the tissue inside the orifice during the use of the syringe.

The fluid was alkaline to litmus. Acid phosphate was absent. It contained a slight amount of proteid coagulating at 63° to 65° C. A few erythrocytes were to be seen under the microscope; some leukocytes and, here and there in the field, epithelial cells also. No crystalline matter was present. A good biuret reaction was obtained with the fluid and a trace of reducing substance was detected in it. The phenylhydrazin test showed that this was due wholly or at least mainly to dextrose. Calcium, magnesium, sodium and potassium salts of phosphoric, sulfuric and hydrochloric acids were present in minute amounts.

Tested by the methods now in vogue, the fluid was found to possess only *slight* tryptic and *scarcely any* lipolytic action; was entirely devoid of milk-curdling and inverting power, but showed comparatively *marked* amylolytic effect. Pepsin was absent. The emulsifying power of the fluid was the same as that of lymph.

The fluid did not contain fibrin—no sign of coagulation manifested itself at any time.

The following percentage results for general composition were obtained:

Water	99.34
Solids	0.66
Organic matter	0.35
Inorganic matter	0.31

Of the total solid matter:

Organic	52.93
Inorganic	47.07

b. Second Portion, 31 cc. — Almost colorless. Less turbid or opalescent than the first portion. Peculiar odor missing. No hemoglobin present. Qualitative factors otherwise were the same as for the first portion except that a proteid coagulation was obtained at 69° to 70° C. The fluid did not contain red-cells and no lipolytic action was induced by it. Further, the amylolytic action was relatively weaker.

The following percentage results were obtained in quantitative analysis:

Water	99.54
Solids	0.46
Organic matter	0.19
Inorganic matter	0.27

Of the total solid matter:

Organic	42.32
Inorganic	57.68

B. February 10, 1902.—The fluid was removed about three hours after a light breakfast. The fluid was 45 to 50 cc. in volume, light yellow in color, alkaline in reaction and turbid, containing small particles. One-half of it was filtered.

a. Filtered Portion.—Slightly opalescent. Qualitatively it was the same as the second portion examined on January 21, except that all ferment tests were negative save that for diastatic enzyme.

The following percentage results for composition were obtained:

Water	99.15
Solids	0.85
Organic matter	0.52
Inorganic matter	0.33

Of the total solid matter:

Organic	61.63
Inorganic	38.37

b. Unfiltered Portion.—Quite turbid with flocculent material. Leukocytes grouped in clusters made up the particles visible to the naked eye. The fluid was the same qualitatively, otherwise, as the filtered portion, except that very weak, almost imperceptible, tryptic action was demonstrated in addition to amylolytic.

The appended percentage composition results were obtained:

Water	99.09
Solids	0.91
Organic matter	0.56
Inorganic matter	0.35

Of the total solid matter:

Organic	61.24
Inorganic	38.76

The striking features of the results under *A* and *B* are (1) the very weak, practically negative, action of the fluid so far as typical pancreatic enzymes are concerned, under conditions which had been made particularly favorable to them; and (2) the very slight amount of solid substance, particularly organic matter, contained in the fluid. The tryptic action was so slight and uncertion that the enzyme may have come from the leukocytes of the fluid. The diastatic action manifested, while vigorous, was no more pronounced than that shown by any serous fluid, a statement applying with equal force to the observed lipolytic effect.

The generally negative results of the preceding tests for typical pancreatic enzymes led us to examine fluid collected at a time when pancreatic activity would be most decided and when, therefore, the probability of diffusion or direct delivery from the gland into the fistula (if either process now occurs at any time) would be greatest. Any duct, or passage, connecting with the cyst would naturally empty more fluid into the fistula during such a period of glandular activity than at any other. Accordingly, the collection was begun at the patient's home just an hour after the completion of the heaviest meal of the day and continued into the third hour after the meal had been taken, with the following results:

C. February 18, 1902.—There was a gradual increase of flow after removal of the tube. At the end of three-quarters of an

hour it was comparatively rapid. In one hour and 10 minutes 125 cc. of the fluid passed from the fistula.

This surprising result is worthy of special notice. Although as much as 20 liters of fluid have been removed from a pancreatic cyst (by Stapper) at the time of operation, no such afterflow as this has been previously observed. Indemans noted a flow of 120–130 cc. per day for a few days after operation, but this soon diminished in quantity.

The fluid above mentioned had only a very faint tinge of yellow, was slightly opalescent, odorless and without appreciable sediment. Its specific gravity was 1,002.8. Qualitatively it was the same as the first portion collected on January 21, except that coagulable proteid was separated at 68° C., red cells were absent and crystals of calcium oxalate were obtained on concentration.

The tests for enzymes were practically negative except for amylopsin, which was present in comparatively active amount. No tryptic action could be shown even with the aid of dilute alkali. The emulsifying action was slight and only such as may be obtained with any serous fluid.

The following results for percentage composition were obtained in duplicate:

	1.	2.	Average.
Water	99.520	99.520	99 520
Solids	0.480	0.480	0.480
Organic matter	0.329	0.321	0.325
Inorganic matter	0.151	0.157	0.155

Of the total solid matter:

Organic	68.50	67.72	68.11
Inorganic	31.50	32.28	31.89

The analyses of February 18 were repeated, after a good interval, with fluid collected under similar conditions, *i. e.*, within 1 to 3 hours after the heaviest meal of the day. Samples of the patient's urine, passed on the same and the previous day, were also carefully examined. The results follow:

D. April 1, 1902.—The silver tube had been kept out of the fistula during part of the day. Later, because of a tendency to closure and retention, a rubber tube had been inserted. In the evening, on removing the rubber tube, the first portions of the fluid withdrawn with a syringe were amber-colored and contained considerable mucus, leukocytes and some oil globules. The insertion of the rubber tube required considerable manipulation, which fact doubtless accounts, in part, for the greater proportion of mucus, etc., in the fluid first collected. The fluid collected in this way is referred to below as the "first portion."

To facilitate further collection a catheter was inserted and the fluid carried directly to a bottle. The flow appeared to be uniformly rapid for more than an hour, when it seemed to slow up somewhat. In 1 hour and 45 minutes, 155 cc. of the fluid was collected. (Compare with result of February 18.) This is referred to below as the "second portion."

a. First Portion.—The fluid collected at first possessed distinct diastatic action, very slight tryptic power, and only a trace of lipolytic influence. It was composed as follows:

Water	97.75
Solids	2.25
Organic matter	1.59
Inorganic matter	0.66

Of the total solid matter:

Organic	70.69
Inorganic	29.31

Compared with previous analyses, the chief difference to be noted is the somewhat increased proportion of solids. This was undoubtedly due to the mucus in the fistula at the time, and which was taken up by the syringe. The catheter delivered the fluid of the second portion as it gathered in the fistula. The comparative analyses given below show that the mucus is a variable and a secondary constituent.

b. Second Portion.—This was given more extended analysis than any of the other portions collected. Its specific gravity was 1,003.6. With the exception of enzyme content, the fluid possessed all of the qualitative characters of that collected on February 18, calcium oxalate, however, being more in evidence.

This fluid possessed comparatively vigorous diastatic action even in the cold. At 40° C. it showed tryptic power *very gradually*, and had *some* lipolytic action. Even when tested with ethyl butyrate and litmus, however, the latter action was seen to be comparatively slight.

The following substances *could not be detected* in the fluid: Bile pigment, proteose, peptone, tryptophan, nuclein base, urea, leucin, tyrosin, creatin, glycogen. These and previous negative results indicate that neither the liver nor a kidney is involved in the production of the fluid.

In addition to the substances already found in each sample of fluid collected, cholesterin crystals were observed in this. The coagulable proteid consisted of both albumin and globulin. On boiling, the fluid gave off an odor suggestive of fatty acid. In the cold, acetic acid precipitated a proteid insoluble in a moderate excess of the acid. This substance, which appears to have been nucleoproteid, was somewhat soluble, however, in a slight excess of hydrochloric acid.

The amount of coagulable proteid was accurately determined. The first separation was made within two hours of the time of collection, the second 12 hours later. The results are practically the same. The original fluid, in the meantime, was kept in a cool place—at 15° C.

First determination	= 0.1896	gram	coagulable	proteid	per	100 cc.
Second "	= 0.2000	"	"	"	"	"
Average	= 0.1948	"	"	"	"	"

The second result for coagulable proteid content, compared with its duplicate, shows, further, how little tryptic action the fluid was able to exert—though, of course, the conditions during the interval were not particularly favorable to such action. At the same time, if there had been any significant quantity of trypsin in the fluid, a good proportion of this small amount of proteid would have been hydrated beyond the coagulable stage.

The following results for general percentage composition were obtained:

Water	99.07
Solids	0.93
Organic matter	0.58
Inorganic matter	0.35
Albumin, globulin	0.19

Of the total solid matter:

Organic	62.51
Inorganic	37.49
Albumin, globulin	20.96

c. Urine of March 31 and April 1, 1902.—The results of our examination of the patient's urine may be summed up in the statement that it was found to be normal for both days. Excepting mucus and a few epithelial cells, no proteids or proteid elements could be detected. Sugar was absent, as shown by negative results with Nylander's solution and with phenylhydrazin.

Fatty stools have never been observed, it should be remarked again—the feces have been normal constantly.

REVIEW OF QUALITATIVE RESULTS.

A general review of our qualitative results shows that the fluid is similar to a simple transudate. In no previous case has the specific gravity been as low as that recorded here—1,002.8. Gussenbauer found it as high as 1,610. Qualitatively, the fluid is like many of those from pancreatic cysts already analyzed. Quantitatively, it is much different than the fluid from some; similar, however, to others. The significant variations from most of the fluids previously analyzed are the low content of organic matter, indicating absence of particular inflammation and the large proportion of water. Unlike a number of such cystic fluids examined previously, it is, further, entirely devoid of constituents representing various stages of tryptic proteolysis. The noteworthy content of oxalic acid (calcium oxalate) brings to mind the similar result obtained by Zdarek in his examination of fluid withdrawn soon after operation.

These later examinations emphasize the deductions drawn from the results of those of January 21 and February 10. All of the data indicate that the case under consideration is not now one of true, permanent pancreatic fistula, although they do not exclude the probability that diffusion from the pancreas constantly takes place to some extent, or that pancreatic tissue makes up part of the wall of the cyst. Certain it is, at all events, that the fluid is not pancreatic juice in the ordinary sense.

SUMMARIES OF QUANTITATIVE RESULTS.

The following summary shows the uniformity of our average quantitative analytic results:

TABLE I.—GENERAL PERCENTAGE COMPOSITION OF THE FLUID FROM THE FISTULA.

Constituents.	January 21.		February 10.		February 18.		April 1.	Av'r-age.
	a	b	a	b	a	b	b*	
Water	99.34	99.54	99.15	99 09	99.52	99 52	99.07	99.32
Solids	0.66	0.46	0.85	0.91	0.48	0.48	0.93	0.68
Organic matter	0.35	0.19	0 52	0.56	0.33	0.32	0.58	0.41
Inorganic matter	0.31	0.27	0.33	0.35	0.15	0.16	0.35	0.27
Of the total solids..								
Organic	52.93	42.32	61.63	61 24	68.50	67.72	62.51	59.55
Inorganic	47.07	57.68	38.37	38.76	31.50	32.28	37.49	40.45

* The results of our analysis of the first portion obtained on April 1, are not included above, because of the exceptional amount of mucus, etc., in the fluid at the outset of its collection.

The significance of the above analytic figures may be fully appreciated at a glance, on comparison of our average results with similar data for various lymphatic or serous fluids given in the appended table:

TABLE II.—GENERAL PERCENTAGE COMPOSITION OF LYMPH AND TRANSUDATES.

	Our own result.	Cerebrospinal fluid.[1]	Aqueous humor.[2]	Spermatocele fluid.[3]	Lymph.[4]	Amniotic fluid.[5]	Peritoneal fluid.[6]	Pericardial fluid.[7]	Hydrocele fluid.[8]
Water	99.32	99.17	98.69	98.68	98.63	98.43	97.89	96.09	93.89
Solids	0.68	0.83	1.31	1.32	1.37	1.57	2.11	3.91	6.11
Organic	0.41	0.32	0.54	.....	0.49	0.98	1.13	3.03	5.18
Inorganic	0.27	0.51	0.77	.....	0.88	0.59	0.97	0.88	0.93

[1], [4], [5], [6] Results summarized by Halliburton: Textbook of Chemic Physiology and Pathology, 1891, pp. 334-356.
[2] Given in Schäfer's Textbook of Physiology, 1898, i, p. 123.
[3], [7], [8] To be found in Mandel's translation of Hammarsten's Textbook of Physiologic Chemistry, 1900, p. 193.

Our results are almost identical with those for cerebrospinal fluid. They show clearly, we think, that the fluid from our patient's fistula has the general characters of a transudate and that it is very much like ordinary lymph. The similarity to the cerebrospinal fluid also suggests that selective cells have somewhat influenced composition—cells probably situated in the wall of the cyst.

All of the analyzed fluids referred to in the above table were samples of the fluid obtained on first withdrawal, which naturally would contain more solid matter, particularly proteid, than such portions as might flow from the body immediately on formation. In the former cases prolonged osmotic influences, particularly resorption of water, would tend to raise the percentage of inorganic products, whereas cellular activity would bring about increase of organic constituents. Our own patient's fluid on retention, would, for the same reason, surely contain a somewhat greater proportion of solid matter—as it did at the time of operation, when its specific gravity was 1,018.

The results summarized in the appended table show that, so far as general composition is concerned, the fluid we have examined is not very similar to pancreatic juice—even such as is collected from a permanent fistula—a further fact in harmony with our qualitative, enzyme results. The figures for blood plasma are also brought into comparison:

TABLE III.—COMPOSITION OF PANCREATIC JUICE AND BLOOD PLASMA.

	Fluid from our own patient's fistula.	Fluid from a *temporary* pancreatic fistula.[1]	Fluid from a *permanent* pancreatic fistula.[2]	Fluid from a *permanent* pancreatic fistula (dog).[3]	Blood plasma.[4]
Water	99.32	86.41	97.59	97.68	90.29
Solids	0.68	13.59	2.41	2 32	9.71
Organic matter	0.41	13.25	1.79	1.64	8.86
Inorganic matter	0.27	0.34	0.62	0.68	0.85

[1] Zawadsky: Centralblatt für Physiologie, 1892. v, p 179
[2] Herter: Zeitschrift für physiologische Chemie, 1880, iv, S. 160.
[3] Schmidt: Hermann's Handbuch der Physiologie, 1881, v-2, S. 189.
[4] Halliburton: Textbook of Chemic Physiology and Pathology, 1891, p. 334.

Only a few quantitative analyses of the fluids from pancreatic cysts have been recorded. The following summary shows the general chemic relationships of the fluid we have examined to those analyzed by previous observers. In each case the analyzed fluid was collected either at the time of operation or shortly after. Our results, it will be seen, are more nearly in accord with those of Zdarek than of any other:

TABLE IV.—GENERAL PERCENTAGE COMPOSITION OF PANCREOCYSTIC FLUIDS.

	Our own results.	Zdarek.	Indemans.	Mörner.	Lenarcic.	Riegener.	Fitz.	Kosinski.
Water	99.32	98 94	98.70	98.55	98 21	98.14	92.68	86.41
Solids	0.68	1.06	1.30	1.45	1.79	1.86	7.32	13.59
Organic matter	0.41	0.19	0.36	0.55	1.00	.....	6.51	13.25
Inorganic matter	0.27	0.87	0.94	0.90	0.79	.....	0 81	0 34
Coagulable proteid	0.19	0.10	0 32	0.27	0.82	.....	1.66	9.21

REVIEW OF CHEMIC OBSERVATIONS IN PREVIOUS CASES.

The published results of chemic analysis of the fluid of various established pancreatic cysts have shown that the enzymes are frequently absent, not only from the fluid withdrawn at the time of operation, but also from that eliminated during the healing of the wound. As Körte suggests, stagnation and consequent prolonged contact with the other constituents of the fluid are doubtless destructive to the enzyme. Analysis has also shown that occasionally the enzymes have been absent from the fluid retained in the cyst, but have appeared for a while in the secretion thrown from the drainage tube, only to again disappear, and that permanently. In such instances it is probable that changes in the cells of the gland due primarily to drainage, as in cases of permanent experimental pancreatic fistula, cause alterations in the character of the fluid and the complete disappearance of the enzymes. In other cases of pancreatic cyst the enzymes were detectable in all samples of fluid withdrawn. In one rather odd case, cited by Körte, the enzymes were absent from all samples of fluid, but could be extracted from the wall of the cyst.

These facts, together with the additional observations by various investigators that lipolytic, proteolytic and amylolytic enzymes are found in various pathologic and lymphatic fluids, seem, in considering the qualities of cystic contents, to lead to the conclusions that (1) the presence of slight amounts of these enzymes does not necessarily imply a pancreatic source of the fluid; further, that (2) the absence of these enzymes does not necessarily mean that the fluid in question has an extra-pancreatic origin.

Körte, in summing up, emphasizes the following as the chief points in our knowledge of the characters of

pancreocystic fluid. It is usually tinged with hemoglobin or its derivatives, reddish to black in color, somewhat slimy, alkaline, rich in proteids, specific gravity 1,010–1,020 and frequently contains enzymes and cellular detritus. When the enzymes are present, *in particularly active quantity* in the "puncture-fluid," the presumption is strong that the fluid is directly derived from the pancreas. The absence of enzymes from such fluid is no evidence, however, that the cyst is not truly pancreatic in nature.

That our own patient's cyst was truly pancreatic was definitely established at the time of operation. That the fluid no longer partakes of the characters of true pancreatic juice harmonizes entirely, therefore, with observations of the past.

GENERAL OBSERVATIONS.

Transudation is mainly a physical, hydrostatic matter. But the permeability and the character of the tissues separating the blood and the transudate naturally determine the selective factors and largely influence composition. The cyst wall is always very vascular. In this case it was, at the time of operation, lined also with a smooth membrane. In retention cysts the wall is frequently the more or less altered wall of the original structure. The vessels are thin and no doubt unduly permeable. Passive congestion has probably become chronic and has doubtless increased permeability. Under these conditions the fluid of the cyst has lost its original qualities and is not easily comparable, except in a general way, with any other. Degenerate cells may also be responsible in part for the character of the transudate.

The walls of pancreatic cysts are usually composed of connective tissue, and incision and drainage has in nearly all cases favored ready granulation and rapid healing. The cystic membrane in some cases has consisted partly of pancreatic tissue, normal or degenerate or both, or has been lined with a secreting epithelium. Such an epithelium naturally interferes with granulation, may entirely prevent closure of the wound and makes the flow of cystic fluid continuous. In our own case, epithelial cells are to be found in the fluid along with leukocytes and mucus, and in all probability the cyst is still lined, in part or throughout, with a secreting mucous membrane. Although the interior of the cyst has been steadily treated with carbolic and nitric acids, etc., healing has appeared to cease and the fistula

persists. The tube was lately kept out of the fistula for several days. The aperture narrowed at once and cumulative retention resulted, much to the physical distress of the patient. The tube has again been replaced, the patient being thereby relieved, and the flow goes on as before.

With the exception of the first of Körte's cases none other like ours appears in the records. In Körte's case a fistula similar to that in our patient remained 2½ years after the operation. Varied treatment repeatedly with caustic substances, heat cauterization, etc., was without result. Several times the fistula closed temporarily, but as often opened up, with continued flow. Finally, 2½ years after operation, complete healing suddenly occurred spontaneously. Several years thereafter Körte saw the patient, found that the closure was permanent and the patient enjoying good health.

Riegener has expressed the opinion that in Gussenbauer's operation of incision and drainage there is little danger of a permanent fistula resulting. The facts Körte recapitulates, as well as the experience our own case affords, show that this possibility is not as remote as Riegener imagined.

It has been frequently observed that during periods of excitement or nervousness the flow from the fistula of our own patient has been particularly abundant. The patient herself has come to associate special elimination with such conditions. Since transudation is determined largely by intracapillary pressure, it seems probable that such periodic increases in the quantity of fluid are dependent on vasomotor changes, with augmented blood-pressure in the splanchnic region in general and the capillaries of the wall of the cyst in particular, rather than on special secretory activity of the pancreas. That the increased flow is not due to formation of true pancreatic juice is very evident from our results. Several of our analyses were made of fluid obtained in abundance during the before-mentioned periods of nervousness.

CONCLUSIONS DRAWN FROM THE RESULTS OF CHEMIC ANALYSIS.

The results of our recent analyses and those made at the time of and shortly after the operation, seem to be in harmony with the following conclusions:

Such connections of the pancreatic gland with the cavity of the cyst as may have existed at the time of

operation have closed and direct secretion from the gland into the fistula has ceased.

The fluid originally contained pancreatic products in abundance. The fluid still leaving the fistula appears, however, to be a transudate, resulting probably from chronic serous inflammation. It is possible, of course, that lymph from the pancreas contributes to the flow and that the lining membrane, in part consisting of abnormal pancreatic tissue, influences the composition of the fluid.

That the pancreas is no longer seriously involved is evidenced by the continued vigorous health of the patient—good appetite, absence of fatty stools, neither sugar nor proteid in the urine. At least sufficient normal pancreas remains to perform all of the observable functions of the gland.

The case is similar to the exceptional one of Körte's, in showing that after incision and drainage of a true pancreatic cyst (1) general recovery may be rapid, (2) the functions of the pancreas remain normal, (3) the patient enjoy excellent health thereafter, (4) with a persistent permanent fistula eliminating a transudate containing (*a*) a minimal proportion of solid matter, (*b*) a maximal percentage of water, and (*c*) little or no pancreatic enzyme.

The case is different than any other on record in (1) the length of time the fistula has persisted, and (2) in the quantity of fluid steadily eliminated from it.

BIBLIOGRAPHY.

Practically all original papers on pancreatic cysts printed before 1898 are listed by Körte. See his monograph for references to the work of observers mentioned above. Körte: Deutsche Chirurgie; Die chirurgischen Krankheiten und die Verletzungen des Pankreas, 1898, Lieferung 45d, p. xv (Literatur).

Mörner, K. A. H.: Skandinavisches Archiv für Physiologie, 1895, v, S 274.

Lenarcic: Centralblatt für innere Medicin, 1898, xix, S. 773.

V. Brackel: Deutsche Zeitschrift für Chirurgie, 1898, xlix, S. 293.

Payr: Wiener klinische Wochenschrift, 1898, xi, S. 629.

Pollard: British Medical Journal, 1899, Pt. I, p 594.

Keitler: Wiener klinische Wochenschrift, 1899, xii, S. 764.

Zdarek: Ibid, 1899, xii, S. 767.

Israel: Deutsche medicinische Wochenschrift, 1900, xxvi, S. 352.

Bessel-Hagen: Verhandlungen der deutschen Gesellschaft für Chirurgie, 1900, xxix, S. 683.

Fitz: American Journal of the Medical Sciences, 1900, cxx, p. 184.

Lazarus: Zeitschrift für Heilkunde, 1901, xxii, S. 165.

Seefisch: Deutsche Zeitschrift für Chirurgie, 1901, lix, S. 153.

Subbotic: Ibid, 1901, lix, S. 197.

Murray: *American Medicine*, 1902, iii, p. 141.

C. MISCELLANEOUS RESEARCHES.

Reprints, Nos. 29-35.

ANTITOXIC ACTION OF IONS.

LOEB & GIES.

Separatabdruck aus dem Archiv f. d. ges. Physiologie Bd. 93, 1902.

Weitere Untersuchungen über die entgiftenden Ionenwirkungen und die Rolle der Werthigkeit der Kationen bei diesen Vorgängen.

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I. Einleitung.

1. Während Ringer[1]) und Howell[2]) die Meinung aussprachen, dass das Calcium der „Reiz" für die Herzthätigkeit sei, indem es die Systole auslöse, wies Loeb[3]) darauf hin, dass das Calcium nicht direct für die rhythmischen Contractionen und die Herzthätigkeit nöthig sei, sondern nur indirect, nämlich um die giftige Wirkung des Kochsalzes im Blut oder in den Geweben aufzuheben. Zwei Gruppen von Thatsachen führten ihn zu dieser Annahme, nämlich erstens, dass ein Zusatz von Calcium zu einer Lösung nur dann günstig wirkt, wenn die Lösung grössere Mengen von Salzen mit einwerthigem Kation, besonders Natriumsalze, enthält. In einer mit dem lebenden Gebilde isotonischen Lösung eines Nichtleiters finden im Allgemeinen keine rhythmischen Contractionen statt, wie viel Calcium man auch zusetzen mag[4]). Die zweite, entscheidende Beobachtung war aber

1) Ringer, Journal of Physiology vol. 3 p. 388. 1880, vol. 4 p. 29, 222, vol. 5 p. 247, vol. 6 p. 154, 361, vol. 8 p. 20, 288, vol. 9 p. 425.

2) Howell, American Journal of Physiology vol. 2 p. 47. 1898.

3) J. Loeb, American Journal of Physiology vol. 3 p. 327, 383, 434. 1900 und vol. 6 p. 411. 1902. Pflüger's Archiv Bd. 80 S. 229. 1900 und Bd. 88 S. 68. 1901.

4) Loeb, Ueber Ionen, welche rhythmische Zuckungen hervorrufen. Festschrift für Fick. Braunschweig 1899. American Journal of Physiology vol. 3 p. 383. 1900. Pflüger's Archiv Bd. 91 S. 248. 1902.

folgende: Die Eier von Fundulus, die sich normaler Weise im Seewasser entwickeln, bilden keinen Embryo, sondern sterben rasch ab, wenn sie in einer reinen Kochsalzlösung sich entwickeln von der Concentration, in der dieses Salz im Seewasser enthalten ist. Fügt man einen kleinen, aber bestimmten Betrag eines Calciumsalzes zu, so entwickeln sich die Eier ebenso gut wie im Seewasser. Dass aber in diesem Falle die Calciumionen nicht direct für die Entwicklung nöthig sind (den „Reiz" bilden), sondern nur indirect (um die giftigen Wirkungen der Kochsalzlösungen aufzuheben), wird dadurch bewiesen, dass die Eier in mehrfach destillirtem Wasser sich völlig normal entwickeln [1]).

Die Rolle der Ionen in diesen Vorgängen stellt sich Loeb folgendermaassen vor. Die Ursachen („Reize") für die rhythmischen Contractionen sowohl wie für die Zelltheilungs- und Entwicklungsvorgänge sind nicht die Ionen, sondern bestimmte chemische (katalytische) Vorgänge und zwar, da für Herzthätigkeit sowohl wie für die Zelltheilung genügende Sauerstoffzufuhr ausnahmslos unerlässliche Bedingung ist, anscheinend Oxydationsvorgänge. Die Betheiligung der Ionen dürfte sich möglicher Weise darauf beschränken, dass dieselben die physikalischen Zustände der lebenden Substanz in einer für die Ausführung der nöthigen Bewegungen günstigen (oder ungünstigen) Weise beeinflussen. Das wäre der Fall, wenn beispielsweise in einer reinen Kochsalzlösung Bestandtheile des Protoplasmas verflüssigt würden, welche fest sein sollten, und wenn ein kleiner Zusatz von Calcium die Verflüssigung verhinderte [2]). Wenn das richtig wäre, so sollte man auch erwarten, dass, wenn die Gewebe zu viel Calcium enthalten, ebenfalls giftige Wirkungen entstehen. Die

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1) Physiologen scheinen im Allgemeinen anzunehmen, dass Kochsalzlösungen die ungiftigsten Lösungen unter den Lösungen von Elektrolyten seien. Das ist nur für gewisse physiologische Vorgänge richtig, z. B. Muskelcontractionen. Für die ersten Entwicklungsvorgänge von Funduluseiern (und anscheinend auch für andere Fischeier und vielleicht auch Froscheier) ist KCl weniger giftig als NaCl. Americ. Journal of Physiology vol. 6 p. 411. 1902.

2) Eine ausführlichere Discussion dieses Zusammenhanges zwischen fermentativen Processen und Ionenwirkungen findet sich in Loeb's Comparative Physiology of the Brain and Comparative Psychology p. 17 ff. New York and London 1900.

Beobachtungen über die Einwirkung von zu viel Calcium bei den Contractionen der Medusen und der Herzthätigkeit stützen diese Anschauung. Wenn das Centrum einer Meduse oder das Herz in Folge einer zu starken Dosis von Calcium zum Stillstand gekommen ist, so kann es wieder anfangen zu schlagen, wenn man es in eine reine Kochsalzlösung oder eine Kochsalzlösung mit weniger Calcium zurücksetzt.

2. Wenn es sich hier in der That um antagonistische Wirkungen von Ionen (auf die physikalischen Zustände gewisser Protoplasmabestandteile) handelte, so war zu erwarten, dass die Rolle von Calciumionen einer reinen Kochsalzlösung gegenüber auch durch andere Ionen übernommen werden könnte; und dass ferner eine kleine Dosis von Calciumionen nicht nur Kochsalzlösungen, sondern auch die Lösungen anderer Salze, namentlich mit einwerthigen Kationen, entgiften müsse.

Hardy's Untersuchungen[1]) über die Fällung suspendirter Theilchen in flüssigen Medien vermittelst Elektrolyten brachte Loeb auf die Vermuthung, dass die antitoxischen Wirkungen der Calciumionen gegenüber einer reinen Kochsalzlösung vielleicht bedingt seien durch die Werthigkeit und positive Ladung des Calciumions, und dass es desshalb möglich sei, dass andere zweiwerthige Metalle ähnliche antitoxische Wirkungen ausüben wie das Calcium. Die Versuche über die Entwicklung von Fundulusеiern bestätigten diese Erwartung auf das Ueberraschendste[2]). Von einer geringen Concentration an sind die Lösungen der Chloride mit einwerthigem Kation für das Fundulusei giftig, d. h. kein befruchtetes Ei kann in einer solchen Lösung einen Embryo bilden, und die befruchteten Eier sterben alsbald. Fügt man aber einen sehr kleinen, aber bestimmten Betrag irgend eines löslichen Salzes mit zweiwerthigem Kation zu (mit Ausnahme von Hg und Cu), so bilden sich im Allgemeinen ebenso viel Embryonen wie im Seewasser. Je höher die Concentration der Lösung des Salzes mit einwerthigem Kation ist, um so mehr Calcium ist auch zur Entgiftung nöthig. Dagegen konnten mit Anionen höherer Werthigkeit die toxischen Wirkungen einer reinen NaCl-Lösung nicht

1) Hardy, Proceedings of the Royal Soc. vol. 66 p. 110. 1900.
2) Loeb, l. c.

aufgehoben werden. Zur völligen Entgiftung von 100 ccm einer $\frac{5}{8}$ *m*[1]) NaCl-Lösung waren beispielsweise nöthig:

ungefähr 4 ccm $\frac{m}{64}$ $CaSo_4$

„ 4 „ $\frac{m}{32}$ $BaCl_2$ (gleiches Anion mit NaCl!)

„ 2 „ $\frac{m}{64}$ $ZnSO_4$

„ 2 „ $\frac{m}{8}$ $CoCl_2$ (gleiches Anion mit NaCl!)

Wenn man die ausserordentlich geringe Quantität des entgiftenden Salzes berücksichtigt, so wird es klar, dass es sich hier nicht um eine directe Wirkung des entgiftenden Salzes auf die Kochsalzlösung handeln kann. Die zur Entgiftung von 100 ccm $\frac{5}{8}$ *m* NaCl nöthigen Calciumionen betragen nur ein Tausendstel der Natriumionen (und Cl-Ionen). Wenn man aber die Concentration der reinen Kochsalzlösung selbst um 20 % verringert (also eine $\frac{m}{2}$ statt einer $\frac{5}{8}$ *m* NaCl-Lösung anwendet), so entwickelt sich in derselben im günstigsten Falle vielleicht ein Procent der Eier. Durch Zusatz von 4 ccm einer $\frac{m}{64}$ $CaSO_4$- (oder $Ca[NO_3]_2$)-Lösung zu 100 ccm einer $\frac{5}{8}$ *m* NaCl-Lösung entwickeln sich in derselben aber ebenso viele Embryonen wie im normalen Seewasser, also ca. 90 % oder mehr aller Eier bilden bei günstigem Material Embryonen. Es muss sich also wohl bei diesen antitoxischen Wirkungen darum handeln, dass die einwerthigen und zweiwerthigen Kationen einen entgegengesetzten Einfluss auf eine im Ei enthaltene Substanz ausüben. Dieser Einfluss ist zum Theil wenigstens eine Function der Werthigkeit der Ionen und ferner wohl auch eine Function des Vorzeichens der Ladung,

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1) Eine $\frac{5}{8}$ *m*-Lösung ist eine solche, welche 5 Grammmoleküle (oder 5 Mol.) der gelösten Substanz in 8 Litern der Lösung enthält. Das Zeichen *m* steht für Mol. Eine *m*-Lösung enthält 1 Mol. der gelösten Substanz in 1 Liter der Lösung. Es ist ohne Weiteres einleuchtend, dass diese Bezeichnungsweise vor der üblichen Bezeichnung der Concentration im Sinne von Normallösungen den Vorzug verdient.

da durch Anionen keine antitoxischen Wirkungen hervorgerufen werden konnten.

Die beschränkte Dauer der Laichzeit erlaubte Loeb nicht, den Gegenstand zu erschöpfen, und so war es nöthig, diese Versuche dieses Jahr weiter zu führen. Die Auswahl der einzelnen Probleme, über die wir im Folgenden berichten, rührt von Loeb her, die Ausführung der Versuche fiel Gies zu, dieselben wurden aber von Loeb genau verfolgt, so dass die folgenden Ergebnisse fast alle von uns beiden verificirt sind. Die Versuche wurden in Woods Holl ausgeführt.

II. Ueber die Gegenseitigkeit der entgiftenden Wirkung zweier Elektrolyte.

In seiner früheren Mittheilung hatte Loeb bereits die Frage aufgeworfen, ob es auch möglich sei, eine giftige Lösung eines Calciumsalzes durch Zusatz eines Salzes mit einwerthigem Kation zu entgiften. Er fand, dass das mit Salzen von K und NH_4 gelang, dagegen nicht mit Salzen von Li und Na[1]). Während man also eine giftige NaCl-Lösung durch kleine Quantitäten eines Calciumsalzes entgiften kann, kann man eine Calciumchloridlösung durch Zusatz eines Natriumsalzes nicht entgiften. Wohl aber ist das durch Kalium- und Ammoniumsalze möglich, aber nur, wenn man ausserordentlich grosse Quantitäten der letzteren anwendet. Loeb fand, dass in einer $\frac{m}{8}$ $Ca(NO_3)_2$-Lösung die frischbefruchteten Fundulus-eier im Allgemeinen keinen Embryo zu bilden im Stande sind. Um 100 ccm einer solchen Lösung zu entgiften, waren 2—4 ccm einer $2^1/_2$ *m* KCl-Lösung nöthig, d. h. die Quantität der toxischen und antitoxischen Substanz mussten von fast derselben Grössenordnung sein. Bei der Entgiftung einer Kl-Lösung durch $Ca(NO_3)_2$ konnte die antitoxische Substanz weniger als ein Tausendstel der toxischen betragen! Auch diese Thatsache ist nur verständlich unter der Annahme, dass es sich hierbei nicht um directe Wirkungen der beiden Elektrolyten auf einander, sondern um gemeinsame Wirkungen auf eine im Ei enthaltene Substanz handelt, wobei das zweiwerthige Kation im Allgemeinen eine viel grössere und entgegengesetzte Wirkung hat wie

1) Loeb, Americ. Journal of Physiology vol. 6 p. 411. 1902.

das einwerthige Kation. Aehnliche Erfahrungen machten wir in Bezug auf Magnesiumsalze. Es lag uns daran, diese Erfahrungen zu erweitern.

Wir wählten dazu ein sehr giftiges Salz, nämlich $ZnSO_4$. In einer $\frac{5}{8}$ m NaCl-Lösung entwickelt sich niemals ein Fundulusembryo, wenn die Eier nicht allzulange nach der Befruchtung in die Lösung gebracht werden. Setzt man zu 100 ccm einer $\frac{5}{8}$ m NaCl-Lösung 4 oder 8 ccm einer $\frac{m}{32}$ $ZnSO_4$-Lösung, so entwickeln sich eine grosse Zahl von Eiern. In einem besonderen Versuche bildeten in 100 ccm $\frac{5}{8}$ m NaCl + 4 ccm $\frac{m}{32}$ $ZnSO_4$ 26 % aller Eier Embryonen, während in 100 ccm $\frac{5}{8}$ m NaCl + 8 ccm $\frac{m}{32}$ $ZnSO_4$ 34 % der Eier Embryonen bildeten. In normalem Seewasser bildeten ca. 46 % der Eier derselben Cultur Embryonen. Die antitoxischen Wirkungen dieser Dosis $ZnSO_4$ sind also gegenüber der grossen toxischen Wirkung der $\frac{5}{8}$ m NaCl-Lösung ganz erstaunlich. Es lässt sich nun zeigen, dass in diesem Falle die Zinksulfatlösung nicht nur die giftige Wirkung der Kochsalzlösung aufhebt, sondern dass auch umgekehrt die Kochsalzlösung die giftige Wirkung der Zinksulfatlösung aufhebt. Die Eier von Fundulus entwickeln sich nämlich, wie schon erwähnt, in destillirtem Wasser ebenso gut wie im Seewasser. Fügt man aber zu 100 ccm destillirtem Wasser 4 ccm (oder 8 ccm) einer $\frac{m}{32}$ $ZnSO_4$-Lösung, so vermag auch nicht ein einziges Ei einen Embryo zu bilden. Das Zinksulfat ist also in der Concentration, in welcher es als Gegengift gegen das Kochsalz angewendet wurde, ein Gift, das die Entwicklung der Eier des Fundulus absolut unmöglich macht und das letztere rasch tödtet. Wir suchten nun festzustellen, was die minimale Dosis von Kochsalz ist, welche die giftige Wirkung des Zinksulfats in der oben erwähnten Concentration völlig aufhebt. Wir verfuhren so, dass wir zu je 100 ccm einer Kochsalzlösung von verschiedener Concentration 4 ccm oder 8 ccm einer $\frac{m}{32}$ $ZnSO_4$-Lösung zusetzten und den Procentsatz der Eier bestimmten, welche Embryonen bildeten.

Tabelle I.

Natur der Lösung	Procentsatz d. Eier, welche Embryonen bildeten
100 ccm destillirtes Wasser	49 %
100 „ destillirtes Wasser + 8 ccm $\frac{m}{32}$ $ZnSO_4$	0 %
100 „ m NaCl + 8 ccm $\frac{m}{32}$ $ZnSO_4$	1 %
100 „ $\frac{7}{8}$ m „ + 8 „ $\frac{m}{32}$ „	6 %
100 „ $\frac{6}{8}$ m „ + 8 „ $\frac{m}{32}$ „	8 %
100 „ $\frac{5}{8}$ m „ + 8 „ $\frac{m}{32}$ „	29 %
100 „ $\frac{4}{8}$ m „ + 8 „ $\frac{m}{32}$ „	34 %
100 „ $\frac{3}{8}$ m „ + 8 „ $\frac{m}{32}$ „	37 %
100 „ $\frac{2}{8}$ m „ + 8 „ $\frac{m}{32}$ „	38 %
100 „ $\frac{1}{8}$ m „ + 8 „ $\frac{m}{32}$ „	44 %
100 „ $\frac{1}{16}$ m „ + 8 „ $\frac{m}{32}$ „	8 %
100 „ $\frac{1}{32}$ m „ + 8 „ $\frac{m}{32}$ „	3 %
100 „ $\frac{1}{64}$ m „ + 8 „ $\frac{m}{32}$ „	0 %

Es ist also klar, dass von einer gewissen Concentration an NaCl die Giftwirkung von $ZnSO_4$ aufzuheben im Stande ist. Das Optimum der antitoxischen Wirkung des Kochsalzes wurde erreicht in einer Lösung von 100 ccm $\frac{m}{8}$ NaCl + 8 ccm $\frac{m}{32}$ $ZnSO_4$. In dieser Mischung kommen auf ein Molekül $ZnSO_4$ 50 Moleküle NaCl. Um aber 100 ccm einer $\frac{5}{8}$ m NaCl zu entgiften, waren nach den früheren Versuchen von Loeb ca. 2—4 ccm einer $\frac{m}{64}$ $ZnSO_4$-Lösung nöthig. Während also 1 Molekül $ZnSO_4$ für die Entgiftung von 1000 Molekülen Kochsalz ausreicht, sind umgekehrt 50 Moleküle Kochsalz zur Entgiftung von 1 Molekül $ZnSO_4$ erforderlich! Das zeigt schlagend die Zunahme der antitoxischen Wirksamkeit eines Kations mit seiner

Werthigkeit[1]). Unsere Tabelle zeigt ferner, dass, wenn die Concentration der Kochsalzlösung höher wird als $\frac{5}{8}$ *m*, der Procentsatz der sich entwickelnden Eier wieder abnimmt, offenbar, weil jetzt Kochsalz im Ueberschuss zugesetzt wird und das Zinksulfat die giftigen Wirkungen des Kochsalzes nicht mehr aufzuheben vermag. Das war nach den früheren Beobachtungen Loeb's zu erwarten, da derselbe gefunden hat, dass die zur Entgiftung von 100 ccm einer Kochsalzlösung nöthige minimale Menge von $Ca(NO_3)_2$ mit der Concentration der Kochsalzlösung zunimmt.

Die in der Tabelle I erwähnten Versuche wurden wiederholt, und um die Constanz der Resultate zu zeigen, wollen wir Tabelle II hier anführen:

Tabelle II.

Natur der Lösung	Procentsatz der Eier, welche Embryonen bildeten
100 ccm destillirtes Wasser[2])	58 %
100 „ „ „ + 8 ccm $\frac{m}{32}$ $ZnSO_4$	0 %
100 „ $\frac{2}{8}$ *m* NaCl + 8 ccm $\frac{m}{32}$ $ZnSO_4$	70 %
100 „ $\frac{1}{8}$ *m* „ + 8 „ $\frac{m}{32}$ „	39 %
100 „ $\frac{1}{16}$ *m* „ + 8 „ $\frac{m}{32}$ „	6 %
100 „ $\frac{1}{32}$ *m* „ + 8 „ $\frac{m}{32}$ „	0 %
100 „ $\frac{1}{64}$ *m* „ + 8 „ $\frac{m}{32}$ „	0 %

Wir schritten nun zur Untersuchung der Frage, ob die antitoxische Wirksamkeit der Salze mit einwerthigem Kation (z. B. Li, K, NH_4) gegen $ZnSO_4$ von derselben Grössenordnung sei wie die von NaCl. Loeb hatte früher gezeigt, dass zur Aufhebung der Giftwirkungen der Chloride, Nitrate oder Acetate von Na, Li, K und NH_4 ungefähr die gleiche, sehr geringe Dosis eines Calciumsalzes nöthig ist. Es zeigte sich in der That eine sehr schöne Uebereinstimmung.

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1) Dass die Anionen höherer Werthigkeit keine antitoxischen Wirkungen haben, hat Loeb früher nachgewiesen. Pflüger's Archiv Bd. 88 S. 68. 1901 und Americ. Journal of Physiology vol. 6 p. 411. 1902.

2) Wenn nicht das Gegentheil erwähnt ist, so wurde in allen Versuchen und Lösungen zwei Mal destillirtes Wasser benutzt.

Tabelle III.

Natur der Lösung	Procentsatz der Eier, welche Embryonen bildeten
100 ccm destillirtes Wasser	68 %
100 „ „ „ + 8 ccm $\frac{m}{32}$ $ZnSO_4$	0 %
100 „ $\frac{m}{128}$ LiCl + 8 ccm $\frac{m}{32}$ $ZnSO_4$	0 %
100 „ $\frac{m}{64}$ „ + 8 „ $\frac{m}{32}$ „	0 %
100 „ $\frac{m}{32}$ „ + 8 „ $\frac{m}{32}$ „	0 %
100 „ $\frac{m}{16}$ „ + 8 „ $\frac{m}{32}$ „	6 %
100 „ $\frac{m}{8}$ „ + 8 „ $\frac{m}{32}$ „	21 %
100 ccm destillirtes Wasser	51 %
100 „ „ „ + 8 ccm $\frac{m}{32}$ $ZnSO_4$	0 %
100 „ $\frac{m}{8}$ LiCl + 8 ccm $\frac{m}{32}$ $ZnSO_4$	33 %
100 „ $\frac{2}{8}$ m „ + 8 „ $\frac{m}{32}$ „	64 %
100 „ $\frac{3}{8}$ m „ + 8 „ $\frac{m}{32}$ „	45 %
100 „ $\frac{4}{8}$ m „ + 8 „ $\frac{m}{32}$ „	21 %
100 „ $\frac{5}{8}$ m „ + 8 „ $\frac{m}{32}$ „	13 %
100 „ $\frac{6}{8}$ m „ + 8 „ $\frac{m}{32}$ „	0 %

Tabelle IV.

Natur der Lösung	Procentsatz der Eier, welche Embryonen bildeten
100 ccm destillirtes Wasser	58 %
100 „ „ „ + 8 ccm $\frac{m}{32}$ $ZnSO_4$	0 %
100 „ $\frac{m}{64}$ KCl + 8 ccm $\frac{m}{32}$ $ZnSO_4$	0 %
100 „ $\frac{m}{32}$ „ + 8 „ $\frac{m}{32}$ „	0 %
100 „ $\frac{m}{16}$ „ + 8 „ $\frac{m}{32}$ „	8 %
100 „ $\frac{m}{8}$ „ + 8 „ $\frac{m}{32}$ „	42 %
100 „ $\frac{m}{4}$ „ + 8 „ $\frac{m}{32}$ „	64 %

Stärkere Lösungen von KCl wirkten nicht besser, sondern sind schlechter als $\frac{m}{4}$-Lösungen.

NH_4Cl war etwas wirksamer. Das Optimum schien erreicht bei einer Mischung von 100 ccm $\frac{m}{16}$ NH_4Cl + 8 ccm $\frac{m}{32}$ $ZnSO_4$, wie die folgende Tabelle zeigt:

Tabelle IV a.

Natur der Lösung	Procentsatz der Eier, welche Embryonen bildeten
100 ccm destillirtes Wasser	68 %
100 „ „ „ + 8 ccm $\frac{m}{32}$ $ZnSO_4$	0 %
100 „ $\frac{m}{512}$ NH_4Cl + 8 ccm $\frac{m}{32}$ $ZnSO_4$	0 %
100 „ $\frac{m}{256}$ „ + 8 „ $\frac{m}{32}$ „	0 %
100 „ $\frac{m}{128}$ „ + 8 „ $\frac{m}{32}$ „	1 %
100 „ $\frac{m}{64}$ „ + 8 „ $\frac{m}{32}$ „	4 %
100 „ $\frac{m}{32}$ „ + 8 „ $\frac{m}{32}$ „	22 %
100 „ $\frac{m}{16}$ „ + 8 „ $\frac{m}{32}$ „	67 %
100 „ $\frac{m}{8}$ „ + 8 „ $\frac{m}{32}$ „	59 %

Es wurde nun untersucht, ob die giftigen Wirkungen der Zinksulfatlösung auch durch Salze mit zweiwerthigem Kation vermindert oder aufgehoben werden können, und ob in diesem Falle die antitoxische Dosis nicht kleiner ist, als wenn das antitoxische Salz ein einwerthiges Kation besitzt. Tabelle V zeigt, dass viel weniger $Ca(NO_3)_2$ als NaCl nöthig ist, um die Giftwirkung von $ZnSO_4$ aufzuheben.

Tabelle V.

Natur der Lösung	Procentsatz der Eier, welche Embryonen bildeten
100 ccm destillirtes Wasser	49 %
100 „ „ „ + 8 ccm $\frac{m}{32}$ $ZnSO_4$	0 %
100 „ $\frac{m}{512}$ $Ca(NO_3)_2$ + 8 ccm $\frac{m}{32}$ $ZnSO_4$	3 %
100 „ $\frac{m}{256}$ „ + 8 „ $\frac{m}{32}$ „	19 %

Natur der Lösuug	Procentsatz der Eier, welche Embryonen bildeten
100 ccm $\frac{m}{128}$ $Ca(NO_3)_2$ + 8 ccm $\frac{m}{32}$ $ZnSO_4$	50 %
100 „ $\frac{m}{64}$ „ + 8 „ $\frac{m}{32}$ „	50 %
100 „ $\frac{m}{32}$ „ + 8 „ $\frac{m}{32}$ „	46 %
100 „ $\frac{m}{16}$ „ + 8 „ $\frac{m}{32}$ „	39 %
100 „ $\frac{m}{8}$ „ + 8 „ $\frac{m}{32}$ „	5 %
100 „ $\frac{m}{4}$ „ + 8 „ $\frac{m}{32}$ „	0 %

Es ist offenbar, dass die antitoxische Wirkung des Calciumions gegen die Giftwirkung des Zinkions ganz erheblich grösser ist als die irgend eines einwerthigen Kations.

$MgCl_2$ verhielt sich dagegen ganz anders, wie Tabelle VI zeigt.

Tabelle VI.

Natur der Lösung	Procentsatz der Eier, welche Embryonen bildeten
100 ccm destillirtes Wasser	25 %
100 „ „ „ + 8 ccm $\frac{m}{32}$ $ZnSO_4$	0 %
100 „ $\frac{m}{128}$ $MgCl_2$ + 8 ccm $\frac{m}{32}$ $ZnSO_4$	0 %
100 „ $\frac{m}{64}$ „ + 8 „ $\frac{m}{32}$ „	0 %
100 „ $\frac{m}{32}$ „ + 8 „ $\frac{m}{32}$ „	0 %
100 „ $\frac{m}{16}$ „ + 8 „ $\frac{m}{32}$ „	0 %
100 „ $\frac{m}{8}$ „ + 8 „ $\frac{m}{32}$ „	14 %
100 „ $\frac{m}{4}$ „ + 8 „ $\frac{m}{32}$ „	1 %

Es ist möglich, dass Magnesiumsalze mit anderem Anion als Cl andere Resultate geben. Weitere Versuche müssen hierüber angestellt werden. Allein es ist auch zu beachten, dass in allen Versuchen Loeb's an Fundulus nur das Kation für die antitoxischen Wirkungen in Betracht kam, während das Anion keine Rolle spielte.

Was für die Aufhebung der giftigen Wirkungen von Zinksulfat gilt, gilt auch für Bleisalze. Loeb hatte schon bemerkt, dass die

giftigen Wirkungen von 100 ccm einer $\frac{m}{2}$ essigsauren Natriumlösung durch Zusatz von ca. 4 ccm $\frac{m}{64}$ essigsaurem Blei aufgehoben werden können. Wir wiederholten diese Versuche mehrfach mit demselben Resultat und geben hier ein Beispiel:

Tabelle VII.

Natur der Lösung	Procentsatz der Eier, welche Embryonen bildeten
100 ccm destillirtes Wasser	46 %
100 „ $\frac{m}{2}$ CH_3CO_2Na	0 %
100 „ destillirtes Wasser + 4 ccm $\frac{m}{64}$ $Pb(CH_3CO_2)_2$ . . .	0 %
100 „ „ „ + 8 „ $\frac{m}{64}$ „ . . .	0 %
100 „ $\frac{m}{2}$ CH_3CO_2Na + 4 ccm $\frac{m}{64}$ $Pb(CH_3CO_2)_2$	23 %
100 „ $\frac{m}{2}$ „ + 8 „ $\frac{m}{64}$ „	31 %

Es ist richtig, dass wegen des gemeinsamen Anions die Dissociation des essigsauren Natriums und essigsauren Bleies in den letzten zwei Lösungen der Tabelle verringert ist. Das könnte möglicher Weise die Giftigkeit des essigsauren Bleies in diesen Versuchen verringern, kann aber nicht für die Giftigkeit des essigsauren Natriums in Betracht kommen, da die Quantität des essigsauren Bleies so verschwindend klein gegen die Quantität des essigsauren Natriums ist, dass die winzige Verringerung der Dissociation des letzteren seine Giftigkeit nicht merklich beeinträchtigt. Dass das nicht eine blosse Argumentation oder theoretische Annahme ist, sondern thatsächlich richtig ist, hat Loeb durch viele Versuche über die Giftigkeit einer reinen NaCl-Lösung festgestellt. Es sei daran erinnert, dass weder kleine noch grosse Mengen von KCl oder LiCl die Giftigkeit einer $\frac{5}{8}$ m-Kochsalzlösung zu verringern im Stande sind, trotz der Verringerung der Dissociation, worauf ja schon in der Einleitung hingewiesen wurde. Dass aber das gemeinsame Anion auch nicht für die Beseitigung der Giftwirkung des essigsauren Bleies verantwortlich ist, wird u. A. durch die vorausgehenden Versuche über die Entgiftung von Zinksulfat bewiesen, in welchen die beiden antagonistischen Salze kein gemeinsames Anion hatten.

III. Haben die Lösungen von Nichtleitern eine antitoxische Wirkung?

Loeb[1]) hatte in seinen ersten Mittheilungen darauf hingewiesen, dass die antitoxischen Wirkungen nur von Elektrolyten und wesentlich nur von den Kationen der letzteren ausgehen. Diese Thatsache war von Interesse, weil sie möglicher Weise auf eine Bedeutung der elektrischen Ladung der Ionen für die antitoxischen und vielleicht auch andere physiologische Vorgänge hinwies. Bei der grossen Rolle, welche die Elektrolyte in der Constitution und Dynamik der lebenden Substanz spielen, war es nöthig, Nichts unversucht zu lassen, um zu entscheiden, ob die Nichtleiter thatsächlich ausser Stande sind, die giftigen Wirkungen eines Salzes zu beseitigen oder zu verringern. Wenn der Nachweis eines gesetzmässigen Verhaltens sich auf negative Resultate stützen muss, wie in diesem Falle, muss die Zahl der Versuche viel grösser sein, als wo es sich um positive Ergebnisse handelt. Wir unternahmen desshalb eine grosse Zahl von Versuchen, um sicher zu stellen, dass die giftigen Wirkungen einer Kochsalzlösung oder Zinksulfatlösung durch die Nichtleiter Rohrzucker, Harnstoff, Glycerin und Aethylalkohol nicht verringert werden. Wir glauben, sagen zu dürfen, dass das zutrifft. Wir wollen einzelne Versuchsreihen etwas ausführlicher besprechen.

Wir wählten als toxische Lösung 100 ccm einer $\frac{5}{8}$ m NaCl-Lösung und suchten festzustellen, ob Zusatz von Harnstoff diese Lösung entgiften könne oder weniger giftig mache.

In der ersten Versuchsreihe werden zu je 100 ccm einer $\frac{5}{8}$ m NaCl-Lösung, $^1/_2$, 1, 2, 4, 8 und 16 ccm einer $\frac{m}{64}$-Harnstofflösung zugesetzt. Die Giftigkeit der Kochsalzlösung wurde nicht verringert. Dann wurde statt der $\frac{m}{64}$-Lösung eine $\frac{m}{8}$-Harnstofflösung gewählt und $^1/_2$, 1, 2, 4, 8 und 16 ccm derselben zu je 100 ccm der $\frac{5}{8}$ m NaCl-Lösung zugesetzt, mit wieder gänzlich negativem Resultat. Dann wurden $^1/_2$, 1, 2, 4, 8, 16 ccm einer 3 m-Harnstofflösung zu je 100 ccm $\frac{5}{8}$ m NaCl-Lösung zugesetzt, mit wieder völlig negativem Resultate,

1) Loeb, l. c.

und das Gleiche war in einer weiteren Versuchsreihe der Fall, in der $^1/_2$, 1, 2, 4, 8 und 16 ccm einer 10 *m*-Harnstofflösung zu 100 ccm $\frac{5}{8}$ *m* NaCl-Lösung zugesetzt wurden. Wir dürfen also wohl sagen, dass es unmöglich ist, mit Harnstoff die giftige Wirkung einer Kochsalzlösung zu verringern. Man kann nicht einwenden, dass der Harnstoff selbst in den Dosen, in denen er zugefügt wurde, giftig ist. Denn erstens vernichtete Zinksulfat in einer an sich giftigen Dosis die Giftwirkungen von NaCl, und zweitens ist, wie Loeb früher schon gezeigt hat, das Fundulusei sehr unempfindlich gegen Harnstofflösung. In einer $\frac{m}{16}$-Harnstofflösung bildeten beispielsweise ebenso viele Eier Embryonen wie in normalem Seewasser oder destillirtem Wasser. Selbst in einer $\frac{m}{2}$-Harnstofflösung wurden noch einzelne Embryonen gebildet.

Unsere Versuche, ob Rohrzucker im Stande sei, die toxischen Wirkungen einer reinen Kochsalzlösung aufzuheben, waren nicht so vollständig. Wir stellten nur zwei Versuchsreihen an. In der einen wurden $^1/_2$, 1, 2, 4, 8 und 16 ccm einer $\frac{m}{8}$-Rohrzuckerlösung zu je 100 ccm $\frac{5}{8}$ *m* NaCl-Lösung zugefügt. In keinem Falle bildete sich ein Embryo. In einer zweiten Reihe wurden zu je 100 ccm $\frac{5}{8}$ *m* NaCl $^1/_2$, 1, 2, 4, 8 und 16 ccm einer $2^1/_2$ *m*-Rohrzuckerlösung zugefügt. Auch diesmal wurden keine Embryonen gebildet. Im Hinblick auf die sogleich zu erwähnenden Versuche mit Zinksulfat müssen wir aber die Frage offen lassen, ob mit sehr grossen Dosen von Rohrzucker nicht am Ende kleine antitoxische Wirkungen zu erzielen wären.

Es gelang uns auch nicht, durch Zusatz von Aethylalkohol oder Glycerin die toxischen Wirkungen einer $\frac{5}{8}$ *m* NaCl-Lösung abzuschwächen. Wir setzten zu je 100 ccm der $\frac{5}{8}$ *m* NaCl-Lösung $^1/_2$, 1, 2, 4, 8 und 16 ccm einer $\frac{m}{32}$, $\frac{m}{8}$, 10 *m* und 20 *m*-Alkohollösung zu, ohne jede Spur einer antitoxischen Wirkung. Glycerin wurde in

$\frac{m}{8}$- und 3 m-Lösungen angewandt, ohne dass antitoxische Wirkungen beobachtet wurden.

Die Versuche mit Zinksulfat als toxische Substanz fielen ebenso negativ aus, mit einer einzigen, aber wie wir glauben, nur scheinbaren Ausnahme. Wir benutzten in diesem Versuche als giftige Lösung 5 ccm einer $\frac{m}{32}$ $ZnSO_4$-Lösung, welche zu 100 ccm H_2O zugefügt wurde. Wir hatten ja gesehen und überzeugten uns von neuem in jedem der folgenden Versuche, dass, wenn man die $\frac{m}{32}$-$ZnSO_4$-Lösung 21 fach durch destillirtes Wasser verdünnt, sie die Entwicklung von Embryonen verhindert. Wir hatten ferner gesehen, dass, wenn man statt 100 ccm destillirten Wassers 100 ccm einer $\frac{m}{8}$ bis $\frac{5}{8}$ m NaCl-Lösung zusetzt, die giftigen Wirkungen geringer werden oder aufhören. Wir versuchten nun, ob auch die giftigen Wirkungen von 5 ccm einer $\frac{m}{32}$-Zinksulfatlösung verhindert werden, wenn man 100 ccm der Lösung irgend eines Nichtleiters zusetzt. Wir setzten in einer Versuchsreihe 100 ccm einer $\frac{m}{64}$, $\frac{m}{32}$, $\frac{m}{16}$, $\frac{m}{8}$, $\frac{m}{4}$, $\frac{m}{2}$ und m-Harnstoff- zu je 5 ccm der $\frac{m}{32}$-Zinksulfatlösung. Kein einziges Ei bildete einen Embryo. In einem analogen Versuch wurden die gleichen Mengen einer Glycerinlösung statt der Harnstofflösung benutzt, ohne dass sich ein Embryo bildete. Auch Lösungen von Aethylalkohol waren nicht im Stande, die toxischen Wirkungen des Zinksulfats aufzuheben. Ganz unerwarteter Weise gab aber ein Versuch mit Zuckerlösung positive antitoxische Wirkungen, wie aus Tabelle VIII hervorgeht.

Tabelle VIII.

Natur der Lösung	Procentsatz der Eier, welche Embryonen bildeten
100 ccm destillirtes Wasser	55 %
100 „ „ „ + 5 ccm $\frac{m}{32}$ $ZnSO_4$	0 %
100 „ $\frac{m}{2}$ Rohrzucker + 5 ccm $\frac{m}{32}$ $ZnSO_4$	42 %
100 „ $\frac{3}{8}$ m „ + 5 „ $\frac{m}{32}$ „	47 %
100 „ $\frac{m}{4}$ „ + 5 „ $\frac{m}{32}$ „	44 %

Natur der Lösung	Procentsatz der Eier, welche Embryonen bildeten
100 ccm $\frac{m}{8}$ Rohrzucker + 5 ccm $\frac{m}{32}$ $ZnSO_4$	4 %
100 „ $\frac{m}{16}$ „ + 5 „ $\frac{m}{32}$ „	1 %
100 „ $\frac{m}{32}$ „ + 5 „ $\frac{m}{32}$ „	0 %

100 ccm einer $\frac{m}{2}$ — $\frac{m}{8}$-Rohrzuckerlösung waren also im Stande, die giftigen Wirkungen von 5 ccm einer $\frac{m}{32}$-Zinksulfatlösung fast ganz aufzuheben. Es ist jedoch zu berücksichtigen, dass die Rohrzuckerlösung ein Jahr alt war. Wir erhielten aber mit einer frischbereiteten $\frac{m}{2}$-Rohrzuckerlösung ebenfalls, wenn auch geringere positive Resultate. Leider war die Laichzeit von Fundulus inzwischen abgelaufen, so dass wir keine weiteren Versuche mehr anstellen konnten. Wir sind geneigt, anzunehmen, dass der Rohrzucker die Entgiftung der Zinksulfatlösung durch die Bildung von Zinksaccharaten und dadurch bedingter Verminderung der Zinkionen zu Stande brachte. Wenn das richtig ist, so können wir allgemein sagen, dass die Nichtleiter nicht im Stande sind, bei Funduluseiern die toxischen Wirkungen von Ionen aufzuheben, es sei denn, dass sie Verbindungen mit denselben eingehen und so die Concentration der toxischen Ionen vermindern.

IV. Können die toxischen Wirkungen eines Elektrolyten durch H- oder HO-Ionen aufgehoben werden?

Die Thatsache, dass die antitoxische Wirksamkeit eines Kations so rasch mit der Werthigkeit desselben zunimmt, bringt diese Beobachtungen in Beziehung zu den Thatsachen, welche auf einen ähnlichen Einfluss der Werthigkeit auf die Fällungserscheinungen in colloidalen Lösungen hinweisen. Dieser Einfluss der Werthigkeit der Ionen auf die Fällung suspendirter Theilchen wird von Bredig anders aufgefasst. Nach ihm ist „der von Linder und Picton, Schulze u. A. gefundene Einfluss der Werthigkeit des Kations wohl auf den grösseren Gehalt an hydrolytisch abgespaltener Säure mehrwerthiger Metalle zurückzuführen“ [1]). Das machte es nöthig,

1) Bredig, Anorganische Fermente. Leipzig 1901.

zu prüfen, ob nicht die giftigen Wirkungen einer reinen Kochsalzlösung durch Zusatz von Säure aufgehoben werden könnten. Zunächst wurden Versuche über die Giftigkeit verschiedener Säuren auf das Fundulusei angestellt. Dieselben ergaben, dass in $\frac{m}{1000}$ und selbst $\frac{m}{2000}$-Lösungen von anorganischen Säuren im Allgemeinen kein Fundulusei einen Embryo zu bilden vermag. So entwickelte sich weder in $\frac{m}{2000}$ HCl noch in $\frac{m}{2000}$ HNO_3 ein Embryo. Es macht den Eindruck, als ob die giftigen Wirkungen der Säuren nicht ausschliesslich auf das Wasserstoffion bezogen werden dürften. Wir wollen eine Versuchsreihe hier anführen. Es kam uns in derselben darauf an, solche Concentrationen zu benutzen, die gerade unter der in voraufgehenden Versuchen gefundenen Schwelle für absolute Giftigkeit liegen.

Tabelle IX.

Natur der Lösung	Procentsatz der Eier, welche Embryonen bildeten
100 ccm destillirtes Wasser	33 %
100 „ $\frac{m}{4000}$ HCl	27 %
100 „ $\frac{m}{8000}$ „	34 %
100 „ $\frac{m}{4000}$ HNO_3	27 %
100 „ $\frac{m}{8000}$ „	37 %
100 „ $\frac{m}{2000}$ H_2SO_4	1 %
100 „ $\frac{m}{4000}$ „	2 %
100 „ $\frac{m}{1000}$ $HClO_3$	3 %
100 „ $\frac{m}{2000}$ „	7 %
100 „ $\frac{m}{2000}$ H_3PO_4	0 %
100 „ $\frac{m}{4000}$ „	1 %
100 „ $\frac{m}{1000}$ H_3AsO_4	2 %
100 „ $\frac{m}{2000}$ „	10 %

Natur der Lösung	Procentsatz der Eier, welche Embryonen bildeten
100 ccm $\frac{m}{1000}$ Essigsäure	9 %
100 „ $\frac{m}{2000}$ „	17 %
100 „ $\frac{m}{2000}$ Milchsäure	1 %
100 „ $\frac{m}{4000}$ „	9 %
100 „ $\frac{m}{4000}$ Weinsäure	7 %
100 „ $\frac{m}{6000}$ „	10 %
100 „ $\frac{m}{4000}$ Citronensäure	16 %
100 „ $\frac{m}{6000}$ „	21 %

Da bei dem hier angewendeten Grad der Verdünnung die Dissociation ziemlich vollständig ist, so ist der auffallende Unterschied in der Giftigkeit z. B. zwischen H_3PO_4 und H_3AsO_4 schwer zu verstehen, es sei dann, dass gewisse Anionen bei der Giftwirkung betheiligt sind. Allein, da die relative Giftigkeit der Säuren nicht unser eigentliches Thema ist, so wollen wir uns lieber gleich der Frage nach den antitoxischen Wirkungen der Säuren zuwenden. Um die (allerdings geringe) Möglichkeit einer antitoxischen Wirkung des Anions der Säure auszuschliessen, benutzten wir Salzsäure als antitoxische Substanz gegen NaCl. Wir fanden, dass Salzsäure oder richtiger Wasserstoffionen in den von uns angewendeten Concentrationen die giftigen Wirkungen einer $\frac{5}{8}$ m-Kochsalzlösung nicht aufzuheben im Stande sind, wie Tabelle X zeigt.

Tabelle X.

Natur der Lösung	Procentsatz der Eier, welche Embryonen bildeten
100 ccm Seewasser	47 %
100 „ $\frac{5}{8}$ m NaCl	0 %
100 „ $\frac{5}{8}$ m „ + ¼ ccm $\frac{m}{100}$ HCl	0 %
100 „ $\frac{5}{8}$ m „ + ½ „ $\frac{m}{100}$ „	0 %

Natur der Lösung	Procentsatz der Eier, welche Embryonen bildeten
100 ccm $\frac{5}{8}$ m NaCl + 1 ccm $\frac{m}{100}$ HCl	0 %
100 „ $\frac{5}{8}$ m „ + 2 „ $\frac{m}{100}$ „	0 %
100 „ $\frac{5}{8}$ m „ + 3 „ $\frac{m}{100}$ „	0 %
100 „ $\frac{5}{8}$ m „ + 4 „ $\frac{m}{100}$ „	0 %

Loeb hatte bereits mitgetheilt, dass die Hydroxylionen bei Weitem nicht so giftig für das Fundulusei sind wie die Wasserstoffionen. In $\frac{m}{200}$-Lösungen von KHO bildeten eine Reihe von Eiern noch Embryonen, während für NaHO und $Ca(HO)_2$ die Grenze etwas niedriger liegt, nämlich $\frac{m}{400}$. Loeb fand, dass Hydroxylionen die toxischen Wirkungen einer Kochsalzlösung nicht aufheben oder vermindern. Wir wiederholten den Versuch ebenfalls mit demselben Resultat.

Tabelle XI.

Natur der Lösung	Procentsatz der Eier, welche Embryonen bildeten
100 ccm destillirtes Wasser	47 %
100 „ $\frac{5}{8}$ m NaCl	0 %
100 „ $\frac{5}{8}$ m „ + ¼ ccm $\frac{m}{10}$ KHO	0 %
100 „ $\frac{5}{8}$ m „ + ½ „ $\frac{m}{10}$ „	0 %
100 „ $\frac{5}{8}$ m „ + 1 „ $\frac{m}{10}$ „	0 %
100 „ $\frac{5}{8}$ m „ + 2 „ $\frac{m}{10}$ „	0 %
100 „ $\frac{5}{8}$ m „ + 3 „ $\frac{m}{10}$ „	0 %
100 „ $\frac{5}{8}$ m „ + 4 „ $\frac{m}{10}$ „	0 %

Wir sehen also, dass die giftige Wirkung einer $\frac{5}{8}$ m NaCl-Lösung weder durch HO- noch durch H-Ionen beseitigt werden kann, soweit unsere bisher angestellten Versuche gehen, und dass es daher wohl einst-

weilen nicht angeht, die antitoxischen Wirkungen, welche durch Elektrolyte mit mehrwerthigen Kationen erzielt werden, auf hydrolytisch abgespaltene Säure zurückzuführen.

V. Weitere Versuche über die Entgiftung von Kochsalzlösung durch mehrwerthige Metallionen.

Loeb hatte gefunden, dass sehr kleine, aber bestimmte Mengen irgend eines Salzes mit zwei- oder dreiwerthigem Metall die giftigen Wirkungen grosser Mengen eines Salzes mit einwerthigem Kation, z. B. Kochsalz, aufheben. Die mehrwerthigen Kationen, mit deren Salzen er bisher antitoxische Wirkungen erzielt hat, waren: Mg, Ca, Sr, Ba, Fe, Co, Zn, Pb, Al, Cr. Negative Resultate erhielt er mit Kupfer- und Quecksilbersalzen. Wir dehnten diese Versuche weiter aus und fanden, dass auch die Mangansalze im Stande sind, die giftigen Wirkungen einer reinen Kochsalzlösung völlig aufzuheben, dass Nickelsalze nur in beschränktem Maasse derartige Wirkungen haben. Wir wollen eine Versuchsreihe hier mittheilen.

Tabelle XII.

Natur der Lösung	Procentsatz der Eier, welche Embryonen bildeten
100 ccm Seewasser	48 %
100 „ $\frac{5}{8}$ m NaCl	0 %
100 „ $\frac{5}{8}$ m „ + 4 ccm $\frac{m}{16}$ $MnCl_2$	52 %
100 „ $\frac{5}{8}$ m „ + 8 „ $\frac{m}{16}$ „	55 %
100 „ $\frac{5}{8}$ m „ + 16 „ $\frac{m}{16}$ „	34 %
100 „ $\frac{5}{8}$ m „ + 2 „ $\frac{m}{8}$ $NiCl_2$	0 %
100 „ $\frac{5}{8}$ m „ + 4 „ $\frac{m}{8}$ „	5 %
100 „ $\frac{5}{8}$ m „ + 8 „ $\frac{m}{8}$ „	0 %

Die Versuche wurden wiederholt und bestätigt. Der Umstand, dass wir Chloride von Mangan und Nickel benutzten, um die Kochsalzlösung zu entgiften und dass so die Dissociaton der Kochsalzlösung verringert wurde, hat nichts mit dem Resultat zu thun, da, wie wiederholt erwähnt, erstens der Zusatz irgend eines Chlorids mit

einwerthigem Kation keine antitoxischen Wirkungen hervorbringt, und da zweitens die zugesetzte Menge des Manganchlorids ausserordentlich klein im Verhältniss zur angewandten Kochsalzmenge ist. Dieser Punkt ist übrigens in den früheren Versuchen von Loeb eingehend geprüft worden. Spuren antitoxischer Wirkung erhielten wir mit $Th(NO_3)_4$ und $UO_2(NO_3)_2$. In einem Falle wurden zu 100 ccm $\frac{5}{8}$ m NaCl 1 ccm $\frac{m}{160}$ $UO_2(NO_3)_2$ zugesetzt, und 3% der Eier bildeten Embryonen. Dieser Versuch wurde wiederholt und bestätigt. Durch Zusatz von 8 ccm von $\frac{m}{192}$ $Th(NO_3)_4$ zu 100 ccm $\frac{5}{8}$ m NaCl erhielten wir ebenfalls eine Andeutung einer antitoxischen Wirkung. Aber alle Versuche, mit Uran- und Thoriumsalzen kräftigere antitoxische Wirkungen zu erzielen, schlugen fehl.

Es gelang uns auch nicht, mit Cadmiumsalzen irgend welche antitoxische Wirkungen zu erzielen. In Bezug auf Kupfer- und Quecksilberionen nahm Loeb an, dass dieselben bereits in derjenigen Concentration tödtlich sind, in welcher sie für die antitoxischen Wirkungen zur Anwendung gelangen müssen. Ob dasselbe auch für Cadmiumionen zutrifft, vermögen wir einstweilen nicht zu entscheiden.

VI. Schlussfolgerungen.

Die vorliegende Arbeit bestätigt die frühere Beobachtung von Loeb, dass jede Lösung eines Elektrolyten von einer gewissen Concentration an die Entwicklung des Funduluseies hemmt und das Ei tödtet, dass aber diese giftigen Wirkungen im Allgemeinen ganz oder theilweise durch Zusatz eines zweiten Elektrolyten aufgehoben werden können.

Die Arbeit bestätigt ferner und liefert neues Material für die von Loeb gefundene Thatsache, dass für den Grad der Wirksamkeit des antitoxischen Elektrolyten die Werthigkeit des Kations derselben eine grosse Rolle spielt, wenn nicht entscheidend ist; und zwar ist im Allgemeinen die antitoxische Wirksamkeit zweiwerthiger Kationen ausserordentlich viel grösser als die einwerthiger. Während beispielsweise ein Molekül Zinksulfat für die Entgiftung von 1000 Molekülen Kochsalz bei der eben giftigen Concentration des letzteren ausreichte, waren umgekehrt 50 Moleküle Kochsalz für die Entgiftung

von einem Molekül Zinksulfat bei der eben giftigen Concentration des letzteren erforderlich.

Unsere Versuche machen es unwahrscheinlich, dass die antitoxischen Wirkungen von Salzen mit mehrwerthigem Kation durch die in gewissen dieser Lösungen enthaltenen freien Wasserstoffionen bedingt sind.

Unsere Versuche endlich bringen, wie wir glauben, überzeugendes Material dafür, dass Lösungen von Nichtleitern, nämlich Harnstoff, Rohrzucker, Glycerin und Alkohol, keine antitoxischen Wirkungen auf die Lösung eines Elektrolyten haben, mit der scheinbaren Ausnahme der Fälle, in denen der Nichtleiter (z. B. Rohrzucker) die Concentration der giftigen Ionen durch Bildung schwer dissociirbarer Verbindungen verringern könnte, (z. B. Saccharatbildungen).

In Bezug auf die Grundlage für die antagonistischen Beziehungen zwischen zwei Elekrolyten und die besondere Bedeutung der Werthigkeit und möglicher Weise der elektrischen Ladung der Ionen sei an die früheren Arbeiten von Loeb erinnert. Derselbe zeigte, dass zwei verschiedene Annahmen hier zulässig sind. Es ist erstens möglich, dass die Metalle dadurch wirken, dass sie Verbindungen mit gewissen Protoplasmabestandtheilen eingehen und so die Eigenschaften des Protoplasmas verändern. Oder es ist möglich, dass die Ionen, vielleicht vermöge ihres elektrischen Feldes, auf gewisse colloidale Lösungen in den Zellen wirken und so die Zustände des Protoplasmas beeinflussen, ohne dass sie chemische Verbindungen mit den Bestandtheilen einzugehen brauchen, deren Eigenschaften sie ändern. Herr Dr. W. Koch hat neuerdings im physiologischen Institut in Chicago gefunden, dass (colloidale?) Lösungen von Lecithin durch kleine Quantitäten eines Elektrolyten mit zweiwerthigem Kation gefällt werden, nicht aber durch Elektrolyte mit einwerthigem Kation; und dass sogar ein Antagonismus zwischen den Salzen mit einwerthigem und zweiwerthigem Metall besteht, indem Zusatz von Kochsalz (oder KCl etc.) zu der Lecithinlösung die zur Fällung des Lecithins nöthige Menge eines Elektrolyten mit zweiwerthigem Kation erhöht. Da Lecithin in allem Protoplasma erhalten ist, so ist immerhin die Möglichkeit vorhanden, dass die antagonistischen Ionenwirkungen zum Theil auf den Einfluss der Elektrolyte auf den physikalischen Zustand der Lipoide in den Zellen zurückzuführen sind. Was aber auch die Ursache dieser antagonistischen Ionenwirkungen sein möge, das Wichtigste ist einstweilen der Nachweis, dass sie bestehen, und dass

wir bei allen Versuchen mit Nährlösungen mit dem von Loeb eingeführten Begriff der physiologisch äquilibrirten Salzlösungen[1]) zu rechnen haben, d. h. solchen Salzlösungen, bei denen die Giftwirkungen sich gegenseitig aufheben, welche jeder einzelne Elektrolyt oder jede einzelne Gruppe von Ionen haben würde, wenn sie allein in Lösung wären.

1) American Journal of Physiology vol. 3 p. 434. 1900.

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A NOTE ON THE EXCRETION OF KYNURENIC ACID.

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IN their paper on the excretion of kynurenic acid, Mendel and Jackson showed that substance to be a direct product of proteid catabolism. They found, further, that excretion of kynurenic acid accompanied accelerated proteid decomposition, whether this condition was brought about by fasting, or the ingestion of proteid food in quantities largely in excess of the needs of the body, or through the action of drugs. These observers also noted that, in conditions of ordinary nitrogenous equilibrium, the kynurenic acid in the urine was greatly diminished or might be entirely absent.[1]

The author, in repeating recently some of Mendel and Jackson's experiments, determined the excretion of kynurenic acid (1) during periods of nitrogenous equilibrium; (2) when proteid catabolism was stimulated, by chemical dosage as well as by excessive ingestion of proteid substance; and (3) when proteid catabolism was diminished by the lack of food. The animal, a healthy mongrel bitch, weighing 15 kilos, was confined in a cage suitable for metabolism work and given daily, at 9 A. M and 6 P. M., in two equal portions, a diet of 250 gms. of hashed meat,[2] 50 gms. of cracker meal, 40 gms. of lard and 700 c.c. of water, containing a total of 9.854 gms. of nitrogen.

The experiment lasted twenty-four days and was divided into three periods. Throughout the first period, of seven days, normal conditions prevailed and the dog was in almost perfect nitrogenous equilibrium. During the second period, ten days, the animal was given

[1] Mendel and Jackson: This journal, 1898, ii, p. 190. See also, Mendel and Schneider: Proceedings of the American Physiological Society. This journal, 1901, v, p. ix.

[2] The hashed meat was prepared in bulk, freed from surplus moisture and kept in bottles, in a cold storage room, the frozen condition maintaining constancy of composition.

several large doses of tellurous oxide, a substance which not only causes slight stimulation of proteid catabolism, but likewise induces vomiting and loss of appetite.[1] In the third period, of seven days, normal conditions were present once more and equilibrium was restored.

On the morning of the second day of the dosage period, when the greatest amount of tellurous oxide was administered (0.5 gm. with the morning meal), all of the food given with it was vomited immediately. The second half of the daily portion of food was vomited in the evening also, so that no food was retained that day.[2] On the following day twice the usual amount of food was given. All of it was eaten and retained. For the remainder of the dosage period no gastric disturbances were induced and nitrogenous equilibrium was restored.

The experimental data[3] in this connection are given herewith in the table on the opposite page.

Nitrogen was determined by the Kjeldahl process; uric acid with Ludwig's,[4] kynurenic acid with Capaldi's,[5] methods. Uric acid was determined in combined urines, which were preserved with powdered thymol; the figures in the tables were recorded on the last days of each separate combination. The nitrogen of the daily food was 9.854 gms. The "total nitrogen balance" includes the nitrogen of the fæces and hair. The nitrogen of the vomit of the ninth day (10.335 gms.) was subtracted from the ingested nitrogen of the period in striking the balance. The total nitrogen in the fæces of the three periods was 2.374, 5.154 and 3.291 gms., respectively; in the cast off hair it was 1.054, 1.232 and 1.184 gm. The amount of tellurous oxide given on the first day of the dosage period was 0.5 gm., on the second, 0 75 gm.; on each of the third and fourth, 0.25 gm.; during the remainder of the period, 0.1 gm. per day. Indican, determined by the Jaffe-Stokvis test,[6] was present in the urine of each period.

[1] MEAD and GIES: This journal, 1901, p. 147.

[2] The quantity of nitrogen in the vomit slightly exceeded that of the daily food, showing that none of the latter had been retained. The excess of nitrogen in the vomit came from gastric mucus.

[3] These results were presented informally at the last annual meeting of the American Physiological Society.

[4] NEUBAUER und VOGEL: Analyse des Harns, zehnte Auflage, 1898, p. 820.

[5] CAPALDI: Zeitschrift für physiologische Chemie, xxiii, p. 92.

[6] NEUBAUER und VOGEL: *Ibid.*, p. 166.

1. Fore Period.				2. Dosage Period.					3. After Period.			
Day. No.	Nitrogen. Grams.	Uric acid. Gm.	Kynurenic acid.	Day. No.	Nitrogen Grams.	Uric acid. Gm.	Kynurenic acid. Gm.	Remarks.	Day. No.	Nitrogen. Grams.	Uric acid. Gm.	Kynurenic acid.
1	10.421	....	trace	8	8.982	....	none	Usual quantity food eaten	18	9.002	....	none
2	10.013	....	trace	9	3.654	....	0.046	All food vomited	19	9.434	....	none
				10	13.117	....	0.180	Double quantity food eaten				
3	9.403	....	none	11	10.031	0.284	0.131	Usual quantity food eaten	20	8.831	....	none
4	8.921	0.242	none	12	12.831	....	0.085	Usual quantity food eaten	21	9.324	....	none
				13	9.982	....	trace	Usual quantity food eaten				
5	9.410	....	none	14	9.674	....	none	Usual quantity food eaten	22	9.238	....	none
6	8.960	....	none	15	8.361	....	none	Usual quantity food eaten	23	8.597	....	none
				16	8.904	....	none	Usual quantity food eaten				
7	8.231	0.199	none	17	9.206	0.328	none	Usual quantity food eaten	24	8.768	0.468	none
Totals	65.359	0.441	trace	Totals	94.752	0.612	0.442	....	Totals	63.194	0.468	none
Daily averages	9.337	0.063	....	Daily averages	9.475	0.061	....	...	Daily averages	9.028	0.067	....
Total nitrogen balance, + 0.191				Total nitrogen balance, — 3.079.					Total nitrogen balance, + 1.309			

The results of this experiment agree entirely with those obtained by Mendel and Jackson. It will be seen from the table that, excepting traces at the very beginning of the experiment when the dog was about to enter into equilibrium, kynurenic acid was eliminated only during the second period and then only on the days when the physiological balance was upset by the circumstances attending tellurium dosage. When the animal drew upon its own store of proteid, as it certainly did on the day of vomiting, kynurenic acid in small quantity was excreted for the first time. On the following day, when fed more than enough to satisfy its immediate needs, kynurenic acid was again eliminated. On the two succeeding days excretion of kynurenic acid continued; but it failed to appear when equilibrium was restored.

That the dog was in almost perfect nitrogenous balance during the second half of the dosage period (five days), when, with the exception of the trace on the thirteenth day, no kynurenic acid was eliminated, is evident from the following summary:

Nitrogen excreted:		
Urine	46.127	
Fæces[1]	2.577	49.320
Hair[1]	0.616	
Nitrogen ingested		49.270
Nitrogen balance		− 0.050

From these figures it is also clear that the increased nitrogenous catabolism, represented by 3.079 gms. of nitrogen (the "total nitrogen balance"), occurred in the first half of the period, during four days of which kynurenic acid was eliminated in appreciable quantity. These results indicate, further, that when nitrogenous equilibrium is completely upset by vomiting, it may sometimes be quickly restored by proper quantitative feeding.

It seems worthy of note, in this connection, that intestinal putrefaction, as indicated by the constant presence of indoxyl in the urine, was normal throughout the experiment. This, since kynurenic acid was excreted only when metabolism was disturbed, suggests, of course, that formation of this substance may occur independently of putrefactive changes in the intestine.[2] It certainly may be entirely absent when putrefaction is quite marked.

[1] The figures for nitrogen of fæces and hair represent one-half of the totals for the period. The quantitative elimination of each was constant daily, so that the above amounts are almost exact values.

[2] See MENDEL and SCHNEIDER: *Loc. cit.*

In conclusion, attention may be drawn to the fact that uric acid was eliminated in constant quantity throughout the experiment and that, therefore, kynurenic acid did not replace it. Excretion of the latter occurred independently of elimination of the former. The results recorded here confirm the observations of Solomin,[1] and also those of subsequent workers in this connection.

[1] SOLOMIN: Zeitschrift für physiologische Chemie, 1897, xxiii, p. 497.

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SOME FACTS REGARDING "UREINE."[1]

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AND

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NEW YORK.

WE were amazed, recently, on reading Dr. William Ovid Moor's account of "The Discovery of Ureine, the Principal Organic Constitutent of Urine, and the True Cause of Uræmia,"[2] to find that such sweeping generalities had been based upon methods so defective chemically. Our faith in the older observations that urea is the chief organic constituent of urine has been so complete, and our confidence in the deductions of far-reaching significance based thereon has been so thorough, that it was impossible, in the absence of real chemical evidence in favor of Dr. Moor's assertions, to accept his inferences that "the human urine contains a liquid [!] organic body, in a quantity superior to urea," and that "this organic liquid (ureine) is the most characteristic component part of urine."

Dr. Moor began his paper with the statement that he found the human urine to contain "a large quantity of some organic substance which gives in a very intense manner" the same characteristic blue reaction with a solution of ferricyanide of potassium and ferric chloride that may be observed when morphine and various alkaloids are treated with a solution of these two

[1] From the Laboratory of Physiological Chemistry of Columbia University, at the College of Physicians and Surgeons, New York.

[2] Communication presented to the Thirteenth International Medical Congress, Paris, 1900. Published in the MEDICAL RECORD, 1900 (Sept. 1st), vol. lviii., No. 9, p. 336.

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salts of iron. "Numerous and exact investigations," he adds, "forced him to the conclusion that none of the known organic or inorganic components of urine could account for this intense blue reaction, and that, therefore, some chemical body, until the present unknown, must be the cause of this strange phenomenon." We do not know, of course, how "large" the quantity of this substance was, nor how "intense" the reaction it caused; but the fact that Gautier,[1] Bouchard,[2] Aducco,[3] and others have found basic bodies in both normal and abnormal urine giving this reaction, makes it seem probable that Dr. Moor's "strange phenomenon" is to be referred, in part at least, to substances of similar character dissolved in urine, and not especially to anything unknown, as he has assumed. There is sufficient reason to believe that each of these observers was dealing with chemically pure, individual substances, and that these products were not contaminated with ureine.

Dr. Moor goes on to say: "It is not surprising that the existence of a metabolism product of such great importance should, until the present, have escaped our knowledge, for every urinary analysis has been made with the firmly rooted idea that urine is a liquid composed of water and of inorganic solid ingredients"! In the light of every-day knowledge regarding the quantity of such organic substances as urea, uric acid, creatinin, hippuric acid, etc., in normal urine and their relation to metabolism, this inaccurate statement is worth considering only because it suggests how much of fact there is in the ureine story.

In one place Dr. Moor concludes that "this organic metabolism product of the human body (ureine) belongs to the group of alcohols of the aromatic series; at a temperature of about 80° C. it begins to split into

[1] Gautier: "Les alcaloïdes dérivés des matières protéiques sous l'influence de la vie des ferments et des tissus." Journ. de l'anat. et de la physiol., 1881, p. 330.

[2] Bouchard: "De l'origine intestinale de certains alcaloïdes normaux ou pathologiques." Revue de méd., 1882, p. 12.

[3] Aducco: "Sur l'existence de bases toxiques dans les urines physiologiques." Arch. ital. de Biol., 1888, p. 203.

several bodies belonging to the class of aromatic oxy-acids." At another, he states "ureine is a ferment, which has a potential energy of at least 130° C." Yet neither of these conclusions is accompanied by any statement of fact upon which to base them, and only imagination seems to account for them. Thus, ureine is said, in this connection, to be "the principal [!] cause of the ammoniacal fermentation of urine, as without its presence urea cannot be decomposed into ammonia and carbon dioxide." Again, "only a temperature of above 130° C., perhaps 140° C., can split urea into ammonia and carbon dioxide." All this in mere dogmatic statement, with no experimental justification and entirely in opposition to the numerous results of very laborious work for years by many careful investigators.

Dr. Moor also says: "Urea, in many respects, is just as indestructible [does he mean undecomposable?] as iron, silver, or any other elements, for the strongest mineral acids do not decompose [!] it, but simply combine with it." Is it not true that one of the properties of all matter is "indestructibility"? Dr. Moor, however, may have intended to use the word in a different sense from that customarily applied to it. If he means to refer to comparative stability, what of the fact that even dilute hydrochloric acid decomposes urea? Everybody knows, also, how quickly urea is broken up when concentrated nitrous acid, for example, acts upon it.

In all of the unaccountable assumption characterizing Dr. Moor's paper, there is nothing, however, to compare with the biological burlesque at the close. "Without ureine," he says, "all organic matter would become converted into urea, which would remain in nature without any use, and thus within a limited period of time all vegetation and animal as well as human life would cease."

Truly, as Dr. Moor remarks, "this wonderful organic fluid," this "mysterious chemical body," ought to receive very careful study. We ourselves have been of the opinion, however, that the method by which it has been made deserves much more attention.

With a view of testing these points experimentally, we have lately made several preparations of ureine, not only by the method given in the paper alluded to at the outset, but also by the improved process outlined in Dr. Moor's latest communication.[1]

Reviewing the method, as improved, the normal human urine, varying in quantity from 1.5 to 49 litres, was evaporated at a temperature not above 50° C., usually at 48° C., until no more vapor could be seen arising from the surface of the fluid, even after the application of Dr. Moor's "indispensable" and "delicate test"; as long as rapid withdrawal of the thermometer from the fluid, following directions, caused "a puff of vapor ascending from the mercury bulb," the evaporation was continued. It stands to reason, of course, that even though no steam can be seen arising from the evaporating fluid under these conditions, insensible vapor may continue to form at this point, and a large proportion of water may be left in the fluid. Certainly, water cannot be completely removed from a urinary residue at such a low temperature—a matter of importance, bearing not only on the separation of urea, etc., farther on, but also influencing the percentage of ureine.

The concentrated fluid, still containing a fairly large proportion of water, of course, was next kept in an ordinary freezing mixture, at 10° below 0° C. for several hours. (Dr. Moor says: "We reduce the temperature of the liquid, if possible, to 0° C." The length of time the mixture is to be held there is not given.) A heavy precipitate, mostly of inorganic matter, quickly separated. Absolute alcohol at 10° below 0° C., equal to half the quantity of fluid, was then added to "facilitate filtration," and this mixture filtered in small quantities (while below the freezing-point), thus removing most, though by no means all, of the urinary salts.

The main bulk of urea was thrown out of this fil-

[1] Moor: "The Discovery of Ureine, the Principal Organic Constituent of Urine." THE MEDICAL RECORD, 1900 (Sept. 22d), lviii. No. 12, p. 471.

trate in the form of urea oxalate with powdered oxalic acid (1 gm. for each 100 c.c. of urine used), and then alcoholic solution of oxalic acid—thirty-per-cent solution—was added "until no further precipitate was formed." By very careful work at this point we found that immediately visible precipitation could not be relied upon as a true indicator of complete separation. Consequently, in order to avoid excess of oxalic acid (Dr. Moor says nothing about this) its alcoholic solution was added in small quantities at intervals of several hours until the filtrate gave only a delicate reaction for oxalic acid, with calcium chloride in the presence of acetic acid. At this stage only a slight permanent precipitate formed on adding a little more alcoholic solution of oxalic acid, even after standing over night. It must not be forgotten in this connection, however, that urea oxalate is somewhat soluble even in absolute alcohol. But under these conditions, with considerable water left in the previously evaporated residue, the alcoholic mixture possessed increased solvent action. In testing with calcium chloride, the positive reaction just referred to was doubtless due in part to the combined oxalic acid in solution in the form of urea oxalate. This method, even at best, therefore, certainly does not suffice, as Dr. Moor seems to assume, for complete and satisfactory removal of urea.

The filtrate from the urea oxalate was next concentrated at 48° C. In a few hours a thick, dark-brown, oily fluid was obtained. This Dr. Moor has the assurance to speak of as a chemical individual—"ureine"—admixed merely with pigment and "some saline matter." He says that at this point "there is nothing but ureine, together with coloring matters." He adds that this ureine "is sufficiently pure to satisfy all exigencies of clinical medicine and of physiology," and then, inconsistently and with little reason, concludes, "for it is evident that the presence of some saline matter cannot influence the qualities of ureine." How, we may ask, were the other organic substances of the urine having physiological influence removed, such as indi-

can, aromatic oxy-acids, toxic basic bodies, etc., etc.? Further, what reason is there for believing that the presence of urinary saline matters cannot influence the toxicological qualities of ureine—potassium salts for example?

In the preparation of chemically pure (?) ureine from this point, following Dr. Moor's recommendations for making ureine for merely chemical purposes, the alcoholic solution was "treated successively with barytes to remove the sulphates and phosphates, with nitrate of silver to separate the chloride of sodium"! This addition was made very carefully in each case, to avoid excess of silver and barium, a precaution not referred to by Dr. Moor. But did all of this result in removal of inorganic salts contained in the urinary residue, or did it effect mainly their transformation? We think we are safe in saying that the potassium chloride, for example, still present in ureine was merely converted into soluble nitrate of potassium and into insoluble chloride of silver. The latter was filtered off later, but the nitrate remained in the ureine, possessing even more toxic influence than the corresponding chloride. In short, these reagents, speaking generally, removed radicles, not salts; the amount of inorganic matter left behind was undiminished, if not increased; and the mixture became in reality more toxic. We fail to see, therefore, how chemical purity was approached by such awkward steps as these. We purposely followed Dr. Moor's directions in detail here, however, merely to obtain results that would be comparable with his. Very heavy precipitates formed on the addition of the reagents, showing how large was the proportion of inorganic substance in the ureine which its discoverer pronounced "sufficiently pure to satisfy all exigencies of clinical medicine and of physiology."

Finally, in the preparation of pure (?) ureine, as Dr. Moor directs, coloring matters were removed with mercuric nitrate. Much of the residual urea and other organic substances, as well as coloring matter, were

precipitated from the mixture by this substance. At first the precipitate with mercuric nitrate dissolved in the mixture, but eventually became permanent. In order to avoid excess of mercury (Dr. Moor gives no suggestion in this connection), addition of the nitrate was discontinued when nearly all color was removed and only very slight precipitation was still obtainable. The bulkiness of the precipitate at this point, as well as its appearance, further emphasized the absurdity of considering the previous fluid anything but a mixture. The final liquid was decidedly acid in all cases, as all the filtrates had been from the beginning. According to the original paper, Dr. Moor neutralized with sodium carbonate at this point. "It is advisable then," he says, "to add a sufficient amount of sodium carbonate, so as to render the liquid slightly alkaline." After all this he described ureine as a "very slightly alkaline, almost neutral," substance. Was its reaction just what Dr. Moor made it? In his second communication Dr. Moor says, why we do not know, "It is better not to neutralize subsequently with sodium carbonate." Consequently, though he does not say so, ureine would have to be an acid substance, if purity had been attained by the procedure he has outlined.

The decolorized ureine was finally evaporated at 48°–50° C., to remove water, and then was carefully analyzed qualitatively. Employing customary methods of separation and detection, "purified" ureine was found to contain sodium, potassium, ammonia, phosphate, urea, creatinin, pyrocatechin, phenol, alkaloidal substances, and nuclein bases. Other urinary substances were present which we did not attempt to identify. In spite of the fact that we avoided excess of mercuric nitrate in the process of decolorization, we always found mercury in ureine. Doubtless a soluble organic compound of mercury that had formed with the nitrate was its immediate source. Nitrate and oxalic acid, also introduced during Dr. Moor's process of "purification," were constant constituents. Bluish to brownish amorphous material

separated as the fluid concentrated after decolorization. Shaken up repeatedly with ether during a period of three months, ureine separated gradually into four permanent layers of different color and varying degrees of transparency, and the ether itself became slightly yellowish. Samples that had been allowed to stand exposed to the air for three months, deposited crystals of urea. The mother liquor yielded additional crystals when its temperature was reduced to, and held at, 0° C. Under these conditions the fluid became semi-solid, so large was the proportion of crystalline material.

Dr. William A. Taltavall, who has had considerable chemical experience in this laboratory with urinary extractive bodies, analyzed several of our preparations and favored us with some of the qualitative data just presented. Mr. A. N. Richards, assistant in physiological chemistry, has also given us valuable help in this connection. We cordially thank both of these gentlemen for their able co-operation.

In a special series of preparations designed to determine the influence of variations of the method, we obtained the crude ureine from forty-nine litres of urine, and then, dividing it into two equal parts, decolorized one half with mercuric nitrate, the other half with plumbic acetate. Since the deductions in Dr. Moor's first paper were based on the qualities of neutralized ureine, we neutralized with sodium carbonate one half of each quantity of the "purified" ureine. That is, of the portion decolorized with mercuric nitrate, one half was neutralized ("M—2"), the other was not ("M—1"). In the same way, one half of that decolorized with plumbic acetate was neutralized ("P—2"), the other remained acid ("P—1"). "M—2," "P—1," and "P—2" solidified after evaporation at 45° C. for about a week, because of crystallization of urea and inorganic matter. They were unlike in appearance and hardness; "M—1," a thick oily fluid at this stage, seemed to contain the least quantity of solid matter, and to hold the smallest amount of crystalline substance in suspension.

The appended table (results in duplicate) shows the loss in weight of samples of each of these preparations, after having been at higher temperatures for prolonged periods; also the ash of each:

Preparation.	Weight of Urine Taken.	On Water Bath 50 Hours.	Total Number of Days in Air Bath at 100–110° C.			Weight of Ash.
			3½.	10½.	25.	
	Grams.	Grams.	Grams.	Grams.	Grams.	Grams.
M—1	3.90	3.36	2.63	2.25	1.88	0.1788
	3.94	3.37	2.64	2.23	1.96	.1818
M—2 (neutralized).	6.49	5.51	4.47	4.02	3.58	.8137
	7.63	6.66	5.49	4.82	4.10	.9566
P—1	5.65	5.03	4.16	3.79	3.41	.3562
	6.80	6.02	5.17	4.73	4.21	.4231
P—2 (neutralized).	5.12	4.58	3.81	3.53	3.08	.7590
	6.09	5.51	4.70	4.33	3.69	.7888

The following figures represent the loss of substance, at the end of the drying process, both in amount and in percentage, of original ureine; they give, also, the proportion of ash in the ureine and in the final dried residue:

Preparation.	Total Loss of Substance.		Percentage of Ash in:	
	Amount.	Per Cent.	Original Ureine.	Final Residue.
M—1	2.02	51.80	4.51	9.51
	1.98	50.25	4.61	9.27
M—2 (neutralized).	2.91	44.84	12.54	22.73
	3.53	46.26	12.53	23.33
P—1	2.24	39.65	6.30	10.44
	2.59	38.09	6.22	10.05
P—2 (neutralized).	2.04	39.84	14.82	24.64
	2.40	39.41	12.95	21.36

These results show at a glance that ureine, as our

qualitative results proved, is not a definite chemical substance, and indicate that with comparatively unimportant modifications of method it varies greatly in composition. If it were "a body belonging to the group of alcohols of the aromatic series," we should expect to find little or no residue after such vigorous heating for so long a period. Except that they were viscid rather than fluid, and a little darker in color, some of the final products were the same in appearance as the original mixture and seemed little affected by the heating. If "at a temperature of about 80° C. it (ureine) begins to split into several bodies belonging to the class of aromatic oxy-acids," what organic substance, may we ask, is left behind that is so resistant to this destructive action of a temperature of 100°–110° C.? There was, of course, some decomposition of contained organic products; but, we believe, most of the loss represented in the above tables was due to elimination of water. The figures in the tables are valuable, then, for the suggestion they make that ureine is a mixture.

In his second communication Dr. Moor says: "If we add nitric acid to ureine, a solid waxlike mass will be formed at once, and this is a fact worth remembering, as otherwise one might think that this solid, waxlike substance was the result of a chemical combination of nitric acid and urea." In all probability it was. This waxlike mass could be obtained immediately with concentrated nitric acid in all of our ureine preparations. When it was broken up mechanically in an excess of concentrated nitric acid, and examined under the microscope, this mass was found to consist almost solely of urea nitrate crystals. Diluted somewhat, each sample of ureine gave an abundant yield of crystals of urea nitrate and urea oxalate, with the corresponding concentrated acids.

We cannot agree that "it is this constitutent (ureine) of urine which is the cause of its specific odor." The longer the period of evaporation in preparing ureine, we have found, the less distinct is the odor remaining with it. The ability of ureine "to take up large quan-

tities of oxygen with great facility" is doubtless equivalent to the total capacity of its various constituents to do the same. The reaction with potassium ferricyanide and ferric chloride given by ureine may be attributed, in part at least, to the reducing substances we have found in it.

Dr. Moor's statements regarding the amount of ureine in urine, and also its specific gravity, cannot be credited. Our own experience in comparative observations shows that each is determined largely by the length of the period of evaporation in preparing ureine. The more prolonged the evaporation, after the time that vapor no longer can be seen arising from the fluid, the less is the volume, and the greater the weight, of residue (ureine).

With respect to the toxicity of ureine, upon which Dr. Moor lays so much stress, and its consequent influence in uræmia, little need be said in view of the chemical facts we have just presented. We tested this matter, however, in two experiments. In the first, in a healthy dog weighing 5 kgm., subcutaneous injection of 8 c.c. of "purified," concentrated ureine caused great restlessness, diarrhœa, diuresis, and vomiting during the first twenty-four hours. Marked local irritation and œdema about the point of injection also resulted. There were no convulsions at any time; no suggestions whatever of uræmia. The dog was chloroformed on the third day after injection.

In the second experiment, on a lively cat weighing 2 kgm., subcutaneous injection of 4 c.c. of the same preparation of ureine, after it had been further concentrated at 45° C. for forty hours, caused marked local irritation and was followed at once by restlessness; later by diuresis, diarrhœa, vomiting, paralysis, and finally by death in convulsions, nine hours after introduction.

The results of our second agree in the main with those of the single experiment, reported in detail by Dr. Moor, on a rabbit with "3½ c.c. of ureine obtained from the urine of a pregnant woman in the ninth month of her pregnancy." We are unable to say, how-

ever, that any single symptom exhibited in our experiments was due to any one definite chemical compound in the mixture Dr. Moor terms ureine. The poisonous action of ureine is doubtless due to the sum of the toxicity of the normal urinary compounds contained in it.

The fact has long been known that the normal urine contains substances of a very toxic character.[1] Potassium compounds, as all of our readers know, are prominent among these; but even more poisonous are the various organic bodies of an alkaloidal nature, present in only minute proportion. Dr. Moor's method of preparing ureine fails to eliminate completely either potassium salts or the normal basic alkaloidal bodies giving the typical reaction with potassium ferricyanide and ferric chloride, and the toxicity ascribed to ureine must undoubtedly be referred, in part at least, to these substances dissolved in it. The evaporation process from the beginning, it is perhaps needless to point out, causes an accumulation in ureine of these various products possessing toxic influence.

Summing up in a few words: Ureine is not a chemical individual. It is a *mixture* containing several of the organic substances and a considerable proportion of inorganic salts ordinarily found in normal urine. Further, its toxicity can be referred to some of these normal urinary constituents. Consequently, our knowledge of the cause of uræmia, we regret to say, has been in no way increased by Dr. Moor's work on ureine, nor can any of his deductions regarding the biological significance of this urinary complex be accepted.

[1] The latest reference to the matter that we have seen is the recent paper by Dresbach, confirming the previously accepted general fact, without, however, isolating or identifying any active substances: "On the Toxicity of Normal Urine," The Journal of Experimental Medicine, 1900, v., p. 315.

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SOME NOTES ON POLLACCI'S NEW METHOD OF DETECTING ALBUMIN IN THE URINE.

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Methods for detecting "albumen" in the urine have accumulated so rapidly in recent years that it is frequently a difficult matter to decide which is best adapted for special clinical purposes. The delicacy of the methods alluded to is so variable and the number of possible fallacies connected with the use of each so numerous, that the difficulties of selection are made all the greater. Then, too, in the use of the various methods, not a little confusion results from the fact that many of the tests show the presence of such amounts of proteid as are of no clinical importance—such, for example, as are contained in the normal urinary mucus.

We have recently investigated the utility of Pollacci's new method for the detection of albumin in urine. The original description appeared not long ago in the *Schweizerische Wochenschrift für Chemie und Pharmacie* (1901, xl, p. 168). We have not had access to the original paper but several abstracts[1] agree in giving the following facts regarding the method:

Pollacci has made a modified Spiegler reagent with the composition indicated below:

A. 1 gram tartaric acid
   5 " mercuric chlorid } dissolved in 100 cc. water.
   10 " sodium chlorid
B. Solution A + 5 cc. formaldehyd (40% solution).

In applying this solution (B) for the detection of albumin, Pollacci uses 2 cc. of his reagent and cautiously adds 3–5 cc. of the urine, as in Heller's test, care being taken to stratify the solutions and to prevent their admixture.

"Should a white zone appear at the line of contact

[1] Chemist and Druggist, 1902, lx, p. 82; Therapeutic Monthly, 1902, ii, p 223; Merck's Report, 1902, xi, p. 236, also 237.

of the two fluids the urine contains pathologic albumin. If this ring or zone appears slowly, after about 10 to 15 minutes, it indicates the presence of only normal quantities of albumin." Pollacci established the limits of sensitiveness of the various albumin reagents now in use, compared with his own, with the following results:

Heat, with acetic or nitric acid	1 in 75,000
Heller's reagent	1 " 78,000
Potassium ferrocyanid and acetic acid	1 " 100,000
Jolles' reagent	1 " 150,000
Roberts' "	1 " 300,000
Sulfosalicylic acid	1 " 300,000
Spiegler's reagent	1 " 365,000
Pollacci's "	1 " 370,000

It did not appear probable to us that this method would show only the presence of albumin. We were inclined to believe that other proteids would be indicated by it. This belief was fully warranted.

We find, as Pollacci states, that the reagent shows the presence of mere traces of albumin, although it does not appear to be so delicate as Pollacci's figures would indicate. But we have also observed that the reagent precipitates minute amounts of other proteids also, such as globulins, proteoses, mucoids, mucus proteids and even gelatins. The test has no differential value, therefore, and the reagent must be regarded as a general proteid precipitant rather than an albumin detector.

We are also unable to agree with Pollacci that the proteid normally present in the urine reacts with his reagent only after a lapse of 10 or 15 minutes. Deductions drawn from the observed breadth of the "zone" and from the lapse of time until the ring appears are not reliable. We have tested numerous samples of urine from individuals apparently in perfect health and in each instance, in less time in this connection than that specified by Pollacci we obtained the white ring at the point of junction of reagent and urine. That these urines were normal in this respect was not merely assumed from the evident good health of the individuals excreting them, but shown experimentally by the fact that none of them gave positive reactions in Heller's test.

We have not attempted to determine, in this connection, the responsiveness of alkaloids and other remedial agents commonly detectable in the urine and frequently affecting the "albumen" tests. The presence of mercury in the acid reagent makes it probable, however, that other substances, nonproteid in character, readily respond to the reagent.

SUMMARY OF CONCLUSIONS.

1. Pollacci's reagent readily precipitates various proteids—simple, compound and albuminoid.

2. The test is too delicate for ordinary clinical purposes, since the normally occurring urinary proteids are precipitated by the reagent.

3. Various nonproteid substances occurring in the urine in health and disease are probably also precipitated by the reagent.

4. The latter possesses little or no advantage over Spiegler's fluid.

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PROTEOSURIA.[1]

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Among the proteid products which occur in the urine under various conditions, are proteoses. The urinary proteoses appear to be chemically identical for the most part with the proteoses formed normally in the gastro-intestinal tract during the digestion of albuminous matter. Proteoses frequently appear in the urine when extensive tissue catabolism occurs, such as takes place in connection with various fevers, intestinal ulceration, carcinoma, apoplexy, gangrene, yellow atrophy of the liver, absorption of pus and of exudates rich in leukocytes, etc. They arise also, occasionally, from spermatic fluid, and may originate from the food in nephritis, and as a result of disease of the walls of the digestive tract. The urine not infrequently contains proteose during pregnancy. When proteose passes into the blood from any cause it is eliminated in the urine.

Before the time of Kühne's classic researches, proteoses were included in the term peptone. In recent years, however, more exact chemic differentiation of primary and secondary proteoses, and peptones, has taken place and it seems highly probable that the urinary peptone of the earlier observers was in reality deuteroproteose. Many researches in the last decade have demonstrated that deuteroproteose is frequently found in the urine in disease, but that true peptone occurs only rarely and apparently only in association with deuteroproteose. In no known case has more than 5 grams of proteose been eliminated in 24 hours. The quantity is usually much less.

[1] We use the generic term "proteosuria" in preference to "albumosuria" merely because in these urinary conditions more than one type of proteose is eliminated. The generic term, therefore, is the more accurate, unless urine containing only albumose is referred to.

The use of the term "peptonuria" in connection with the proteoses of the urine is not only inaccurate, in the light of our present knowledge, but confusing as well. It should be restricted to the occurrence of true peptone as we now understand the term.

In addition to the various proteoses, another substance of similar qualities, known as histon, sometimes appears in the urine, which was doubtless also formerly detected and designated peptone. Histon has been detected in the urine in cases of peritonitis, pneumonia, erysipelas, scarlet fever and in lymphemia. "Bence Jones' proteid," which repeatedly appears in the urine in association with multiple myelomas of the bones, and which for a long time was regarded as albumose, is in reality a coagulable substance. Recent researches have shown that it is not a proteose. Its exact nature is still undetermined.

Numerous methods for the detection of the proteoses and other proteids in the urine have recently been suggested. Freund[1] has lately communicated a "method for the detection of peptone in the urine and feces." Freund shows throughout his paper, however, that he has taken the usual liberty with the term peptone. He seems to have had proteose in mind, not peptone.

His method for the detection of proteose is very simple, and may be summarized as follows: 10 cc. of urine is first acidified with 2–3 drops of 2% acetic acid, and then treated with 20% neutral or basic lead acetate—5 cc. The milky mixture is thoroughly boiled and the precipitate of proteid, inorganic matter, etc., is filtered off. The filtrate is next treated with potassium hydroxid as long as a precipitate of lead hydroxid continues to form, when the mixture is again boiled for a moment or two. The filtrate, it is claimed, is entirely free from urobilin, and contains a little more than 90% of the proteose originally present in the urine. The presence of the proteose in this filtrate may finally be detected with the biuret reaction. The filtrate is always water-clear, says Freund, pigments such as uroerythrin, uro-bilin, bilirubin and hematoporphyrin being completely precipitated.

All of these results, adds Freund, are obtainable with proteose-containing feces. He states that in a large number of experiments with this method, normal feces were found to be entirely free from proteoses ("peptone").

Not only is the title of Freund's paper rather mis-

[1] Freund: Centralblatt für innere Medicin, 1901, xxii, p. 647.

leading, but his conclusions, also, are hardly warranted. The method he uses for preparing the final proteose-containing filtrate does not exclude peptone, and if gelatin were present, by accident or otherwise, it also would be contained in the filtrate.[1]

We have made numerous experiments with urine and feces to test the validity of Freund's method. Moderate amounts of various proteids or their concentrated solutions were dissolved in, or mixed with, urine and feces from individuals who had been in perfect health continuously for a long time. The samples thus prepared, together with the corresponding normal urines and feces as controls, were very carefully subjected to Freund's method, and the biuret reaction applied finally as he directs. The normal feces, and the feces with proteid admixture, were extracted for a few minutes in hot water and the filtrates treated the same as the urine. Care was taken to effect extraction speedily, so as to prevent hydration of any contained proteid. Basic lead acetate was used for precipitative purposes with both urine and feces.

Positive results were repeatedly obtained by this method in samples of normal urine which had been treated with the following substances:

(1) "Witte's peptone" (containing proteoses).

(2) Pure peptones, made by us from tendomucoid, fibrin and ligament elastin.

(3) Commercial gelatin (containing gelatose).

(4) Pure gelatins, made by us, from tendon, bone, and ligament.

(5) Pure primary and secondary proteoses, of our own make, from tendomucoid, fibrin, and ligament elastin.

(6) Aqueous extract of sheep pancreas (containing nucleoproteid, proteose, and peptone).

(7) Egg albumen: commercial products, also from fresh eggs, (containing ovomucoid, Neumeister's "pseudopeptone").

(8) Ox blood (containing seromucoid).

Among the proteid substances which gave negative results under similar conditions were:

(1) Mucus from the gastrointestinal tract (containing nucleoproteid and mucin.)

(2) Mucoids from tendon, cartilage and bone.

[1] The frequent use of gelatin in solution in the sick-room makes it highly probable that sometimes small quantities of it by accident get into the vessels used for collecting urine. Commercial gelatin contains gelatoses. A very slight quantity of gelatin or gelatose will give a strong biuret reaction.

(3) Various animal and vegetable albumins and globulins.

Many of the final filtrates were quite yellowish to red in color, contrary to Freund's experience, although in a majority of cases all of the urinary pigment was removed. When large excess of blood was present in the first place, the final filtrate contained soluble, pigmented derivative of hemoglobin. Further addition of lead acetate, however, entirely removed it.

The same positive and negative results with nearly all of the above proteids were also obtained when these substances were admixed with dog feces. The latter normally contained nothing that gave a biuret reaction in the final filtrate. Every sample of normal human feces tested by us, however, gave a positive result. The reaction was stronger in the presence of the above substances. Further, the final filtrates were usually highly colored. Our biuret tests were made on one-half of each portion; the other half serving for comparison. "Peptone," it is said, does not occur in the feces normally, although it is probable that peptone, as well as proteose, occasionally appears in the feces in health, particularly as a result of the normal bacterial action on undigested proteid such as muscle fibers or on mucus. Possibly the coloring matter present accounted for the biuret reaction in the fecal extracts we examined, just as urobilin in the urine may affect it.

These results show, we think, that Freund's method is not a differential process, and that it cannot be safely applied to the urine or feces as a peptone test. They prove that peptones, proteoses and gelatins in urine and feces may each give positive results with it. They indicate, further, that seromucoid in the urine might also affect the final reaction.

Since the foregoing was completed we have seen Ito's paper on the occurrence of true peptone in the urine. He gives improved methods of detecting proteose and peptone in urine in the presence of each other. (See *Deutsches Archiv für klinische Medicin*, 1901, lxxi, p. 29.)

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ON THE QUANTITATIVE DETERMINATION OF ACID-ALBUMIN IN DIGESTIVE MIXTURES.

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I. INTRODUCTORY

IN many of the experiments which have been carried out to determine quantitatively the proteolytic power of pepsin under various conditions, the chief deductions have been drawn directly from the amounts of undigested or residual matter rather than from the proportions of the digestive products themselves. In a majority of these cases the figures for undigested matter have doubtless suggested approximately correct conclusions in this regard, but it seems probable that, in some instances at least, quantitative studies of the albuminates, proteoses and peptones formed would have furnished more accurate and acceptable data.

The writer has recently been engaged in a study of the action of pepsin under varying degrees of acidity with a number of acids, and in the presence of different ions, the results of which will be reported later. In experiments of such character the increasing

or decreasing amounts of acid associated with the pepsin, to say nothing of its quality, variously affect the proteid indicator, irrespective of the influence on the latter of the enzyme. Different proportions of acidalbumin would be formed, also, with variations in the chemical character and physical condition of the proteid used to test relative zymolysis. If correct comparative deductions are to be drawn from the results of such experiments, it would seem that determining the amounts of albuminate present in each case would be almost if not quite as important as ascertaining the quantity of undissolved or undigested substance. It is conceivable that in comparative cases where, for example, the undigested matter might be decreased, the proportion of acidalbumin formed by the mere solvent action of the acid might be correspondingly larger. To assume from the fact of diminished quantity of original proteid, in such an instance, that zymolysis had been greater in the one case than in the other obviously would be unwarranted.

In the first of the writer's ion experiments, previously alluded to, purified fibrin was used as the indicator. At the end of the digestive interval the residue was filtered on a weighed paper and a given portion of the filtrate carefully neutralized for the precipitation and quantitative determination of the albuminate. After standing from twelve to twenty-four hours the precipitate was filtered on a weighed paper and, after washing and drying, estimated in the customary manner. Later, however, it was discovered that boiling the digestive fluid from which the neutral precipitate had been filtered, caused a further precipitate, presumably of albuminate, which was not separable by neutralization in the cold. The amount of this precipitate seemed comparatively small, but of course, for accuracy's sake, could not be ignored. The boiled fluid was either permanently turbid or minute flocks separated from it. The precipitate was obtained on boiling, in spite of the most careful neutralization of the digestive fluid. It likewise occurred independently of the character of the alkali used in neutralizing, the acid associated with the pepsin, the length of time between neutralization and filtration, and the volume of the digestive fluid.[1]

These facts led us to make a special inquiry into the accuracy of the neutralization method for directly precipitating and determining the quantity of acidalbumin in digestive mixtures. This simple method is desirable and convenient not only for the special experi-

[1] See page 485 for further reference to the influence of volume.

ments in progress in this laboratory, but also for various other proteid studies. Further, such separation by neutralization alone is particularly advantageous in digestive experiments because it can be made without affecting the associated proteid products. Direct determinations of any substance, when they can be made accurately, always possess advantages over indirect determinations.

Very little attention has been given to the quantitative determination of acidalbumin. In those cases in which its approximate determination when present among other proteids has been desired, neutralization in the cold has been effected and then the precipitate has been filtered as in the writer's experiments just referred to. In most instances, however, acidalbumin has been determined as a part of albumin or globulin in the form of coagulated proteid; or, by reason of small amount or relative unimportance, has been ignored altogether.

As an example of direct determination quantitatively the process recently referred to by Effront[1] may be cited. In a general way this method has been in occasional use for years. In the experiments by Effront the acidalbumin ("syntonin") in a fluid mixture of proteoses, peptone, etc., was precipitated by careful neutralization. The neutral mixture was allowed to stand for two hours and the flocculent precipitate which had then separated was filtered on a weighed paper. Boiling was not a part of the process.

As we have already indicated, boiling the filtrate from the acidalbumin obtained in the cold fluid usually yields an additional flocculent proteid precipitate, an occurrence suggesting that mere neutralization is not sufficient for effecting separation if particular quantitative accuracy is desired.[2] Several theories to account for this fact suggest themselves.

It is usually stated that albuminates are insoluble in neutral salt solutions, although not all observers are agreed on this point.[3] It

[1] EFFRONT: Chemisches Centralblatt, 1899, ii, p. 457.

[2] UMBER, among others, has noted, in cases where only a slight amount of acidalbumin, or none at all, could be precipitated on neutralizing, that the neutral filtrate remained clear on boiling, but additional "acidalbumin" separated on evaporation of the fluid to one-half its volume. See Zeitschrift für physiologische Chemie, 1898, xxv, p. 263.

[3] HEYNSIUS: Chemisches Centralblatt, 1876, p. 807; MÖRNER: Jahresbericht der Thier-Chemie, 1877, p. 10; SAVIN: *Ibid.*, 1887, p. 2; NIKOLJUKIN: *Ibid.*, p. 5; HALLIBURTON: Text-book of chemical physiology and pathology, 1891, p. 128.

may be admitted that these derived proteid substances, and particularly the *dried* products, are for the most part insoluble in neutral saline media, but the moist, *freshly precipitated* digestive albuminate is clearly soluble in solutions of various salts, as we ourselves have definitely ascertained.[1] Consequently, on neutralization of its acid solutions, a portion of the acidalbumin remains in the salt solution formed in the process. On boiling the filtrate, however, some of this last residual portion is precipitated because albuminates are coagulable by heat in neutral saline fluids.[2]

It appeared probable, also, that in the experiments in mind the associated proteoses and peptones exerted solvent action on the albuminate, thus increasing the retaining power of the solution and thereby helping to prevent complete precipitation on simple neutralization.[3]

We were inclined to believe for a time that carbon dioxide in the fluid, which might have influenced the indicators (litmus and lacmoid papers), was driven out on boiling and its possible solvent action done away with, so that the rest of the albuminate was then thrown down.[4]

That the precipitate obtained on boiling was not due to earthy phosphate impurity in the reagents was definitely ascertained.

As the neutral point is approached in such experiments as these, it is possible that portions of the albuminate which have already been precipitated are redissolved and perhaps modified by the dilute alkali, added drop by drop to the nearly neutral fluid. These dissolved portions are not precipitated again in the cold, possibly,

[1] See page 487. Most of the statements regarding insolubility of acidalbumin refer to the dried product prepared from muscle tissue. By many writers these statements have been accepted as including the acidalbumin formed during pepsin proteolysis. Acidalbumin prepared from muscle tissue begins to diminish in solubility in dilute acid, even after standing under water for only a few minutes. Acidalbumin formed during peptic digestion is very different in this respect, for its solubility in dilute acid remains essentially the same, no matter how often it is washed, nor is its solubility altered by drying at 40° C. Even then it is soluble to a certain extent in dilute salt solution. Myosin albuminate under these conditions is quite insoluble. See pages 464, 472, and 486.

[2] We do not say above that *all* of the albuminate is precipitated on boiling, for the reasons given on page 474.

[3] See page 479.

[4] The alkaline fluids used in neutralizing were dilute KOH and NaOH. These naturally introduce some carbonate, no matter how carefully the pure solutions are handled in such experiments. See page 487.

because of the lack of acidity or on account of the solvent action of the increasing quantity of salts formed in the neutralization process.

We are not aware of any combinations of albuminate with proteose or peptone, nor of transformations of these substances under the conditions of these experiments, which would account for the precipitate thrown down when the neutral fluid is boiled.[1]

It seemed desirable, then, to determine the influence of the various factors referred to and, particularly, to ascertain the proportions of albuminate lost on neutralizing, as well as the proportion thrown down on boiling the filtrate from which the neutralization precipitate had been removed. The facts we have ascertained, bearing on the sufficiency of the precipitation method of direct determination of acidalbumin, are indicated in the summaries of our experiments on pages 470–484.

II. Experimental.

Preparation of acidalbumin. — Two varieties of acidalbumin were used in these experiments. One was prepared from muscle with 0.2 per cent HCl at room temperature, the other from fibrin with pepsin — HCl (0.2 per cent) at 40° C.

Acidalbumin from muscle. — **A.** Several pounds of fresh, lean meat was finely minced in a meat chopper and the hash thoroughly washed in running water for thirty-six hours. After straining the last washings through cloth the hash was placed in an excess of 0.2 per cent HCl and kept there for twenty-four hours. At the end of that time the acid extract was filtered and the "syntonin" separated by neutralization with dilute KOH. The separated precipitate was redissolved in 0.2 per cent HCl and reprecipitated three times with dilute KOH for the complete removal of impurities. The final precipitate obtained from the filtered solution was frequently washed during twenty-four hours by decantation in seven to eight litres at a time, at first with ordinary water, at last with distilled water. All of the washings contained substance yielding the biuret reaction and causing very faint turbidity on boiling. A trace of this substance persisted in the washings, indicating a slight solubility of the freshly precipitated material

[1] The solutions were not sufficiently concentrated for the separation of heteroproteose, nor was there any acidity for the precipitation of acroproteose. The precipitate bore no resemblance to "coagulated" heteroproteose. There is no reason for believing that dysproteose separated under these conditions.

even in water. The precipitate was finally filtered off, spread on a glass plate in a thin layer and dried in a few hours in warm air at a temperature slightly under 40° C.[1] The dried material was eventually ground to a very fine powder before using. About 50 gms. were prepared.

B. A second preparation from washed meat was made by essentially the same method as that used for the separation of the first. This preparation was not dried, but the moist substance after thorough reprecipitation, washing, filtration, etc., was used in the sixth experiment, as indicated on page 478. In this preparation, also, the washings, in spite of their volume and frequency, contained, to the last, a trace of substance separable by boiling. Phosphates were absent from the later washings.

Acidalbumin from fibrin. — C. A considerable quantity of fibrin, which had been kept in 95 per cent alcohol for some time, was put through a meat chopper and the alcohol thoroughly washed out in running water. After the completion of the washing process the fibrin was placed in a moderate amount of HCl (0.2 per cent) containing only a very small proportion of pepsin and was kept at 40° C. for about an hour — until practically all of the fibrin had dissolved. The amount of pepsin selected was small, and the period of digestion short, so that the proportion of albuminate at this stage should be large. The digestive mixture was now brought quickly to the boiling-point, to destroy the pepsin; was kept at the boiling-point for a minute or two, and then immediately cooled to about 25–30° C. The cold filtrate was next neutralized with dilute KOH, and the heavy flocculent precipitate redissolved in 0.2 per cent HCl and reprecipitated once with dilute KOH, after which it was repeatedly and very thoroughly washed by decantation in large excess of ordinary water and, finally, in distilled water. The substance settled quickly and could be washed repeatedly in twenty-four hours. Even to the last, the washings gave biuret reactions and became turbid on boiling, just as with the product obtained from muscle. It seems necessary to conclude that in this case also the freshly precipitated material was slightly soluble in the water.

The freshly precipitated substance was used in the first experiment, as stated on page 470.

D. The second preparation of acidalbumin from fibrin by digestive process was made in essentially the same manner as the previous one. Fibrin boiled in water, and then extracted in alcohol and ether was used. The precipitate was washed in about fourteen litres of water frequently during twenty-four hours. Even to the last, the washings again became slightly turbid on boiling and on the addition of picric acid. On warming, the turbidity with the latter appeared to diminish somewhat and to increase

[1] The time required for the drying was too short for any perceptible bacterial changes to have occurred.

again on cooling, facts indicating the presence of proteose with acid-albumin.[1]

The moist substance was finally dried in a thin layer[2] in warm air at a temperature below 40° C. The dried substance was finely powdered. It weighed 2.5 gms.

E. A third preparation of acidalbumin was made from fibrin by enzyme action. The fibrin had not been boiled, although it had been thoroughly washed in alcohol. This sample also was made by the general method just outlined. The neutralization precipitate was redissolved in 0.2 per cent HCl four times and as frequently reprecipitated with dilute KOH. Extreme care was taken to wash thoroughly and frequently. The precipitate was whipped up repeatedly in as much as fourteen litres of water at a time. The final washings in distilled water were almost entirely free from substance giving the biuret reaction and yielding turbidity on boiling or on treatment with picric acid. At this point the substance was divided roughly into two portions.

a. The first portion was dissolved in 0.2 per cent HCl, the solution diluted with an equal volume of water and filtered. The filtrate was precipitated with dilute KOH and the proteid, after thorough washing for a few hours, was spread on a glass plate and quickly dried below 40° C. as usual. Twelve grams were obtained.

b. The second portion was dissolved in 0.2 per cent HCl, allowed to stand several hours, without dilution, and then precipitated, washed and dried as was the first portion. It weighed about 30 gms.

The washings of both portions at first showed an increased content of acid-albumin. Merely a trace was present in the final washings in distilled water.

F. The fourth preparation of acidalbumin from fibrin was made from several hundred grams of the proteid which had not been boiled in water, but which had been very thoroughly extracted in alcohol and in ether. The method of preparation was the same as that for the previous products. The acidalbumin was thoroughly washed in fourteen litres of water eight times during forty-eight hours. At the end of the process only a mere trace of coagulable substance was detectable in the distilled water washings, and in the last two washings no satisfactory biuret reaction could

[1] The washing was done frequently during twenty-four hours, and there was hardly time enough for bacteria to develop and form proteose. It is possible that in the course of twenty-four hours slight bacterial changes did occur without our knowledge. The water used in washing all these preparations was kept free of antibacterial substances so as to prevent possible transformations of the desired products through such chemical agencies. See page 486.

[2] Higher temperature was avoided to prevent possible transformation into a less soluble product.

be obtained without concentration. The moist substance, after it had been allowed to drain, and after excess of moisture had been expressed from it through hard filter paper, was used in the experiment referred to on page 487.

G. A mixture of residues of **D** and **E** was used in the eleventh experiment, described on page 484.

Acids employed (salts formed). — In order to test the precipitation method as thoroughly as possible the following acids were used: hydrochloric, nitric, chloric, sulphuric, arsenic, phosphoric, acetic, lactic, oxalic, tartaric and citric. All these were carefully titrated with standard alkali and appropriate indicators, and made equivalent to $\frac{m}{10}$ NaOH.[1]

In our experiments the acidalbumin was transferred to the acid and, after solution of the substance, most of the fluids were carefully made neutral to litmus. Mixtures in which acid salts were formed were tested with lacmoid paper. Alkali was added to these until all free acid was exactly transformed to *acid* salt.

In all cases permanent, bulky precipitates were formed even while some free acid still remained in the fluid, a fact in harmony with previous observations by various investigators.[2] The maximum effects were obtained at the neutral point, however, or when free acid was present only in inappreciable traces.[3]

[1] $\frac{m}{10}$ HCl contains 0.36 per cent HCl, $\frac{m}{20}$ H_2SO_4 contains 0.49 per cent H_2SO_4, $\frac{m}{30}$ H_3PO_4 contains 0.33 per cent H_3PO_4. These strengths of acid are approximately equivalent to those used in representative peptic digestive experiments.

[2] The salts formed on neutralization help precipitation. The more saline matter present in the fluid the greater the acidity may be without the exertion of solvent action on the part of the acid. This fact accounts for the heavy turbidity observed in some of the fluids, while free acid was still detectable in them. This precipitation occurred earlier in some than in others, doubtless because of the different influence of the anions. It appeared in the sulphate solution as quickly as in any, SO_4 seemingly being helpful to the precipitation of acidalbumin.

[3] In reprecipitating several of our main products it was observed that when the solution was carefully carried from acid to *exact neutral reaction*, the supernatant fluid over the main bulk of the precipitate remained somewhat milky. On adding a little more dilute alkali the substance causing the turbidity became flocculent and settled out quickly under a perfectly clear fluid. The latter still remained neutral to litmus. In our quantitative experiments neutralization was carried to the point of flocculation in a perfectly clear fluid. This point corresponds very closely with the point of neutralization of acid and alkali. The filtrates from the neutral precipitates were "water-clear." See footnote, page 469.

Proteoses and peptones used. — Witte's peptone was used in all of the experiments in which we determined the influence of the digestive products on the precipitation of acidalbumin.

In such experiments weighed amounts of dry Witte's peptone and our acid-albumins were dissolved in given quantities of each of the acids above-mentioned. With most of the acids all of the substance comprising the "peptone" completely dissolved. In others, however, a permanent precipitate was formed, either immediately on admixture or later on neutralizing. In each case we determined accurately the amounts of the precipitates of such extraneous matter and made corresponding corrections in the data subsequently obtained.

The following summary gives our results in this connection, no precipitate as bulky or as heavy as that in HCl having been obtained with the acids not mentioned below.

Summary. — 100 c.c. of each acid was taken. All were equivalent to $\frac{m}{10}$ NaOH. Weighed peptone (Witte's) was dissolved in each.[1] The solutions were left standing several hours, and then neutralized with dilute KOH, litmus or lacmoid the indicator — in some cases before boiling, in others while the fluid was at the boiling point. After neutralization each mixture was allowed to stand over night and then was filtered. Subsequent boiling of the neutral filtrate failed to cause turbidity; neither did longer standing result in further separation of solid matter.

Acid.	Amount of peptone dissolved. Gram.	Time of neutralization.	Amount of neutralization precipitate. Gram.
A. Oxalic	*a* 0.5	Before boiling	0.0214
	b 0.5	After "	0.0201
	c 1.0	Before "	0.0392
	d 1.0	After "	0.0376
B. Phosphoric	*a* 1.0	Before "	0.0146
	b 1.0	After "	0.0158
C. Citric	*a* 1.0	Before "	0.0091
	b 1.0	After "	0.0058
D. Tartaric	*a* 1.0	Before "	0.0118
	b 1.0	After "	0.0076
E. Hydrochloric	*a* 0.5	Before "	0.0032
	b 1.0	" "	0.0058

[1] Samples of the preparation used in the succeeding experiments. See page 469.

Compounds with calcium and with phosphoric acid doubtless account for the greater portion of the above precipitates. Witte's peptone contains both of these. The boiling process does not appear to have increased the quantity of the precipitated matter, but rather decreased it in most cases.

Quantities of solids and fluids taken. — Unless otherwise stated, 100 c.c. of acid was used for each experiment of a series. The quantities of acidalbumin were usually between 0.05 and 0.5 gm.; of peptone, between 0.5 and 1.0 gm.

We purposely used small amounts of both solids and fluids, believing that the method could be tested most effectively by so doing. With comparatively large quantities of the proteids and fluid, defects of manipulation are apt to cause appreciable errors. Small quantities are more easily and accurately handled. The amounts and proportions employed were such as have figured in the past in typical digestive experiments. In our main series of experiments we used portions of the same general supplies of the acids and Witte's peptone throughout. No variations were introduced, therefore, by reason of differences in the character of the materials used. In all cases where the dry acidalbumin was used we refer to the product dried below 40° C. The amount of water in the "air-dried" preparations was accurately determined by drying to constant weight at 100–105° C. in the usual manner and due correction made as indicated below.

Precipitation of acidalbumin. — The albuminate was first dissolved in the acids alone as already indicated, or in the acids with their content of Witte's peptone. The mixtures were usually allowed to stand in this condition for an hour or more, when careful neutralization was begun and completed as soon as possible.[1]

The neutralized fluids with their precipitates were allowed to stand undisturbed until the following morning, when the solid matter was filtered on weighed papers, washed with water until free of soluble matter[2] and eventually dried in the air-bath in the customary manner. In the summaries farther on we give the corrected final

[1] The combining power of the "peptone" for the acid did not, as will be seen, appreciably influence the effect of neutralization. Acid combined by the peptone was doubtless too slight in amount to be of significance in this connection.

[2] In only a few instances were the filtrates turbid. By repeated filtration the solid matter was retained. The washings, also, on several occasions manifested initial turbidity, but the solid substance of these was likewise held after several filtrations. As these washings were always neutral it is difficult to account for the turbidity on any other than mechanical grounds.

weights in each case.[1] Other matters of method are indicated with the summaries of each experiment.

The completeness of precipitation of acidalbumin from its acid solutions by neutralization depends largely on the amount of saline matter present.[2] In our own experiments the acid was dilute and the final content of salt in the fluid on neutralizing was small, though sufficient for the precipitation of the amounts used. We were careful to carry the addition of acid to the point of exact neutrality or disappearance of free acid, which method, by cautious manipulation, was found to give the maximum amounts of precipitate.[3] Various observers, among them recently Spiro and Pemsel,[4] have noted the difficulty of completely precipitating acidalbumin on neutralizing and it is, perhaps, a fact not generally appreciated.

First experiment. — In this experiment we endeavored primarily to get an accurate idea of the relative proportion of substance thrown from the various neutral filtrates on boiling.

Summary. — Fibrin acidalbumin of preparation **C** was employed. Several grams of the latter was dissolved in a few c.c. of $\frac{m}{10}$ HCl and 3 c.c. of this solution added to each of the acids — 100 c.c. $\frac{m}{10}$ or equivalent thereto — and also to 100 c.c. H_2O. Neutralization was made with dilute KOH in this and the four succeeding experiments. The neutral filtrate was brought to the boiling-point and maintained there about a minute. The gravimetric results are shown in the table on page 471.

All the above filtrates that were obtained after boiling gave the biuret reaction and yielded slight amounts of proteid substance with alcohol.[5] This precipitate dissolved in water and gave the proteose reaction with picric acid. This fact suggested that proteose was contained in the substance in spite of the thorough washing to which it had been subjected.[6] Possibly, however, some of the proteose was derived from the albuminate on boiling.[7]

[1] See facts regarding precipitates of extraneous matter from the peptone, page 468. No allowance was made for the ash of the neutralization precipitates. The quantities of ash were entirely too slight to affect the figures given.

[2] See footnote 2 on page 467.

[3] The formation of alkaline salts was avoided. See page 467.

[4] SPIRO and PEMSEL: Zeitschrift für physiologische Chemie, 1898, xxvi, p. 236.

[5] A large proportion of this precipitate was inorganic matter.

[6] See page 486.

[7] See page 489. The amount of saline matter present in the fluid was quite sufficient for complete precipitation of the acidalbumin.

In this experiment the precipitate obtained on boiling the neutral filtrate amounted, usually, to from 5 to 25 per cent of the quantity separated in the cold with dilute alkali. It is to be noted that no precipitate was obtained, on boiling, from the "control" nor from the solution of citrate. Further, it will be observed that the amount of albuminate recovered from the "water control" was greater than from any other solution. In this slightly acid mixture the amount of saline matter was comparatively small and solvent action on the acidalbumin greatly reduced therefore.[1]

Acid.	Amount of neutralization precipitate. Gram.	Quantity of precipitate on boiling the neutral filtrate. Gram.
Sulphuric	0.031	0.003
Hydrochloric	0.031	0.004
Nitric	0.033	0.003
Lactic	0.033	0.008
Chloric	0.035	0.003
Oxalic	0.035	0.004
Phosphoric	0.036	0.003
Tartaric	0.036	0.004
Acetic	0.036	0.005
Arsenic	0.037	0.002
Citric	0.039	none
Water (control)	0.040	"
Average	(*a*) **0.035**	(*b*) **0.003**
Ratio. $a : b = 12 : 1$		

Second experiment. — In the preceding series we did not know the exact amount of substance taken to begin with. Further, by dissolving the albuminate in HCl, and distributing it in such solution, we

[1] It will be remembered that 3 c.c. of $\frac{m}{10}$ HCl solution of acidalbumin was added to the water. The very slight proportionate acidity resulting thereby was sufficient to keep all of the substance dissolved.

introduced a small amount of the HCl into all of the acids and in the water, and thus, perhaps, tended to complicate matters. In this experiment we began with accurately weighed amounts of dry substance.

Summary. — 0.2 gm. portions of fibrin albuminate of preparation **D** were weighed carefully and transferred to the acids. The weight of this amount of albuminate at 100–105° C. was found to be 0.181 gm.[1]

Acid.	Amount of neutralization precipitate. Gram.	Quantity of precipitate on boiling the neutral filtrate. Gram.	Total amount of acidalbumin recovered. Gram.
Sulphuric	0.109	0.018	0.127
Oxalic	0.114	0.007	0.121
Chloric	0.114	0.017	0.131
Nitric	0.117	0.009	0.126
Tartaric	0.123	none	0.123
Hydrochloric	0.126	0.008	0.134
Lactic	0.133	0.001	0.134
Acetic	0.134	0.011	0.145
Citric	0.135	none	0.135
Phosphoric	0.139	0.003	0.142
Arsenic	0.143	none	0.143
Average	(*a*) **0.126**	(*b*) **0.007**	(*c*) **0.133**

Average total quantity of acidalbumin lost, 0.048 gm. = 26.5 per cent.
Average quantity of acidalbumin lost on neutralizing, 0.055 gm. = 30.4 per cent.
Ratio. $a : b = 18 : 1$.
$b = 3.87$ per cent of the original acidalbumin and 5.26 per cent of c.

Each final filtrate gave the biuret reaction and, when treated with 95 per cent alcohol, yielded a slight amount of substance, which appeared to be proteose in part. These and the above results seem to indicate that some of the original acidalbumin remains in solution. The proteose probably came from some of the dissolved albuminate on boiling. The average proportion of substance separable on boil-

[1] See footnote 2, page 466.

ing was somewhat less in this experiment than in that preceding. In several cases, however, it was unusually large, probably because of inaccurate neutralization in the first place. The variations in the total amounts recovered are slight, and within the limits of unavoidable experimental errors, which shows that there are only insignificant differences in the precipitative influences of the various salts formed from the acids under these conditions.[1]

Third experiment. — The preceding experiment was repeated, but with myosin albuminate. Preparation A was used for the purpose.

Acid.	Amount of neutralization precipitate. Gram.	Quantity of precipitate on boiling the neutral filtrate. Gram.	Total amount of acidalbumin recovered. Gram.
Hydrochloric	0.216	0.006	0.222
Tartaric	0.222	0.008	0.230
Oxalic	0.228	0.009	0.237
Acetic $\frac{n}{10}$	0.229	0.002	0.231
Nitric	0.229	0.004	0.233
Citric	0.232	0.005	0.237
Sulphuric	0.233	0.005	0.238
Chloric	0.233	0.007	0.240
Arsenic	0.237	0.002	0.239
Lactic	0.239	0.008	0.247
Phosphoric	0.243	0.003	0.246
Average	(*a*) **0.231**	(*b*) **0.005**	(*c*) **0.236**

Average total quantity of acidalbumin lost, 0.039 gm. = 14.2 per cent.
Average quantity of acidalbumin lost on neutralizing, 0.044 gm. = 16.0 per cent.
Ratio. $a : b = 46 : 1$.
$b = 1.82$ per cent of the original acidalbumin, and 2.12 per cent of c.

Summary. — 0.3 gm. portions of "syntonin" were carefully weighed and transferred to the acids. The weight of this quantity of substance after drying in the air-bath was 0.275 gm. Much of the material failed to dissolve in the acids, even after twenty-four hours with frequent stirring.

[1] This will be found the case in all our experiments. See footnote, page 467.

The drying had materially affected its solubility. The freshly precipitated, *moist* substance, however, is very easily dissolved in acids much more dilute than those used here. The sulphuric acid seemed to have the least solvent action. The *washings* from the precipitates obtained on neutralization of the hydrochloric and oxalic acid solutions gave respectively 3 and 6 mgms. of substance on boiling. These amounts were included in those for the neutralization precipitates, given in the table on page 473.

It will be observed that the average amount of precipitate obtained on boiling is very nearly the same as in the previous experiment, but that its proportion of the neutralization precipitate is less than before. The acidalbumin prepared from muscle is less soluble in neutral saline solution than that obtained from fibrin through the action of pepsin. The proportion of total substance recovered is large, 85.8 per cent, but, nevertheless, a slight loss resulted — a fact doubtless due, in great part at least, to transformation on heating.[1]

The final filtrates gave typical biuret reactions and precipitates with alcohol which, when dissolved in water, responded faintly though distinctly to the proteose reactions with picric acid, potassio-mercuric iodide, etc. This fact emphasizes the conclusion stated above, and further convinces us that in the boiling process some of the albuminate held in solution is converted into proteose.

Fourth experiment. — The preceding experiments made it clear that a small though appreciable quantity of acidalbumin remains in solution when the acid holding it is neutralized; further that, on boiling the neutral filtrate, a part of this remaining albuminate is precipitable, whereas the larger portion appears to be converted into non-coagulable material. We next endeavored to ascertain the influence of proteoses and peptone on the precipitability of acidalbumin.

Summary. — 0.2 gm. samples of fibrin albuminate, preparation **E**, portion **a**, were used. This amount was equivalent to 0.180 gm. of substance dried at 100–105° C. to constant weight. The weights of Witte's peptone in this and subsequent experiments are for substance as it was received in the original package. The fluid in the first of each pair of experiments with oxalic and hydrochloric acids (**a**) was neutralized at the boiling point, after nearly all of the acid had been previously transformed to salt; that in the second (**b**) was neutralized as usual before boiling. Neutralization in all of the others was made as before in the cold.

[1] We do not overlook the fact that the usual errors of manipulation might account for the observed difference between the quantity taken and that recovered. Our weighings, filtrations, etc., were very carefully conducted, however, and such errors were reduced to an inappreciable minimum.

Acid.		Weight of peptone. Gram.	Amount of neutralization precipitate.[1] Gram.	Quantity of precipitate on boiling the neutral filtrate. Gram.	Total amount of acidalbumin recovered. Gram.
A. Hydrochloric	1 *a*	....	0.133	none	0.133
	1 *b*	....	0.154	"	0.154
	2 *a*	0.5	0.129	"	0.129
	2 *b*	0.5	0.127	0.006	0.133
	3 *a*	1.0	0.130	none	0.130
	3 *b*	1.0	0.127	0.007	0.134
B. Oxalic	1 *a*	....	0.146	none	0.146
	1 *b*	....	0.145	"	0.145
	2 *a*	0.5	0.141	"	0.141
	2 *b*	0.5	0.146	0.005	0.151
	3 *a*	1.0	0.129	none	0.129
	3 *b*	1.0	0.143	0.007	0.150
C. Tartaric	1	....	0.145	none	0.145
	2	0.5	0.138	"	0.138
	3	1.0	0.137	0.004	0.141
D. Phosphoric	1	....	0.149	none	0.149
	2	0.5	0.139	0.007	0.146
	3	1.0	0.155	none	0.155
E. Sulphuric	1	....	0.153	"	0.153
	2	0.5	0.158	0.010	0.168
	3	1.0	0.147	0.005	0.152
F. Nitric	1	....	0.148	none	0.148
	2	0.5	0.156	"	0.156
	3	1.0	0.144	0.008	0.152
G. Citric	1	....	0.161	none	0.161
	2	0.5	0.159	0.006	0.165
	3	1.0	0.175	none	0.175
H. Lactic	1	....	0.152	"	0.152
	2	0.5	0.151	0.001	0.152
	3	1.0	0.140	0.001	0.141
Average[2]	**1**	....	(I) **0.151**	(II) **none**	(III) **0.151**
	2	**0.5**	**0.147**	**0 004**	**0.151**
	3	**1.0**	**0.146**	**0.004**	**0.150**

Average total quantity of acidalbumin lost, 0.029 gm. = 16.1 per cent.
Average quantity of acidalbumin lost on neutralizing (cold), 0.032 gm. = 17.8 per cent.
Ratio. I : II (for 2 and 3) = 37 : 1.
II (for 2 and 3) = 2.22 per cent of the original acidalbumin and 2.65 per cent of III.

[1] Due correction has been made, as indicated on page 468.

[2] These averages do not include any of the figures for *a* in *A* and *B*. They represent, therefore, the average precipitation under uniform conditions throughout. See references in this connection on page 474.

The final filtrates from those fluids into which peptone had not been introduced gave the biuret reaction, faintly though distinctly. It was strongest in the chloride and oxalate fluids. The delicate precipitate obtained on treatment with alcohol was composed in part of proteose. The alcoholic turbidity also was greatest in the chloride and oxalate filtrates.

These results are in harmony with the preceding in showing slight losses of albuminate.[1] The peptone appears to be without any particular influence. The quantities of acidalbumin recovered seem to be below the average in the chloride solution and somewhat above it in the citrate. These data accord with the facts, however, that acidalbumin is fairly soluble in chlorides and less soluble in equivalent amounts of citrates. The quantity recovered from the citrate solution has been relatively high in the preceding experiments, also.

A singular occurrence in this experiment, one rather difficult to account for in the light of the results of succeeding series, was the fact that all of the cold neutral filtrates which were free from peptone, failed to yield a further precipitate on boiling. Most of the cold filtrates containing peptone, on the other hand, gave appreciable quantities of coagulum.

Fifth experiment. — In this experiment we repeated parts A and B of the fourth experiment. Fibrin albuminate from the second portion of preparation **E** was used.

Summary. — 0.2 gm. portions of the substance (**E**, b) were weighed into each beaker. This quantity corresponded to 0.179 gm. of substance dried to constant weight at 100–105° C.

The general results and conclusions of this experiment are the same as those of the fourth. It will be observed that second precipitates were obtained in only those fluids which had not been previously boiled. There is no particular difference in the action of the chlorides and oxalates. The proportion of unrecoverable substance in this experiment is practically the same as that of the preceding,

[1] The amount of saline matter contained in the original albuminate was small. Portion **b** of Preparation **E**, for example, contained only 0.86 per cent ash. It is hardly possible, therefore, that the loss of substance was due to removal of inorganic admixture on reprecipitation. All of our albuminate preparations, it will be recalled, were originally reprecipitated several times and frequently washed before drying, in which process inorganic matter was very thoroughly removed. See footnote 2, page 478.

although different preparations of acidalbumin were used. The final filtrates gave the usual proteose reactions.

Acid.		Weight of peptone. Gram.	Amount of neutralization precipitate.[1] Gram.	Quantity of precipitate on boiling the neutral filtrate. Gram.	Total amount of acidalbumin recovered. Gram.
A. Hydrochloric	1 *a*[2]	....	0.133	none	0.133
	1 *b*	....	0.128	0.004	0.132
	2 *a*	0.5	0.141	none	0.141
	2 *b*	0.5	0.164	0.005	0.169
	3 *a*	1.0	0.154	none	0.154
	3 *b*	1.0	0.131	0.014	0.145
B. Oxalic	1 *a*	....	0.146	none	0.146
	1 *b*	....	0.145	0.002	0.147
	2 *a*	0.5	0.149	none	0.149
	2 *b*	0.5	0.148	0.002	0.150
	3 *a*	1.0	0.152	none	0.152
	3 *b*	1.0	0.144	0.009	0.153
Average	1 *a*	....	(I) **0.140**	(II) none	(III) **0.140**
	2 *a*	**0.5**	**0.145**	"	**0.145**
	3 *a*	**1.0**	**0.153**	"	**0.153**
	1 *b*	....	**0.136**	**0.003**	**0.140**
	2 *b*	**0.5**	**0.156**	**0.003**	**0.160**
	3 *b*	**1.0**	**0.137**	**0.012**	**0.149**

Average total quantity of acidalbumin lost, 0.029 gm. = 16.2 per cent.
Average quantity of acidalbumin lost on neutralizing (cold), 0.035 gm. = 19.5 per cent.
Average ratio I : II (*b*) = 24 : 1.
The average for II *b* (1–3) = 3.35 per cent of the original acidalbumin and 4.0 per cent of the average for III.
Averages. *A*. I, 0.142; II, 0.004; III, 0.146 gm.
B. I, 0.147; II, 0.002; III, 0.149 gm.

[1] See footnote, page 475.
[2] As in the previous experiment, *a* signifies *after* boiling; *b*, *before* boiling. See page 474.

Sixth experiment. — The preceding experiment was repeated. Muscle albuminate was used, instead of the product from fibrin. The freshly precipitated substance was taken because of the insolubility of the "air-dried" product.

Summary. — Preparation **B** was used. A little over 4 gms. of the moist substance was dissolved in 425 c.c. of each acid, giving about 1 gm. of the freshly precipitated material to each 100 c.c. Of this solution, 100 c.c. was taken, as usual, for each of the four tests of a series. The amount of solid substance in the moist syntonin was not determined directly. Dilute NaOH was used to neutralize the acids[1] in this and all subsequent experiments.

Acid.		Weight of peptone. Gram.	Amount of neutralization precipitate.[1] Gram.	Quantity of precipitate on boiling the neutral filtrate. Gram.	Average total amount of acidalbumin recovered. Gram.
A. Hydrochloric	1[2]	....	0.054	0.002	
	2	....	0.051	none	
	3	0.5	0.064	"	
	4	1.0	0.066	"	**0.059**
B. Tartaric	1	....	0.066	0.001	
	2	....	0.070	none	
	3	0.5	0.077	"	
	4	1.0	0.079	"	**0.073**
C. Phosphoric	1	....	0.074	0.002	
	2	....	0.074	none	
	3	0.5	0.081	"	
	4	1.0	0.079	"	**0.077**
D. Oxalic	1	....	0.080	0.001	
	2	....	0.084	none	
	3	0.5	0.089	"	
	4	1.0	0.095	"	**0.087**

[1] See footnote, page 475.

[2] The first of each series was neutralized *before* boiling, the rest *after* the boiling point had been reached.

Each of the final filtrates from the fluids which had not received Witte's peptone gave delicate biuret reactions and slight precipitates in alcohol. These possessed proteose qualities.[2] The biuret reactions, as usual, were strongest in the chloride and oxalate solutions.

[1] No differences were observed in the effects of the alkalies used in the neutralization process. The anions of salts of the alkali metals vary somewhat in their effects. In these experiments, however, their influences have not been particularly appreciable. See page 467.

[2] We cannot believe that a trace of active pepsin adherent to the original acid-albumin caused the appearance of proteose at this point in all these experiments. The boiling of the digestive mixture before the first precipitation of the acid-albumin surely sufficed for the destruction of all of the enzyme. See methods of preparation, page 465.

The comparatively high results for acidalbumin precipitated from the phosphate and oxalate fluids are doubtless due in great measure to phosphate and calcium impurities in this particular preparation of the proteid. The amount of precipitate obtained in this experiment from the neutral filtrate on boiling is perceptibly less than in any heretofore. The failure to obtain such turbidity in the peptone mixtures may mean that the peptone has actually aided complete precipitation. On the other hand, there is just as much reason for assuming that the peptone holds the slight quantities referred to in solution. Only the first fluid of each series — neutralized before boiling — yielded a second precipitate.

It might be assumed that the peptone aids precipitation from the fact that the precipitates from the peptone mixtures are slightly greater here in each case than the precipitates not associated with peptone. We have just suggested a reason for this. Aside from the explanation already offered, the extreme difficulty of washing out last traces of peptone makes us still more doubtful that these slightly higher figures should be regarded as particularly significant.

We are justified, we think, in concluding from this and the foregoing experiments that the peptone has little if any constant, appreciable influence. Our further results harmonize with this deduction.

Experiments 7–10. — These experiments were carried out to ascertain the influence of increase and decrease in the quantities of digestive albuminate present in the fluids to begin with, the volumes of the latter remaining the same. The methods of the previous experiments were followed in detail.

Summary (7). — 0.1 gm. samples of fibrin albuminate of portion a, preparation E were used. This amount of substance at 100–105° C. = 0.090 gm. Results are tabulated on page 480.

Although the quantity of albuminate was reduced in this experiment, compared with the results of those in which 0.2 gm. was used, little proportionate difference is to be noted in the amount of precipitate obtained on boiling. Appreciable loss of acidalbumin was observed as usual. Preliminary boiling here did not seem to favor the highest quantitative precipitation. The process of first separating the neutralization precipitate and then boiling the filtrate appears to be best. In all probability preliminary boiling results in increased hydration.

Acid.	Weight of peptone. Gram.	Amount of neutralization precipitate.[1] Gram.	Quantity of precipitate on boiling the neutral filtrate. Gram.	Total amount of acidalbumin recovered. Gram.
A. Hydrochloric 1 *a*[2]	....	0.065	none	0.065
1 *b*	....	0.068	0.003	0.071
2 *a*	0.5	0.067	none	0.067
2 *b*	0.5	0.072	0.010	0.082
3 *a*	1.0	0.065	none	0.065
3 *b*	1.0	0.063	0.006	0.069
B. Oxalic 1 *a*	....	0.071	none	0.071
1 *b*	....	0.074	"	0.074
2 *a*	0.5	0.063	none	0.063
2 *b*	0.5	0.068	0.004	0.072
3 *a*	1.0	0.067	none	0.067
3 *b*	1.0	0.059	0.004	0.063
Average. 1–3–*A*	....	(I) **0.067**	(II) **0.003**	(III) **0.070**
B	....	**0.067**	**0.001**	**0.068**

Average total quantity of acidalbumin lost, 0.021 gm. = 23.3 per cent.
Average quantity of acidalbumin lost on neutralizing (cold), 0.023 gm. = 25.5 per cent.
Average ratio. I : II *b* = 34 : 1.
The average for II *b* (1–3) = 2.22 per cent of the original substance and 2.90 per cent of the average for III *b*.

[1] See footnote 1, on page 475. [2] See footnote 2, on page 477.

Summary (8). — 0.4 gm. samples of fibrin albuminate, portion **b**, preparation **E**, were used. Dried to constant weight, this amount contained 0.358 gm. substance.

Acid.		Weight of peptone. Gram.	Amount of neutralization precipitate.[1] Gram.	Quantity of precipitate on boiling the neutral filtrate. Gram.	Total amount of acidalbumin recovered. Gram.
A. Hydrochloric	1 *a*[2]	....	0.281	none	0.281
	1 *b*	....	0.284	0.009	0.293
	2 *a*	0.5	0.288	none	0.288
	2 *b*	0.5	0.321	0.005	0.326
	3 *a*	1.0	0.296	none	0.296
	3 *b*	1.0	0.280	0.017	0.297
B. Oxalic	1 *a*	....	0.315	none	0.315
	1 *b*	....	0.305	0.008	0.313
	2 *a*	0.5	0.303	none	0.303
	2 *b*	0.5	0.312	0.009	0.321
	3 *a*	1.0	0.309	none	0.309
	3 *b*	1.0	0.291	0.014	0.305
Average 1–3–*A*		....	(I) **0.292**	(II) **0.005**	(III) **0.297**
	B	....	**0.306**	**0.005**	**0.311**

Average total quantity of acidalbumin lost, 0.054 gm. = 15.1 per cent.
Average quantity of acidalbumin lost on neutralizing (cold), 0.059 gm. = 16.5 per cent.
Average ratio, I : II *b* = 60 : 1.
The average for II *b* (1–3) = 1.40 per cent of the original substance and 1.64 per cent of the average for III *b*.

[1] See footnote 1, on page 475. [2] See footnote 2, on page 477.

In this experiment four times as much acidalbumin was taken as in the previous one, yet the actual amount of precipitate obtained on boiling was only slightly increased; its proportion decreased. As in the preceding and some earlier experiments, the precipitate thrown down on boiling was obtained only from those fluids which had not been heated previous to their neutralization. In all the final filtrates, biuret reacting substance could be detected — doubtless proteose formed in the boiling process.

The amount of albuminate recovered from the chloride solutions was slightly less than from the oxalate, although in the previous experiment, and before that, little difference between the two was noted. Such differences as have been observed have not been at

all constant, probably for the reason that the variations are within the limits of unavoidable experimental error.

The conclusions drawn from experiments 7 and 8 will be found to hold for the results of the two following ones.

Summary (9). — 0.1 gm. samples of acidalbumin from fibrin were used, portion **a**, preparation **E**. This quantity of substance was equivalent to 0.090 gm., dried at 100–105° C.

Acid.		Weight of peptone. Gram.	Amount of neutralization precipitate.[1] Gram.	Quantity of precipitate on boiling the neutral filtrate. Gram.	Total amount of acidalbumin recovered. Gram.
A. Oxalic	1	....	0.069	0.003	0.072
	2	....	0.069	none	0.069
	3	0.5	0.067	0.006	0.073
	4	1.0	0.057	none	0.057
B. Tartaric	1	....	0.064	0.004	0.068
	2	....	0.067	0.004	0.071
	3	0.5	0.068	none	0.068
	4	1.0	0.064	"	0.064
C. Phosphoric	1	....	0.072	0.003	0.075
	2	....	0.070	none	0.070
	3	0.5	0.058	0.007	0.065
	4	1.0	0.068	0.006	0.074
D. Hydrochloric	1	....	0.066	0.004	0 070
	2	....	0.070	0.003	0 073
	3	0.5	0.068	0.006	0.074
	4	1.0	0.068	none	0.068
Average (1–4)–*A*		....	(I) **0.065**	(II) **0.002**	(III) **0.067**
B		....	**0.066**	**0.002**	**0.068**
C		....	**0.067**	**0 004**	**0.071**
D		....	**0.068**	**0 003**	**0.071**

Average total quantity of acidalbumin lost, 0.021 gm. = 23.3 per cent.
Average quantity of acidalbumin lost on neutralizing (cold), 0.023 gm. = 25.5 per cent.
General average ratio. I : II = 22 : 1.
General average for II = 3.33 per cent of the original substance and 4.35 per cent of the general average for III.

[1] See footnote, on page 475.

Summary (10). — 0.340 gm. samples of fibrin albuminate, portion **b**, preparation **E**, were used. This quantity of substance corresponded with 0.340 gm. substance dried to constant weight at 100–105° C.

Acid.		Weight of peptone. Gram.	Amount of neutralization precipitate.[1] Gram.	Quantity of precipitate on boiling the neutral filtrate. Gram.	Total amount of acidalbumin recovered. Gram.
A. Tartaric	1	....	0.275	0.002	0.277
	2	....	0.259	none	0.259
	3	0.5	0.257	0.007	0.264
	4	1.0	0.256	0.009	0.265
B. Oxalic	1	....	0.257	0.005	0.262
	2	....	0.266	none	0.266
	3	0.5	0.265	0.005	0.270
	4	1.0	0.264	0.008	0.272
C. Hydrochloric	1	....	0.274	0.002	0.276
	2	....	0.261	0.002	0.263
	3	0.5	0.267	none	0.267
	4	1.0	0.264	"	0.264
D. Phosphoric	1	....	0.284	0.004	0.288
	2	....	0.279	0.006	0.285
	3	0.5	0.272	0.006	0.278
	4	1.0	0.268	0.006	0 274
Average (1–4)–*A*		....	(I) **0.262**	(II) **0.004**	(III) **0.266**
B		....	**0.263**	**0.005**	**0.268**
C		....	**0.266**	**0.001**	**0.267**
D		....	**0.267**	**0.005**	**0.281**

Average total quantity of acidalbumin lost, 0.033 gm. = 10.9 per cent.
Average quantity of acidalbumin lost on neutralizing (cold), 0.037 gm. = 12.2 per cent.
General average ratio. I : II = 67 : 1.
General average for II = 1.31 per cent of the original substance and 1.85 per cent of the general average for III.

[1] See footnote, page 475.

The percentage of substance recovered on boiling was unusually low in this and in the eighth experiment, in which larger quantities of acidalbumin were taken, a fact suggesting that the loss is proportionately greatest with the least amounts of substance.[1]

[1] See tables, pages 488 and 490.

Eleventh experiment. — The foregoing results show that in these determinations a small though appreciable amount of albuminate invariably was lost. The quantity of substance separated on boiling was slight and approximately the same throughout. Such differences as were perceptible appeared to depend mainly on the quantities of albuminate present to begin with. Thus, the proportion of this precipitate in the hot fluid to that on neutralization in the cold was usually greater the smaller the amount of albuminate originally taken.

This result would indicate that the method of neutralization in the cold is the more satisfactory the larger the quantity of albuminate involved. On the other hand, because the volumes and quantities of acid were uniform in all of these experiments, it might be assumed, that the solvent action of the salts formed was much the same, even though the amounts of substance used did vary somewhat. For this reason, also, the total loss of material noted may have been uniformly slight.

In order to test these points the following special experiment was carried out.

Summary. — 0.5 gm. samples of preparation G of fibrin albuminate (0.450 gm. substance dried at 105° C.) were dissolved in different amounts of $\frac{m}{10}$ HCl, varying from 50 c.c. to 800 c.c. The solutions were allowed to stand as usual for an hour or two, then were exactly neutralized with dilute NaOH, as before, and the bulky precipitate permitted to settle until the following morning. The weights of the acidalbumin recovered are given below. The filtrates were then brought to the boiling point and kept there a moment or two. Each became turbid. The turbidity was least in the smallest volume of fluid and most pronounced in the largest quantity. The precipitates soon settled under perfectly clear fluid and were easily filtered off, with the gravimetric results appended.

Volume of $\frac{m}{10}$ HCl.	Amount of neutralization precipitate.	Precipitate obtained on boiling the neutral filtrate.		Total amount of acidalbumin recovered.	Total quantity of substance lost.
c.c.	Gram.	Gram.	Per cent.	Gram.	Gram.
50	0.399	0.005	1.1	0.404	0.046
100	0.346	0.012	2.7	0.358	0.092
200	0.348	0.014	3.1	0.362	0.088
400	0.312	0.035	7.8	0.347	0.103
800	0.303	0.041	9.1	0.344	0.106

The results of this experiment show quite conclusively that, other conditions being equal, an increasing proportion of acidalbumin is lost as the volume of neutral fluid (NaCl here) becomes larger. We have no doubt it increases somewhat, also, with a rise in the proportion of saline matter and, vice versa, falls in amount with a decrease in the proportional content of neutral salt. Although the albuminate here was the same in amount throughout the series, an increasing quantity of coagulum was separable from this neutral filtrate, a result still further emphasizing the fact of solubility of acidalbumin in cold neutral saline solution.

Each of the filtrates gave the usual biuret and proteose reactions. The increasing loss of acidalbumin above was seemingly due to the greater hydration, inevitably induced by boiling, in the larger volumes.[1]

In considering the value of this method, therefore, the volume of the digestive mixture as well as the percentage content of albuminate and neutral salts cannot be overlooked.

It appeared quite clear from this and each of the previous series of experiments that at least a small amount of acidalbumin was soluble in the cold neutral fluids containing it. Further, it was impossible to recover all of the albuminate used at the beginning of the experiment. It seemed desirable at this point, therefore, to ascertain definitely the solvent power of the various saline fluids made throughout these experiments in the process of neutralizing the acids.

Solubility of acidalbumin in saline solutions. — In the first of our special tests of this matter we ascertained merely the solubility of the

[1] The larger the volume the longer the time required, with a given flame of course, to raise the fluid to the boiling-point, and, therefore, the greater the exposure of the soluble substance to hydrating influence. Some hydration must occur before the solution reaches the boiling-point. In all probability the material which separates earliest and causes the initial turbidity is hydrated in part as the precipitate increases with the rise in temperature. Doubtless some of the material in solution is also hydrated before it can be precipitated. Perhaps heating to only 70–80° C. would have resulted in diminished loss of acidalbumin.

It would be natural to inquire in this connection why, on boiling, a small, fairly constant amount of substance usually remained as a coagulated precipitate, although hydration of the larger proportion, dissolved in the neutral filtrate, invariably occurred. The fact, however, that occasionally no such coagulation was observed, although loss of substance occurred, would indicate that *all* of the substance in the neutral filtrate was transformable into hydration products, and that, perhaps, the sameness of conditions attending the boiling process accounted for the similarity in the quantitative results. The very short boiling period was sufficient now and then to effect complete hydration of the dissolved residue.

dried fibrin albuminate in water and in 0.5 per cent NaCl, with the following results.

Summary (A). — Finely powdered samples of fibrin albuminate of portions **a** and **b**, preparation **E**, were used. 0.2 gm. was weighed for each test. This amount, dried to constant weight at 100–105° C., was equivalent to 0.180 gm. for portion **a**, 0.179 gm. for portion **b**. 100 c.c. of fluid was used as throughout all but the previous experiment. The mixtures were frequently stirred. They were allowed to stand over night, then filtered, etc., as in the previous experiments.

Solution used.		Weight of substance recovered. Gram.	
		Portion **a**.	Portion **b**.
A. Distilled water	*a*	0.1792	0.1738
	b	0.1779	0.1747
Average		**0.1786**	**0.1743**
B. 0.5% salt solution	*a*	0.1767	0.1663
	b	0.1701	0.1655
Average		**0.1734**	**0.1659**
Total substance taken in each of *A* and *B*		**0.180**	**0.179**
Average loss in *A*		0.001	0.005
Average loss in *B*		0.007	0.013

On boiling, the aqueous filtrates remained clear; but the saline fluids became opalescent. Practically nothing seems to have dissolved in water. In salt solution, however, a slight loss resulted in each test. The results with water show, if we grant that the dry acidalbumin is practically insoluble in water, that our preparations contained at most the merest traces of soluble salts or proteoses [1] — obviously not in sufficient quantity to account to any extent for the loss of substance noted throughout all of our experiments. Consequently this experiment is particularly valuable in showing that such disappearance of substance as has been noted in all our previous tests has been due to loss of albuminate itself and not merely to removal of soluble impurity.

[1] See footnote, page 476.

We next tried the solvent action of the salts formed in the neutralization of the various acids previously employed; also the solubility in water alone and in water containing peptone.

Summary (B). — Preparation **F** of our fibrin albuminate (moist substance) was taken. 100 c.c. of various acids used in the preceding experiments were carefully neutralized to litmus and lacmoid with dilute NaOH, as already described. As indicated below, some of these neutral fluids were thoroughly boiled for a few minutes, without material loss by evaporation, for the removal of carbonic acid gas. Weighed amounts of our moist, freshly precipitated acidalbumin were transferred to the neutral fluids (the boiled ones had been cooled). The mixtures were repeatedly stirred and allowed to stand over night as in all of the experiments. At intervals samples from the main bulk of the moist precipitate were weighed into crucibles for the determination of dry solid matter, as indicated below.[1]

It seems obvious, from the results on page 488, that acidalbumin is somewhat soluble in the salts formed on neutralizing acid fluids for its precipitation. Although practically insoluble in distilled water the acidalbumin appeared to be slightly soluble in water to which proteose and peptone had been added. We are not sure, however, that this result is not due to the solvent action of the saline matter present as impurity in Witte's peptone. The proportion of the amounts which dissolved in the cold neutral saline fluids to the total quantities originally taken is slight, however. With more decided acidity to begin with, and therefore more salts formed on neutralization than was the case in these experiments, doubtless the more decided would be the solution of substance, and the greater the quantity recoverable by coagulation.

The data just obtained also indicate that such slight amounts of carbonic acid gas as remained in the fluids on neutralization had little or no measurable influence on the results. The proportionate amounts of substance soluble in and recovered from the fluids which had been thoroughly boiled before the albuminate was put into them[2] and from which, therefore, the carbon dioxide had been removed,

[1] The moist substance was kept in a covered mortar. Before each sample was removed, the whole mass was thoroughly mixed. Errors caused by the slight evaporation of water under the circumstances were thus greatly minimized, and probably made inappreciable.

[2] The second of each series in the summary on page 488.

Solution.	Weight of substance taken.		Dry substance recovered.				Total average amount of substance lost (calculated).
	Fresh.	Dry (calculated).[1]	Insoluble.	Precipitate from the filtrate on boiling.	Total.	Ratio of *b* to *a*.	
	Gm.	Gm.	Gm.	Gm.	Gm.	Per cent.	Gm.
1. Distilled water[2]	3.163	1.232	(*a*) 1.2184	(*b*) none	1.2184		
	3.415	1.330	1.3397	none	1.3397		0.004
2. Distilled water with 0.5 gm. peptone	4.250	1.656	1.6822	0.0076	1.6898		
Distilled water with 1 gm. peptone	4.456	1.736	1.7685	0.0121	1.7806		+0.078[3]
3. Chloride	2.491	0.971	0.7834	0.0138	0.7972	1.76	
	3.240	1.262	1.0552	0.0066	1.0618	0.63	0.187
4. Oxalate	4.574	1.782	1.5796	0.0276	1.6072	1.75	
	3.178	1.238	1.0634	0.0212	1.0846	1.99	0.164
5. Phosphate	2.701	1.052	0.9008	0.0152	0.9160	1.69	
	2.482	0.967	0.8618	0.0148	0.8766	1.71	0.113
6. Tartrate	2 480	0.966	0.8350	0.0188	0.8538	2.25	
	2.682	1.045	0.9275	0.0216	0 9491	2.33	0.104
7. Nitrate	2.911	1.134	1.0176	0.0168	1.0344	1.65	
	3.678	1.434	1.3136	0.0286	1.3422	2.18	0.095
8. Lactate	3.447	1.343	1.2398	0.0124	1.2522	1.00	
	3.743	1.458	1.3834	0.0220	1.4054	1.59	0.072
Average[4]	....	**1.221**	(*a*) **1.080**	(*b*) **0.0180**	**1.098**	**1.67**	**0.123**

Average amount of substance lost = 10.07 per cent of that originally taken (average) and 11.39 per cent of the insoluble portion (average).

The average amount of precipitate obtained on boiling = 1.47 per cent of the original albuminate (average) and 1.67 per cent of the average quantity insoluble in the neutral fluids.

Ratio. $a : b = 60 : 1$.

[1] Portions of the moist substance were taken at the beginning of the experiment and after the third, fifth, and seventh series. The quantities of fresh material used for this purpose varied between 1.7334 and 3.9908 gms. The percentages of dry matter were found to be 38.38, 38.97, 39.20, and 39.29. See footnote, page 476.

[2] The second fluid of each pair throughout the series had been thoroughly boiled before receiving the albuminate.

[3] This figure represents a gain of substance; peptone not completely washed out. See page 479.

[4] The averages do not include the figures for the first two pairs of determinations.

were slightly greater than the others in some cases, but the same or less in others.

The results above likewise show an appreciable loss of substance even after the addition of the precipitate obtained from the boiled filtrate to that previously filtered off. This loss is doubtless due to formation of proteose in the process of boiling, as seems to have been the case in all of our previous experiments. It will be seen from the tabulated data, that this loss occurs in all of the tests, excepting the water alone and the water with peptone. The actual increase in amount recovered in the latter case is very probably due to adherent peptone which was very difficult to wash completely from the bulky precipitate. Since, also, the amount of solid matter in each quantity of the moist precipitate was calculated from special determinations of the dry substance contained in the fresh material, and not ascertained directly, we cannot lay too much stress upon it. All the results for "dry substance taken" may be a little high or low by reason of the unavoidable errors which usually accompany calculated data under such conditions, no matter how careful the experimenter may be to attend to every detail of manipulation in the comparative determinations. That appreciable loss occurred as usual, however, was clearly shown by the proteose content of the final filtrates.

The greatest losses appear, from the figures, to have been associated with the chlorides and oxalates. In our previous experiments, also, we noted that the biuret reactions in the final filtrates were usually strongest in the chloride and oxalate fluids.

III. General Summary of Results.

The table on page 490 summarizes the more important average data of nearly all of our experiments.

IV. Summary of General Conclusions.

We conclude from these experiments that acidalbumin may be almost completely precipitated from acid digestive mixtures at ordinary temperatures by careful neutralization. The later stages in the neutralization process should be conducted with particularly dilute alkali.

The absolute quantity of acidalbumin remaining in such fluids after neutralization in ordinary experiments is small, its proportion to the

GENERAL SUMMARY OF RESULTS.

Experiment.[1]	Amount of acidalbumin taken.	Acidalbumin recovered.					Acidalbumin lost.		
		Average quantity of neutralization precipitate.	Average amount of precipitate obtained on boiling.[2]	Ratio of neutralization precipitate to that obtained on boiling.	Precipitate obtained on boiling.		Total.		On neutralizing.
					Proportion of the total substance taken.	Proportion of the total substance recovered.	Average amount. Gram.	Per cent.	Per cent.
	Gram.								
Seventh	0.090	0.067	0.002	34 : 1	2.22	2.90	0.021	23.3	25.5
Ninth	0.090	0.067	0.003	22 : 1	3.33	4.35	0.021	23.3	25.5
Second	0.181	0.126	0.007	18 : 1	3.87	5.26	0.048	26.5	30.4
Fifth	0.179	0.145	0.006	24 : 1	3 35	4.00	0.029	16.2	19.5
Fourth	0.180	0.148	0.004	37 : 1	2.22	2.65	0.029	16.1	17.8
Third	0.275	0.231	0.005	46 : 1	1.82	2.12	0.039	14.2	16.0
Tenth	0.304	0.267	0.004	67 : 1	1.31	1.85	0.033	10.9	12.2
Eighth	0.358	0.299	0.005	60 : 1	1.40	1.64	0.054	15.1	16.5
B, p. 487[3]	1.221	1.080	0.018	60 : 1	1.47	1.67	0.123	10.1	11.4

[1] Only those are summarized for which data in each column were obtained. The first and sixth experiments gave incomplete results in this connection. The data of the eleventh experiment and under Summary *A* on page 486 are hardly comparable here. The results are so tabulated as to show at a glance the relations of amounts taken to those recovered.

[2] The averages in several cases do not include entirely negative results, as in the fourth experiment, for example.

[3] Total average results for all of these experiments, with the original amounts of acidalbumin so different, would be at best only rough approximations, and also in some ways misleading.

main bulk of the albuminate depending largely on the amount of the latter, also on the volume of the fluid containing it and on the percentage of associated saline matter.

Some of this residual portion of acidalbumin may be obtained on boiling, although in this process the larger part, sometimes all, is retained permanently in solution, apparently because of its hydration into noncoagulable forms.

Proteoses and peptones, even when admixed in comparatively large proportion, do not materially affect the quantitative separation of the albuminate.

Neutralization at the boiling point does not insure the greatest quantitative precipitation of albuminate because of the increased hydration thereby resulting. The largest yield is obtained by neutralizing in the cold, heating the neutral filtrate and combining the two precipitates.

Acidalbumin, particularly that formed through the action of pepsin on fibrin and in the freshly precipitated condition, is somewhat soluble in various saline fluids.

The sodium and potassium salts formed on neutralizing various common acid solutions appear to exert approximately equal quantitative solvent action on the contained albuminate. Only insignificant differences in solvent power were noted.

Such quantities of carbon dioxide as are present in fluids neutralized with freshly prepared solutions of potassium or sodium hydroxides containing ordinary, minute amounts of carbonate, do not appear to materially affect the quantitative separation of acidalbumin by the neutralization process.

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EXPERIMENTS TO DETERMINE THE POSSIBLE ADMIXTURE OR COMBINATION OF FAT OR FATTY ACID WITH VARIOUS PROTEID PRODUCTS.[1]

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IN one of the recent papers from this laboratory on the qualities of connective tissue mucoid, attention was drawn to the "lack of particular uniformity in percentage composition" of osseomucoid preparations. Since the analyzed products had been made with the greatest care, we were led to the deduction "that the mucin substance of bone varies in composition just as does the glucoproteid from other sources . . . a conclusion not only in accord with our analytic results, but in harmony, also, with the deductions drawn under similar conditions, for other tissues and products, by various observers."[2]

Later, and for the same reasons, we came to identical conclusions regarding tendomucoid.[3] In discussing the suggestion of Chittenden and Gies,[4] that possibly "mucin obtainable from tendon is prone to carry with it a certain amount of some other form of proteid matter which the ordinary methods of purification are not wholly adequate to remove," we indicated that there is "no longer any reason to believe that proteid impurity is responsible for the observed variations." It was further stated, at that time, that "we know of no other substance in tendon which would resist the washing treatment and, by mechanical admixture or chemical combination, account for the orderly variations observed in the analytic series."

After the latter account of our experiments had gone to the printer,[5] Nerking's paper on "fat-proteid compounds" reached us. His results

[1] Posner and Gies: Proceedings of the American Physiological Society, This journal, 1902, vi, p. xxix.

[2] Hawk and Gies: This journal, 1901, v, p. 415.

[3] Cutter and Gies: *Ibid.*, vi, pp. 167 and 169.

[4] Chittenden and Gies: Journal of experimental medicine, 1896, i, p. 194.

[5] Cutter and Gies: *Loc. cit.*, p. 169 (foot-note).

and views made it seem possible that the variations noted in analytic results for mucoids as well as for other proteids, may have been due to combinations or intimate admixtures of the proteid substance with fat or fatty acid.[1]

Recalling Dormeyer's[2] physical explanation for the retention of that portion of fat which can be removed from tissues only after their digestion, Nerking suggested that there is quite as much reason for concluding that such fat is chemically united in the tissue as that it is held mechanically, and, therefore, that it is removable with difficulty merely because of such intimate combination. Bogdanow's[3] observation, that the fat obtained in the later tissue extracts contains an increased proportion of free fatty acid, might seem to give strength to such a view, were it not for the probability that the increased amount of free fatty acid under such circumstances results by hydration of fat in the long-continued extraction process in boiling ether.

That fatty or fatty acid radicles are combinable with proteid is clearly evidenced in the example of the so-called lecithalbumins,[4] which do not yield their fatty radicles to ordinary extraction with ether, but can be broken up into fatty and non-fatty matter by appropriate methods.

Obtaining results which seemed to point to the conclusion that blood serum contains combined fat, non-extractable with ether until after digestion in pepsin-hydrochloric acid, Nerking, at Pflüger's suggestion, looked for similar combinations in various proteid products as they are now commonly prepared.

His results indicated that several proteid substances, which had been prepared and purified by the usual methods, contained varying amounts of fat or fatty acid in close combination. Further, this fatty radicle could be broken off and determined quantitatively by Dormeyer's method. No such combination with ovomucoid was shown, but about three per cent of extractive matter was separated from sub-maxillary mucin, among other products. Most of the proteids examined gave negative results. Albuminoids were not studied.

The quantities of substance extracted, and the amounts of extract obtained in the process, were comparatively small in each of the posi-

[1] NERKING: Archiv für die gesammte Physiologie, 1901, lxxxv, p. 330.

[2] DORMEYER: *Ibid.*, 1895, lxi, p. 341; 1896, lxv, p. 102.

[3] BOGDANOW: *Ibid.*, 1896, lxv, p. 81; 1897, lxviii, p. 408.

[4] A résumé of the literature concerning these bodies is given by COHNHEIM: Chemie der Eiweisskörper, 1900, p. 203.

tive cases. When the ordinary unavoidable sources of error in work of this kind are kept in mind it is difficult to lay very much stress upon extractive quantities as slight as those obtained in Nerking's experiments. It should be noted, further, that in no case was more than one sample of each particular proteid analyzed.

Nerking does not make it clear to the reader of his paper that his products were given the great care in preparation, particularly the extended extraction in hot alcohol-ether, which is necessary for their complete purification. He does not state that he was careful to use anhydrous ether, nor, indeed, that the samples of ether he employed had even been distilled by him previous to their use. Possibly he was not certain, therefore, that the extractive fluid itself would not sometimes yield residual matter on evaporation. He states nothing regarding the quantity of fatty material contained in the samples of enzyme used in his digestions. Preparations of pepsin such as he employed contain appreciable proportions of ether-soluble material.

Toward the end of his paper Nerking himself comments on the obvious weakness of his experimental evidence on the existence of "fat proteid compounds." He adds, also, that all his efforts to effect special combinations of proteid with fat have resulted negatively.

With such doubts in our minds as were raised by the omissions above referred to, and at the same time appreciating the suggestiveness of Nerking's results, particularly in connection with the mucoids, we set to work to ascertain the facts regarding the proteids referred to below.

Proteid Products Investigated.

Preparation. — All of the proteids worked with in these experiments, with the few exceptions to be noted, had been prepared and purified very carefully by improved or accepted methods for special research in other connections, some of the data of which have already been published. This fact is emphasized at this point to show that such results as were obtained in these experiments were not dependent on unusual care in this particular instance, in the separation of the proteids, but are typical for these substances as we are accustomed in this laboratory to prepare them.

Method of estimating extractive substance. — Care was taken to follow Nerking's general extractive procedure. The substance, dried at 100–105° C. to constant weight, was extracted for fifteen to twenty days continuously in a Soxhlet apparatus with anhydrous ether pre-

pared in bulk by us and freshly distilled in glass apparatus before use. On evaporation, large quantities of the ether completely disappeared without leaving a residue.

After preliminary extraction, the proteid was digested in a moderate excess of 0.2 per cent hydrochloric acid containing 0.8 gram of commercial pepsin scales per litre. Digestion in this fluid was rapid and complete. This quantity of the pepsin preparation (0.8 gm.) contained 2 to 4.8 milligrams of extractive material. After the digestion the extractions were conducted as in Dormeyer's method.

All ether extracts, those obtained before as well as after digestion, were filtered, the papers thoroughly washed with ether and the washings added to the main filtrate. Separation of the ethereal extract from the fluid digestive mixture was always made exactly, in a separatory funnel. There was no tendency to persistent emulsion at this point in any of our experiments. The amount of indigested matter was at most very slight, even with the mucoids.

The ether extracts, after filtration, were evaporated in vacuo in small beakers. Care was taken entirely to exclude dust particles after filtration. A very small amount of water was left behind on evaporating the ether which had been in contact with the acid fluid. The amount of solid matter dissolved in it must have been very slight in absolute quantity, although forming an appreciable proportion of the weight of the extract. See table, pages 338 and 339.

Mucoids. — It has been known for a long time that a certain amount of ether-soluble matter is admixed with connective tissue mucoid when the latter is first precipitated from its solution in alkali by acids.[1] The difficulties in the way of removing this admixture have been appreciated by various observers, but no one has determined the chemical nature of the extractive substance. These glucoproteid products therefore appeared to offer particularly interesting objects for study in this connection also.

Tendomucoid. — Our mucoids from tendon were prepared for the experiments recently described by Cutter and Gies.[2] We used samples of their analyzed preparations Nos. 1 to 5 inclusive. Our extractive results were practically negative for each of these.

A portion of preparation No. 1, which by accident had been left in the air-bath for a few days — a somewhat longer period than was necessary to carry it down to constant weight — had become slightly

[1] Loebisch: Zeitschrift für physiologische Chemie, 1886, x, p. 58.

[2] Cutter and Gies: *Loc. cit.*

brownish (oxidized?) just as filter paper does, for example, under similar circumstances. On extracting this material the ether became yellowish at first, then reddish yellow in color.[1] The extracted substance was very slight in quantity, however, the high tinctorial effect having suggested a greater amount of solid matter in solution than was actually found.[2]

A sample of the same preparation when dried to constant weight in vacuo, instead of in the air bath, gave essentially the same negative results. Of course, no pigment was developed.

The results with purified tendomucoid having been negative, we determined next the amount of extractive matter in the crude material, which various observers, as we have already noted, have found it very difficult to remove in the purification process. For this purpose we used a sample of crude tendomucoid prepared originally for digestive experiments now in progress. This product was obtained in the usual way from the Achilles tendon of the ox. After its first precipitation from lime-water the substance was washed free of acid, then partly dehydrated in 50 per cent alcohol and dried in the air in thin layers on plates. 10.8 grams of this finely powdered product, in spite of the treatment with alcohol in its preparation, yielded 0.3 gram of extractive matter, a large part of which persisted in the substance even after two weeks' extraction.[3]

The extractive matter thus obtained was yellow in color and oily in consistency, but did not contain any fat crystals. Even when dissolved in ether and allowed to evaporate spontaneously at room temperature, crystals failed to develop. That it contained fat, however, was shown by the yield of fatty acid. It did not contain cholesterin or lecithin in sufficient quantity to respond to the familiar tests.

Our result in this connection emphasizes the need of thorough extraction in the purification of tendomucoid.

[1] The previous extracts were colorless. So were all subsequent ones except that from crude mucoid.

[2] That the usual treatment in an air-bath at 100–110° C. for the removal of water from proteids is an unsatisfactory method has long been recognized. Such results as the above, which indicate gradual decomposition, also emphasize the desirability of an improved method of drying proteid products for analysis.

[3] This fact may have been due to the compactness of the powder particles, since the product had been dried before it was completely dehydrated. It was not light and fluffy, as is the dry, purified, dehydrated mucoid. All of this extractive substance, it will be remembered, can be eliminated from the freshly precipitated mucoid without the aid of the digestive process.

Osseomucoid and chondromucoid. — These preparations had been analyzed by Hawk and Gies.[1] The former was their preparation No. 6; the latter, preparation "b." Like the tendomucoid, these products were found to be free from fatty material.

Albuminoids. — Each of our albuminoid products was prepared by improved method. All were found to be practically free from extractive material.

Collagen. — One sample of collagen from the femur of the ox had been made by us from ossein shavings for other experiments not yet reported. Osseomucoid, etc., had been removed with lime-water and the albumoid[2] eliminated by digestion in alkaline trypsin solution.[3] A sample of tendon collagen from the Achilles tendon of the ox had been made in the same way, for the same purpose, and was available for these experiments.

Gelatin. — Products prepared from bone, for other experiments in progress for some time, were used. They were made from ossein shavings obtained from the rib and the femur of the ox, after removal of the mucoid and albumoid as above. The ligament gelatin used by us was analyzed by Richards and Gies.[4] Through the kindness of the writer's former colleague, Dr. W. G. Van Name, we were able, also, to use samples of two of his preparations of tendon gelatin — C and D.[5]

Elastin. — Our samples of elastin were prepared and analyzed by Richards and Gies.[6] Their preparations Nos. 7 and 8 were used.

Simple proteids. — These also gave practically negative results in the two experiments with purified products.

Globulin. — We used a sample of cocoa edestin obtained by Kirkwood and Gies[7] — their preparation No. 5. The endosperm of the cocoanut, from which this preparation of edestin was made, contains large proportions of fat and fatty acids, a condition particularly favorable to admixture or combination with proteid, if such had occurred.

Alkali albuminate. — This product had been made by Fried and Gies[8] from a mixture of myosin and muscle "stroma substance." It

[1] HAWK and GIES: *Loc. cit.*

[2] HAWK and GIES: This journal, 1902, vii, p. 340.

[3] EWALD and KÜHNE: Jahresbericht der Thier-Chemie, 1877, vii, p. 281.

[4] RICHARDS and GIES: This journal, 1902, vii, p. 128.

[5] VAN NAME: Journal of experimental medicine, 1897, ii, p. 124.

[6] RICHARDS and GIES: *Loc. cit.*, p. 104.

[7] KIRKWOOD and GIES: Bulletin of the Torrey Botanical Club, 1902, xxix, p. 343.

[8] FRIED and GIES: Proceedings of the American Physiological Society. This Journal, 1901, v, p. xi.

had not been thoroughly extracted with ether in the purification process.

Commercial products. — These substances were dried egg albumen, Witte's peptone, somatose, and chloralbacid. An appreciable quantity of extractive matter was separable from the albumen, but the proportion of such substance obtained from it was not as great as that from crude mucoid.

Discussion of Results.

The table on pages 338 and 339 summarizes the data obtained in these experiments. It will be observed that the figures for composition of the purified products agree with the accepted average data for each class of substances. Further, it is seen that the absolute amounts of extractive substance are very slight — so minute, in fact, as to be practically nothing except for the crude products with their usual extractive impurities. The influence of ordinary, unavoidable defects of manipulation on such small quantities of residual substance is obvious.

The perceptible decrease in the weight of many of the extracts during the drying process in the air-bath might be interpreted as indicating a loss of volatile fatty acid. This decrease, however, is seen to be very slight in absolute amount. It is much more probable that the loss was water only. The small beakers in which the ethereal extracts were evaporated were light in weight but of a capacity of 80 c.c. While even this size was somewhat disadvantageous as far as drying and weighing were concerned, smaller vessels could not have served very well in other respects. It is probable that, in their stay in the desiccators over sulphuric acid, not all of the moisture was removed from them. In the air-bath it was, of course, driven off and the total weight thereby reduced somewhat.

Conclusions.

We conclude from the data of these experiments that the above proteids of the simple, compound and albuminoid types, which were prepared by the best methods now in use, are not "fat-proteid compounds."

It is obvious, also, that these substances bear no resemblance to products of the lecithalbumin type.

Proteid substance examined.						
Nature.	Percentage composition.					Amount used.
	C	H	N	S	O	Grams.
Tendomucoid — 1 . . .	47.47	6.68	12.58	2.20	31.07	4.8327
2 . . .	47.46	6.56	11.78	1.81	32.39	2.2376
3 . . .	47.80	6.60	12.66	1.85	31.09	4.8879
4 . . .	48.92	6.83	12.64	2.80	28.81	2.7916
5 . . .	48.54	6.68	12.69	2.34	29.75	2.0688
Tendomucoid — 1 . . .	47.47	6.68	12.58	2.20	31.07	
a. Slightly oxidized .	....	....	....	....	....	4.0910
b. Dried in vacuo . .	....	....	....	....	....	2.8149
Tendomucoid — crude .	....	....	12.82	....	....	10.8250
Osseomucoid	46.53	6.81	11.99	2.55	32.12	4.3211
Chondromucoid	45.58	6.80	12.38	2.55	32.69	5.7899
Bone collagen	....	....	18.39	....	....	4.1949
Tendon collagen . . .	...	....	18.01	....	....	3.8240
Tendon gelatin — 1 . .	50.16	6.63	17.83	0.21	25.14	4.3821
2 . .	50.15	6.50	17.71	0.26	25.38	4.9536
Bone gelatin — rib . . .	....	....	18.20	....	....	2.9991
femur . .	....	....	18.12	....	....	3.5150
Ligament gelatin . . .	50.49	6.71	17.90	0.57	24.33	5.7211
Elastin — 1	54.47	7.30	16.64	0.14	21.45	5.6747
2	53.84	7.31	17.00	0.14	21.71	8.7429
Cocoa edestin	....	....	18.24	....	....	4.2191
Albuminate (myosin) . .	....	....	16.39	....	....	4.8249
Egg albumen	....	....	....	....	....	8.2194
Witte's peptone . . .	....	....	....	....	....	8.1876
Somatose	....	....	....	....	....	13.2002
Chloralbacid	....	....	....	....	....	12.3439

Preliminary extraction in ether, 15–20 days.			Extraction for 7 days in ether after digestion in pepsin — HCl.			Total extract.			
Extract dried over H_2SO_4 24 hrs.	Extract dried in air-bath at 100° C.		Extract dried over H_2SO_4 24 hrs.	Extract dried in air-bath at 100° C.		Dried over H_2SO_4 24 hrs.		Dried in air-bath at 100° C. 24–48 hrs.	
	24 hrs.	48 hrs.		24 hrs.	48 hrs.				
Milligrams.						Mgms.	%	Mgms.	%
....	1.0	....	....	0 8	....	....	....	1.8	0.037
1.2	0.7	0.8	0.9	0.7	0.6	2.1	0.09	1.4	0.062
....	0.7	0.7	1.7	1.1	1.3	....	....	2.0	0.041
1.8	0.8	0.5	1.5	1.0	1.4	3.3	0.12	1.9	0.070
1.1	0.2	0.3	0.7	0.4	0.6	1.8	0.08	0.9	0.044
5.9	2.5	2.3	3.6	2.0	1.8	9.5	0.23	4.1	0.100
2.3	1.1	1.3	0 5	0.0	....	2.8	0.10	1.3	0.046
114.2	**108.9**	**107.5**	**202.6**	**195.7**	....	**316.8**	**2.93**	**303.2**	**2.800**
0.7	0.6	0.7	0.7	0.1	0.0	1.4	0.03	0.7	0.016
2.3	1.5	1.7	0.8	0.2	0.0	3.1	0.05	1.7	0.030
2.7	0.7	....	1.7	0.7	....	4.4	0.10	1.4	0.033
1.2	0.8	....	0.5	0.3	....	1.7	0.04	1.1	0.029
1.7	1.3	0.8	1.8	....	1.0	3.5	0.08	1.8	0.041
1.6	1.2	0.4	2.2	....	1.3	3.8	0.08	1.7	0.034
0.4	0.1	0.0	1.2	....	0.5	1.6	0.05	0.5	0.017
1.5	1.1	1.2	0.4	0.0	....	1.9	0.06	1.2	0.034
1.5	1.0	0.9	1.5	....	0.6	3.0	0.05	1 5	0.026
1.5	0.7	0.1	1.7	....	0.8	3.2	0 06	0.9	0.016
1.3	0.7	0.0	1 3	....	0.5	2.6	0.03	0.5	0 006
1.2	0.8	0.8	2.7	....	1.8	3 9	0.09	2.6	0.061
1.1	0.5	0.7	6.8	....	5.4	7.9	0.16	6.1	0.127
24.1	12.7	0.7	17.6	17.2	....	41.7	0.51	17.9	0.218
9.3	6.5	4.6	15.6	14.7	....	24.9	0.30	4.6	0.056
2.4	1.6	0.0	1.0	0.0	....	3.4	0.26	0.0	....
8.0	3.2	3.0	6.5	4.1	3.9	14.5	0.12	6.9	0.056

D. BOTANICAL STUDIES.

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Chemical Studies of the Cocoanut with some Notes on the Changes during Germination *

BY J. E. KIRKWOOD AND WILLIAM J. GIES

(WITH PLATE 19)

[From the Laboratory of Physiological Chemistry of Columbia University, and the New York Botanical Garden, New York.]

SYNOPSIS

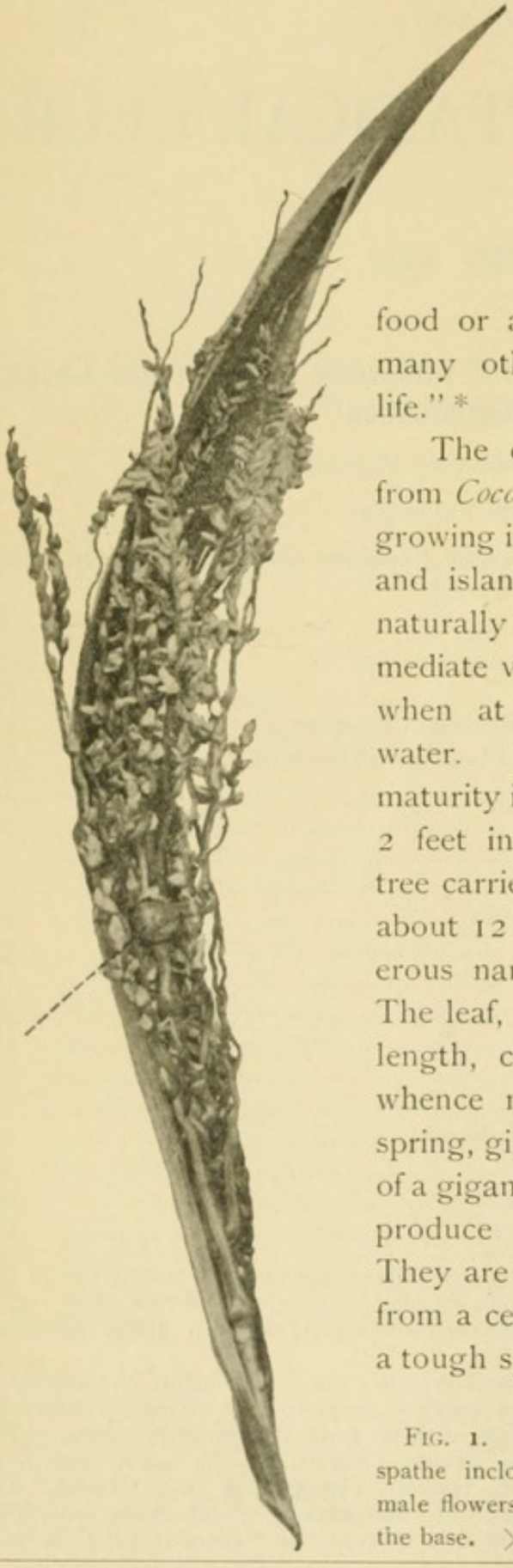
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* Preliminary accounts of some of the results of this research were given in the Proceedings of the American Association for the Advancement of Science,— : 275. 1900, and in the Proceedings of the American Physiological Society. 1900: American Journal of Physiology, 5: 14. 1901.

The term "coco" appears to be derived from "coc" or "cocus," a local name for the "Indian nut," the fruit of *Cocos nucifera*, given to it on account of a fancied resemblance of the base of the endocarp, with its three circular impressions, to the face of a monkey when it utters a cry having a sound like the word. See *f. 1, pl. 19.* The term "cocoa" should be carefully distinguished from "cacao," the product of *Theobroma cacao*, from "coca" the derivative of *Erythroxylon coca*, from "coco," the coco-kola of commerce, and from "cocco" or "cocoa root" (*Colocasia esculenta*).

I. INTRODUCTION



"Of the whole class of seeds having the character of luxuries rather than of necessaries, the cocoanut is by far the most important to mankind, whether considered as a delicious and nutritious food or as supplying valuable oil and many other articles useful in social life." *

The common cocoanut is derived from *Cocos nucifera*, a species of palm growing in practically all tropical coasts and islands. The cocoa palm grows naturally on the seashore or in its immediate vicinity and does not bear well when at a great distance from salt water. (See analyses, p. 335.) At maturity it has a cylindrical stem about 2 feet in diameter. At its apex the tree carries a tuft of leaves, which are about 12 feet long. These have numerous narrow, rigid and long leaflets. The leaf, which may attain to 20 feet in length, consists of a strong mid-rib, whence numerous long acute leaflets spring, giving the whole the appearance of a gigantic feather. The flowers which produce the nut are yellowish-white. They are arranged in spikes, branching from a central axis, and inclosed with a tough spathe usually a meter or more

FIG. 1. Inflorescence of the cocoanut showing spathe inclosing the spikes, each with numerous male flowers above and a single female flower near the base. $\times \frac{1}{5}$. Winton.

* Smith: Food, 226. 1873.

in length. Their appearance and arrangement is shown in Fig. 1, on the opposite page. The tree grows to a height of about 60–100 feet and usually bears 80–100 nuts arranged on the tree in bunches of 10–20. It continues to bear during two generations of men.

The fruit is subtriangular-ovoid in form, about 12 inches long and 6 inches broad. It is composed of a thick, fibrous "husk" (exocarp) and thin, hard "shell" (endocarp), containing a white fleshy seed, the "meat" (endosperm), with a thin integument (testa). (See *Fig. 3*, p. 324.) The thick husk is remarkably adapted to the preservation of the seed, whilst the nut is tossed about by the waves until it reaches some shore, it may be, far distant from that on which it grew. While immature the nut is without the solid endosperm, but is filled with a milky fluid. As it ripens, however, the endosperm gradually develops and the milky juice diminishes in quantity. The temperature of this juice when fresh is always comparatively low. (See page 349 for further reference to the parts of the nut.)

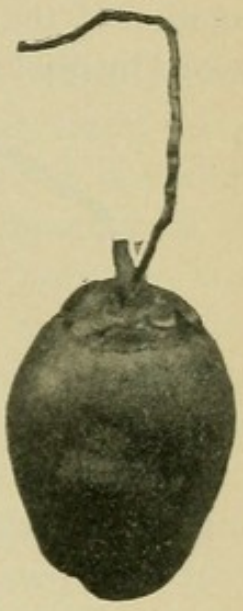


FIG. 2. Half-grown cocoanut fruit with calyx, and axis from which the male flowers have fallen. $\times \frac{1}{5}$. Winton.

Figures 1, 2 and 3 are from cuts loaned to us by Dr. A. L. Winton, who used them lately in the account of his very valuable histological study of "The Anatomy of the Fruit of the *Cocos nucifera*."* We are greatly indebted to Dr. Winton for his kind assistance.

The cocoanut forms the chief food of the inhabitants of Ceylon, the South Sea Islands, the coast of Africa and other tropical coasts and islands.† The flesh is not only eaten as it comes from the tree, both ripe and unripe, but it is also prepared and served in various ways. In India the "copra" is much used as an ingredient of curries. It forms an accessory part of the diet, and is found

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* Winton : American Journal of Science, IV. 12 : 265. 1901.

† The cocoanut is agreeable to the taste of various domestic and other animals, and is eagerly eaten by them. The cocoanut-crab (*Birgus latro*, suborder *Macrura* ; anomalous form, approaching the *Brachyura* and closely related to the hermit crab) feeds almost entirely on the kernel of the cocoanut. Its powerful claws enable it to easily peel off the husk and open the hard shell.

in many of the confections, of civilized man all over the globe.*

Immoderate use of the fruit, which according to the people of the tropics is highly refrigerant, causes, it is said, rheumatic and other diseases.† The milk is considered an agreeable cooling beverage in the tropics. It has been known for some time that irritation of the mucous membrane of the bladder and urethra is caused by drinking too freely of the cocoanut milk.‡ It is strongly

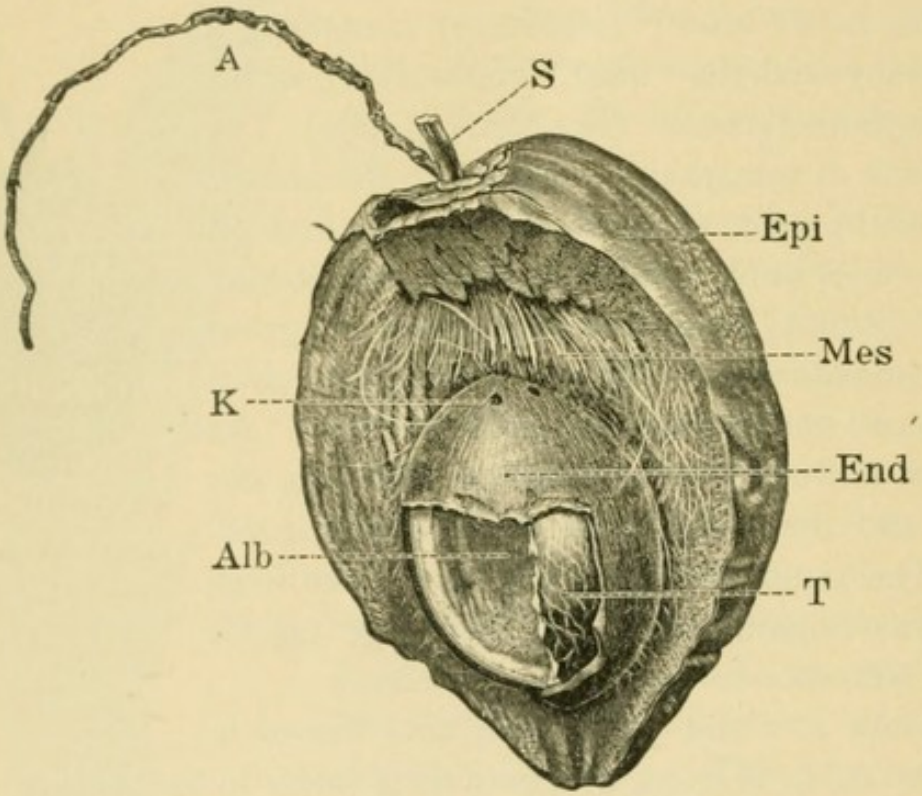


FIG. 3. Ripe cocoanut. *S*, lower part of axis forming the stem; *A*, upper end of axis with scars of male flowers; *Epi*, epicarp; *Mes*, mesocarp with fibers; *End*, endocarp or hard shell; *T*, portion of testa adhering to endosperm; *Alb*, endosperm surrounding cavity of the nut; *K*, germinating eye. × ⅓. Winton.

diuretic. Parisi has used the cocoanut therapeutically as an anthelmintic with uniformly satisfactory results.§ He states that the meat of the nut is a powerful *taenicide*, the milk sharing the prop-

* In the Annals of the New York Academy of Science, 13: 490, 1900–1901, the following may be found: "Dr. Gies in answer to a question stated that the food content of the cocoanut is small." This answer is quoted incorrectly. The question referred to the *nitrogenous* food content. It was stated on that occasion that the "content of *proteid* food-stuff is small." See page 340.

† Pavy: A Treatise on Food and Dietetics physiologically and therapeutically considered, 488. 1878.

‡ Curtis: Annals of the New York Academy of Sciences, 13: 490. 1900–1901.

§ See Liebreich: Encyklopaedie der Therapie, 1: 744. 1896.

erty.* The cocoanut has been used as a vermifuge in India for probably forty generations by the beef eaters of that country and is there well known as a means of expelling the flat worm.† The small, green and immature nut is grated fine for medicinal use, and when mixed with the oil of the ripe nut becomes a healing ointment.

The fibrous husk (coir) is widely used for the construction of ropes, brushes, bags, matting, etc. The compact fleshy edible portion (copra), closely lining the hard shell and which is entirely fluid or gelatinous when young, contains a large proportion of fat, which is extracted and used for various commercial purposes, such as the manufacture of fine soaps and candles and frequently as a substitute for butter. Cocoanut oil and resin melted together yield a substance capable of being used with success in filling up the seams of boats and ships, and in tropical countries for covering the corks of bottles as a protection against the depredations of the white ant. A quart of the oil may be obtained from six to ten nuts. The hard shell is easily polished and lends itself to the formation of various utensils and ornaments. It also has a high fuel value.

Although considerable is known of the constituents of the cocoanut, of its nutritive value and commercial uses, little has been done to ascertain the nature of the changes which the nut undergoes during germination. At the suggestion of Dr. MacDougal we have undertaken such a study, more especially from the chemical standpoint, and although our work in this particular connection has not been quite as fruitful as we had hoped it might be, our results are not without some interest.

II. Chemical Composition of the ungerminated Cocoanut

Before beginning our work on the germinating seed we felt it desirable to make ourselves thoroughly familiar with the chemical qualities of the ungerminated nut. This seemed all the more desirable because of the incomplete as well as the disconnected chemical data thus far recorded in this connection. This purpose was accomplished in a large number of analyses of numerous

* United States Dispensatory, 1619. 1899.

† American Journal of the Medical Sciences, 67 : 281. 1889.

samples. We record the more important of these results, with comparative data from the work of others, on the following pages.

Most of the nuts subjected to the analyses referred to farther on were furnished to us for this work by Hon. Wm. Fawcett and the United Fruit Co., who sent them in their husks from Jamaica. They were ripe, fresh and of about the average size. A few determinations were made with material from nuts bought in the markets in this city—source unknown, though doubtless of West Indian origin. These were of ordinary size, appeared to be ripe and fresh, and gave essentially the same analytic results as those obtained directly from Jamaica.*

We wish at the outset of this paper to thank Dr. MacDougal not only for the supply of material with which he favored us, but also for the suggestions which led us to undertake this work and for the kind encouragement he has given us from the beginning.

PROPORTIONS OF MILK, ENDOSPERM AND SHELL IN THE HUSKED NUT.—The weights and proportions of the main parts of the nut without its husk were carefully ascertained in special observations, as well as incidentally in other experiments.† The milk was removed as indicated on page 328. The empty nut was quickly broken with a hammer, the endosperm and germ, with the thin seed coat, carefully and promptly removed with a knife, and the fresh moist parts weighed at once. The results given on the opposite page were obtained in this connection.‡

The only results recorded on these gross relationships that we have been able to find were those obtained in a single experiment by v. Ollech, and those by Bachofeu.§ The parts of a single cocoanut, except the milk, were dried in the air by v. Ollech.‖

* A few showed signs of deterioration, such as "popping" on opening, free acid in the milk, etc. These were, of cour-e, discarded.

† The weight of the fibrous husk varies considerably, as the amount of moisture increases by absorption or decreases by evaporation. The weights of the other parts are ordinarily not subject to such fluctuations.

‡ The weights of the germ and the thin seed coat enveloping the endosperm were included with the latter.

§ See also Berzelius: Lehrbuch der Chemie. Translated by Wöhler, 7: 533. 1838.

‖ v. Ollech: Quoted by König, Die menschlichen Nahrungs- und Genussmittel, etc., 2: 495. 1893.

FRESHLY IMPORTED NUTS (FROM JAMAICA).

Number.	Weights in Grams.				Percentage of total Weight of husked Nut.			Milk.	
	Fruit without Husk.	Shell.	Endosperm with Germ and Integument.	Milk.	Shell.	Endosperm with Germ and Integument.	Milk.	Vol. c.c.	Specific Gravity.
1	845	255	437	153	30.2	51.7	18.1	150	1018
2	771	198	379	194	25.7	49.2	25.1	190	1017
3	658	168	371	119	25.5	56.4	18.1	117	1020
4	718	199	351	168	27.7	48.9	23.4	164	1019
5	597	152	327	118	25.5	54.8	19.7	113	1022
6	463	127	251	85	27.4	54.2	18 4	83	1019
7	622	195	334	93	31.3	53.7	15.0	90	1023
8	563	144	329	90	25.6	58.4	16.0	87	1027
9	633	166	374	93	26.2	59.1	14.7	90	1027
10	530	156	282	92	29.4	53.2	17.4	90	1021
11	637	150	363	124	23.5	57.0	19.5	121	1024
12	497	144	267	86	29.0	53.7	17.3	85	1014
13	538	162	283	93	30.1	52.6	17.3	90	1021
14	413	123	256	34	29 8	62.0	8.2	33	1030
15	511	158	309	44	30.9	60.5	8.6	43	1037
16	578	190	320	68	32.8	55.4	11.8	67	1016
17	568	142	350	76	25.0	61.6	13.4	74	1026
18	495	140	293	62	28.3	59 2	12.5	60	1024
19	813	221	392	200	27.2	48.2	24.6	194	1021
20	758	208	393	157	27.4	51.9	20.7	150	1022
21	584	148	339	97	25 4	58.0	16 6	94	1028
Aver.	609	169	333	107	27.8	55.2	17.0	104	1023

NUTS FROM THE MARKET (NEW YORK CITY).

1	1070	250	558	262	23.4	52.2	24.4	254	1018
2	1009	251	506	252	24.9	50.1	25.0	246	1015
3	728	202	417	109	27.7	57.3	15.0	106	1027
4	800	226	450	124	28.2	56.3	15.5	120	1026
5	688	191	385	112	27.8	56.0	16.2	110	1015
6	565	131	316	118	23.2	56.0	20.8	116	1017
7	639	210	382	47	32.9	59.8	7.3	46	1024
8	638	210	311	117	32.9	48.8	18.3	115	1017
9	480	125	304	51	26.0	63.3	10.7	48	1034
10	561	158	307	96	28.2	54.7	17.1	92	1024
11	733	204	414	115	27.8	56.5	15.7	110	1024
12	762	176	380	206	23.1	49.9	27.0	202	1020
Aver.	722	194	394	134	27.1	55.1	17.8	130	1021

They represented the following proportions of the total weight, which was 1,133 grams:

Fibrous Husk.	Shell.	Endosperm with Germ.*	Milk.
30.45 per cent.	19.59 per cent.	37.78 per cent.	12.18 per cent.

Of the total weight of the husked nut, which, by calculation, must

* Including, doubtless, the seed coat as well.

have been 788 grams, the percentages of the parts were (calculated by us):

Shell.	Endosperm with Germ.	Milk.
28.17 per cent.	54.32 per cent.	17.51 per cent.

These results, it will be observed, harmonize closely with the averages of our own determinations.

The data obtained by Bachofeu in this connection will be found in the table on page 335.*

COMPOSITION OF THE MILK.—The milk was poured from the nut through an opening made in the "eye" of the fertile carpel (see page 350) with a cork-borer. Extraneous matter could easily be kept out of the milk by this procedure and, besides, the fluid could be obtained when desired in a perfectly fresh, unevaporated condition.

The milk was found to be faintly turbid and opalescent in each case, and always contained a few oil globules and occasionally crystalline matter. It was acid in reaction to litmus although, as shown by lacmoid, no free acid was present in the normal fluid. The reaction is due to acid phosphate. Both alkali and earthy phosphate are present. The latter can be precipitated, in part at least, on boiling. An abundant precipitate of phosphate is obtained when the milk is made alkaline. The average specific gravity, determined with the aid of a hydrometer, was, as already noted on page 327, 1,023 and 1,021. The average specific gravity of the mixed milk of 15 nuts not included in the table on that page was 1,023. Of eight additional nuts not referred to there, and examined at another time, the figures for the mixed milk were 1,022.

The milk reduces Fehling's and Nylander's solutions and it ferments. It contains some monosaccharide which, from the characters of the phenylosazone derivatives, appears to consist of either dextrose or galactose, probably of both. Disaccharide in the form of cane-sugar is also present in good quantity, as might be inferred from the sweet taste of the milk.

* Results having some relation to these are given by Atwater: Report of the Storrs (Conn.) Agricultural Experiment Station, 123. 1899. Hammerbacher (Landwirtschaftlichen Versuchs-Stationen, etc., 18: 472. 1875) found that the endosperm of two nuts weighed 835.8 grams; the milk, 303.95 grams. See also, pages 331 and 356.

On standing the milk turns sour, becomes thicker, and has much the odor and physical appearance of soured cow's milk. The milk ferments readily. As it does so the acidity increases with a production of acid from the sugar. Alcohols are also produced in the process. The distillate from the fermented milk has an agreeable taste and an alcoholic odor.*

Chlorides are prominent with phosphates among the inorganic substances of the milk. It contains only a very small quantity of proteid, coagulating above 80° C., and also traces of a proteose-like body. Very faint biuret and xanthoproteic reactions were obtainable with the fresh fluid. A snow-white precipitate consist-

GENERAL COMPOSITION OF THE MILK

No.	Milk Used.		Percentage of Fresh Milk.				Percentage of Solids.	
	Specific Gravity.	Grams.	Water.	Solid Matter.			Organic Matter.	Inorganic Matter.
				Total.	Organic.	Inorganic.		
1–*a*	1019	28.815	95.52	4.48	3.98	0.50	88.84	11.16
b		27.280	95.43	4.57	4.05	0.52	88.60	11.40
2–*a*	1020	25.403	95.28	4.72	4.27	0.45	90.58	9.42
b		27.837	95.44	4.56	4.14	0.42	90.70	9.30
3–*a*	1022	30.382	94.73	5.27	4.78	0.49	90.57	9.43
b		28.528	94.62	5.38	4.90	0.48	91.02	8.98
4–*a*	1016	25.958	95.73	4.27	3.88	0.39	90.81	9.19
b		25.823	95.65	4.35	3.96	0.39	90.92	9.08
c		26.298	95.68	4.32	3.91	0.41	90.57	9.43
5–*a*	1021	29.416	95.11	4.89	4.47	0.42	91.38	8.62
b		29.467	95.23	4.77	4.36	0.41	91.39	8.61
c		24.667	95.24	4.76	4.35	0.41	91.31	8.69
6–*a*	1024	23.119	95.44	4.56	3.82	0.74	83.68	16.32
b		23.886	95.33	4.67	3.92	0.75	84.04	15.96
7–*a*	1028	22 540	94.80	5 20	4.18	1.02	80.38	19.62
b		26.690	94.94	5.06	——	——	——	——
8–*a*	1027	28.722	95.02	4.98	4.21	0.77	84.45	15.55
b		28.409	94.97	5.03	4.26	0.77	84.69	15.31
Aver.	1022	26.847	95.23	4.77	4.21	0.56	88.47	11.53

ing in part of earthy phosphate is obtained on warming the milk on the water-bath at 70° C. The filtrate from this product when boiled yields a delicate turbidity of coagulated proteid which becomes flocculent on addition of a slight excess of acetic acid. The filtrate from this coagulum gives only a very faint biuret reaction. Cocoanut milk is said to contain malate of lime.†

* Cocoa beer, containing 3.4 per cent. "Extractive," has been made by Calmette: Chemisches Centralblatt, 2: 394. 1894.

† Harley and Harley: Proceedings of the Royal Society of London, 43: 464. 1887–88.

On evaporation to a small volume on a water-bath the fresh milk becomes darker in color, takes on an odor characteristic of sugar syrups and looks not unlike molasses. Cane-sugar crystallizes from it in abundance on cooling.

The analytic data given on page 329 were obtained in our study of the general composition of the perfectly fresh milk of the Jamaican nuts.*

Percentage results in this connection had been obtained previously as follows:

Water.	Solids.	Organic Matter.	Inorganic Matter.	Nitrogenous Substance.	Fat.	Nitrogen-free Extractives	Carbohydrates.
91.50†	8.50	7.31	1.19	0.46	0.07	6.78	—
91.37‡	8.63	7.50	1.13	0.38	0.11	—	7.01§

These results were obtained with milk from nuts grown in the eastern hemisphere. The milk from the Jamaican nuts appears, as we have seen, to contain less solid matter, both organic and inorganic. This difference is emphasized by Hammerbacher's¶ observations on the specific gravity of cocoa-milk. He describes the milk as a colorless, slightly opalescent fluid with a specific gravity at 20° C. of 1044.** The milk from two nuts weighed 303.95 grams. From the nitrogen-free extractive substance in 77.8 grams of milk contained in a third nut, 0.8504 gram of dextrose was obtained. When milk was warmed with dilute sulphuric acid an odor of volatile fatty acid became perceptible. A crystalline barium salt was prepared from the distillate of the acidified milk which was found to consist of barium propionate.

The milk contains a small amount of diastatic ferment and also oxidase.†† We were unable to detect any other enzymes.

The following results were obtained by van Slyke‡‡ in his comparative studies of the milk of six *unripe* nuts and of one ripe one:

* The methods of analysis used for this and similar purposes, throughout our work, were those commonly employed in the laboratory. See Vandegrift and Gies: American Journal of Physiology, 5: 287. 1901.

† Hammerbacher: Landwirtschaftlichen Versuchs-Stationen, etc., 18: 472. 1875.

‡ König: Menschlichen Nahrungs- und Genussmittel, etc., 2: 308. 1893. See also Bizio: Pharmaceutisches Centralblatt, 756. 1833.

§ Including 4.42 per cent of cane-sugar. See page 328.

¶ Hammerbacher, *loc. cit.*

** See our large number of determinations of specific gravity on page 327. Also references on pages 328 and 329.

†† Hunger: Journal of the Society of Chemical Industry, 20: 1030. 1901.

‡‡ Van Slyke: Chemisches Centralblatt, 1: 595. 1891. Compare with results on page 329.

Constituents.	Milk of unripe Nuts.							Milk of ripe Nut.
	1	2	3	4	5	6	Average 1–6.	
Weight in grams. . .	230.5	378.6	347.0	383.7	350.0	330.0	336.6	109.6
Specific gravity . . .	1,024.6	1,023.0	1,022.3	1,023.0	1,022.1	1,021.5	1,022.8	1,044.0
Water (per cent.)	94.37	94.48	94.59	94.89	95.27	96.43	95.01	91.23
Total solid matter (%)	5.63	5.52	5.41	5.11	4.73	3.57	4.99	8.77
Inorganic substance.	0.575	0.635	0.675	0.611	0.658	0.602	0.626	1.06
Glucose	4.58	3.83	3.45	4.06	4.36	3.56	3.97	Trace.
Cane-sugar.	Trace	Trace.	Trace.	Trace.	Trace.	Trace.	Trace.	4.42
"Albuminoid" . .	0.120	0.126	0.114	0.205	0.140	0 095	0.133	0.291
Fat . .	0.084	0.100	0.138	0 131	0.145	0.120	0.120	0.145

The chief chemical differences induced by growth, as indicated by the above results, are an increase in the proportion of solid matter, including ash, fat and nitrogenous substance. Glucose almost entirely disappears from the milk of the ripe nut, cane-sugar replacing it—a fact evidencing synthetic production of disaccharide from monosaccharide.

Hammerbacher, believing that the endosperm develops directly from the milk, determined the quantitative relationships of the saline matters contained in each part from the same nut. He gives the following as his percentage results:

	Ash of the Milk.	Ash of the Endosperm.*
Potassium,	55.200	43.882
Sodium,	0.728	8.392
Calcium,	3.679	4 628
Magnesium,	6.606	9.438
Chlorine,	10.373	13.419
Phosphoric acid,	20.510	16.992
Sulphuric acid,	5.235	5.091
Silicic acid,	——	0.500
	102.331	102.342
Minus oxygen for chlorine,	2.338	3.024
	99.993	99.318

The above results indicate a particular increase of the content of sodium chloride in the ash of the developing endosperm and a corresponding decrease of potassium phosphate. See pages 322 and 335. The amount of silicic acid in the endosperm is also noteworthy. See page 335.

ENDOSPERM. *General Composition.*—The pure white kernel

* Compare with results of Bachofeu's analysis, given on page 335. Our own results were the same as these qualitatively. See also Schaedler, Technologie der Fette und Oele des Pflanzen- und Thierreichs, 840, 1892, who found 3.60 per cent. of iron in the ash of the endosperm in addition to the above constituents.

or "meat" of the nut is fibrous in structure, closely lines the shell, is from 1 to 2 cm. thick, and contains a very large proportion of fat. It is the part used most frequently for dietetic purposes. It possesses a characteristic and pleasant odor and is very agreeable to the taste. The endosperm cells do not contain starch granules, but fat needles and proteid lumps are present in them. The proteid particles are partly crystalline.*

After the kernel has been finely divided in a meat chopper, the resultant hash may be subjected to increasing pressure, when an

GENERAL COMPOSITION OF THE ENDOSPERM

No.	Endosperm used.	Percentage of fresh Endosperm.				Percentage of Solids.	
	Grams.	Water.	Solid Matter.			Organic Matter.	Inorganic Matter.
			Total.	Organic.	Inorganic.		
1–*a*	8.467	47.70	52.30	51.19	1.11	97.88	2.12
b	9.728	42.10	57.90	56.79	1.11	98.09	1.91
c	10.900	46.60	53.40	52.34	1.06	98.01	1.99
2–*a*	11.885	48.31	51.69	50.65	1.04	98.01	1.99
b	12.151	48.90	51.10	50.01	1.09	97.87	2.13
c	11.707	52.29	47.71	46.61	1.10	97.69	2.31
3–*a*	8.762	43.90	56.10	55.20	0.90	98.39	1.61
b	8.185	47.73	52.27	51.20	1.07	97.95	2.05
c	8.923	46.31	53.69	52.71	0.98	98.18	1.82
4–*a*	11.511	47.89	52 11	51.05	1.06	97.97	2.03
b	9.501	46.90	53.10	52.05	1.05	98.02	1.98
c	9.244	47.50	52.50	51.43	1.07	97.96	2.04
5–*a*	8.942	42.80	57.20	56.17	1.03	98.21	1.79
b	9.312	43.79	56.21	55.21	1.00	98.23	1.77
6–*a*	10.214	50.30	49.70	48.68	1.02	97.95	2.05
b	10 624	48.70	51.30	50.28	1.02	98.02	1.98
7–*a*	10.746	42.21	57.79	56.83	0.96	98.34	1.66
b	10.142	39.60	60.40	59.46	0.94	98.45	1.55
Aver.	10.052	46.31	53.69	52.66	1.03	98.07	1.93

oily juice is obtained from it. The filtrate from this turbid mixture has a higher specific gravity than the milk of the nut, is acid in reaction, reduces Fehling's solution, contains a dextrin-like body and the milk salts, gives the proteid color reactions, yields coagulable proteid, and on dilution with water becomes turbid from precipitated globulin.

The data given above were obtained for general composition of the endosperm immediately after the nuts were opened.†

* See pages 342 and 352.

† The methods were the same as those employed with the milk. The thin seed-coat was trimmed off and the pieces of kernel cut into small, thin pieces with a knife. The material was taken from all parts of the nut.

Comparison of the averages given on the opposite page may be made with the following previously recorded results for the fresh endosperm from nuts of eastern origin : *

Fresh Endosperm				Dry Endosperm	
Water.	Total Solids.	Organic Matter.	Inorganic Matter.	Organic Matter.	Inorganic Matter.
46.64	53.36	52.39	0.97	98.20	1.80

The agreement is seen to be very close.

By reason of the dietetic and commercial values of the various constituents of the endosperm of the cocoa fruit, numerous products of the kernel have been made and analyzed. The air-dried endosperm, or so-called "copra," is shipped in large quantities from the tropics. Cocoa-oil is obtained from the copra by various methods in countries distant from the tropics, the solid residues remaining after extraction serving various purposes. This residue makes up the so-called "cocoa-cake" obtained in the process of expressing the oil at various degrees of temperature. It is also ground into "cocoa-meal." In both forms, the residual substance

Products Analyzed.	Water.	Total Solids.	Organic Matter.				Inorganic Matter.
			Nitrogenous Substance.	Fat.	N-free Extractive.	Crude Fiber.	
Air-dried endosperm or copra.†	5.81	94.19	8.88	67.00	12.44	4.06	1.81
Endosperm, perfectly dried.‡	——	100.00	10.29	67.35	15.11	5.42	1.83
Endosperm, free from fat and water.‡	——	100.00	31.49	——	46.25	16.69	5.57
"Cocoa-cake." ⁒	10.30	89.70	19.70	11.00	38.70	14.40	5.90
"Cocoa meal." ‖	11.12	88.88	17.94	10.88	35.34	17.40	7.32
"Cocoa-meal," after extraction of oil. ¶	4.55	95.45	23.20	1.85	64.45		5 95

* Hammerbacher : Landwirtschaftlichen Versuchs-Stationen, etc., 18 : 472. 1875. See also Bizio : Pharmaceutisches Centralblatt, 757. 1833.

† König : Menschlichen Nahrungs- und Genussmittel, etc., 2 : 652. 1893. Also p. 308.

‡ Hammerbacher : Landwirtschaftlichen Versuchs-Stationen, etc., 18 : 472. 1875.

⁒ Dietrich und König : Zusammensetzung und Verdaulichkeit der Futtermittel; 2 : 1031. 1891.

‖ Dietrich und König : *ibid.*, 1 : 725.

¶ Schaedler : Technologie der Fette und Oele des Pflanzen- und Thierreichs, 624. 1892. (*a*) For references to digestibility and nutritive value of cocoa-cake see results of experiments on pigs and sheep given by Dietrich and König, 2 : 1031, 1036, 1040, 1123. (*b*) Compare above results with the table for general composition on the opposite page.

is used as food for cattle and as a fertilizer, having special value in both these connections.* It is sometimes also used illicitly as a food adulterant.

The analytic percentage results on page 333 have been reported by various agricultural chemists for such products from nuts grown in the eastern hemisphere.

The following summary of facts connected particularly with food value was given several years ago by Woods and Merrill: †

	Refuse.	Water.	Proteid.	Fat.	Total carbohydrates.	Ash.	Fuel value per pound; calories.
Edible portion,	——	14.1	5.7	50.6	27.9	1.7	2,986
As purchased,	48.8	7.2	2.9	25.9	14.3	.9	1,529
Without milk, as purchased,	37.3	8.9	3.6	31.7	17.5	1.0	1,872
Cocoanut milk, as purchased,	——	92.7	.4	1.5	4.6	.8	97
Shredded cocoanut,	——	4.3	6.5	63.7	24.1	1.4	
Shredded cocoanut,	——	2.8	6.0	51.0	39.0	1.2	
Edible portion,	——	5.8	8.9	67.0	16.5	1.8	
Cocoanut milk,	——	91.5	.5	.1	6.8	1.2	

Through the kindness of Dr. MacDougal we have been able to examine an account of "The cocoanut and plant vitality" in the Bulletin of the Botanical Department of Trinidad (July, 1900, p. 249). Reference is therein made to the report of Bachofeu in the Queensland Agricultural Journal for April, 1900. Bachofeu says: "Though there exist several analyses of parts of the cocoanut, no one seems to have undertaken the task of getting a complete analysis made with the view of ascertaining the actual demand made by the cocoanut upon the mineral constituents of the soil."

The results obtained by Bachofeu for a single nut are so complete, and so general in their interest and application that we quote, on page 335, his general summary in its entirety. ‡

Bachofeu's results indicate that sodium chloride and potassium phosphate are the chief inorganic matters drawn upon in the development of the cocoanut—chemical data in harmony with the fact

* v. Knieriem: Chemisches Centralblatt, 2: 672. 1898.

† Woods and Merrill: Bulletin, Maine Agricultural Experiment Station, No. 54; 81. 1899.

‡ The analyses were made in Ceylon. Native nuts were used.

that the cocoa palm does not thrive away from the coast or where salt is lacking in the soil. See second table, page 331.

BACHOFEU'S ANALYSIS OF THE COCOANUT

	Husk.	Shell.	Kernel.	Milk.
Total weight in lbs.	2.702	0.546	0.875	0.593
" " in per cent.	57.28	11.59	18.54	12.58
* Moisture in per cent.	65.56	15.20	52.80	——
* Dry matter in per cent.	34.44	84.80	47.20	——
Pure ash in per cent., containing viz :	1.63	0.29	0.79	0.38
Silica, SiO_2.	8.22	4.64	1.31	2.95
Oxide of iron and alumina, $Fe_2O_3Al_2O_3$.	0.54	1.39	0.59	Trace.
Lime, CaO.	4.14	6 26	3.10	7.43
Magnesia, MgO.	2.19	1.32	1 98	3.97
† Potash, K_2O.	30.71	45.01	45.84	8.62
Soda, Na_2O.	3.19	15.42	——	——
† Potassium chloride, KCl.	——	——	13 04	41.09
Sodium chloride, NaCl.	45.95	15.56	5.01	26.32
Phosphoric acid, P_2O_5.	1.92	4.64	20.33	5.68
Sulphuric acid, SO_3.	3.13	5.75	8.79	3.94
	100.00	99.99	99.99	100.00
† Containing total potash, K_2O.	30.71	45.01	54.05	34.54
* Containing nitrogen, N.	0.137	0.100	0.504	——

Thus of the more important ingredients of the soil 1,000 nuts remove the following :

In Lbs.	Husk.	Shell.	Kernel.	Milk.	Total Lbs.
Nitrogen, N.	3.7017	0.5460	4.4100	——	8.6577
Phosphoric acid, P_2O_5.	0.8456	0.0735	1.4053	0.1279	2.4523
Potash, K_2O.	13.5255	0.7127	3.7362	0.7783	18.7527
Lime, CaO.	1.8234	0.0991	0.2143	0.1674	2.3042
Sodium chloride, NaCl.	20.2375	0.2464	0.3563	0.5431	21.4233

Fat.—The striking chemical characteristic of the endosperm is its large content of oil. This may readily be extracted with fluids like ether. It can also be obtained in large proportion by pressure, particularly at the tropical temperatures. The fat has the consistence of butter in northern countries and possesses, when fresh, a fragrant and characteristic odor and an agreeable taste. It is snow white, sometimes cream-colored and readily crystallizes in large rosettes from the molten condition or from its alcohol or ethereal solutions. These crystals closely resemble those of palmitic acid. They melt at about 20–23° C., and congeal again several degrees below the melting point. They are fairly soluble in cold alcohol. Although cocoa-fat differs somewhat in composition in different countries, it has been found that the variations are comparatively slight. The temperature at which the oil is expressed influences

these variations by increasing or decreasing the proportion of fats melting only at higher temperatures. These facts account for the variations in the figures given for melting point. Its specific gravity is 0.9+.

On heating to about 170° C., the oil gives off the odor of lactic acid; at a temperature of 300° C. acrolein may be detected. On long-continued heating with nitric acid the following dibasic acids are formed: succinic, adipic, pimelic, suberic and azelaic. Nitrocaproic acid is also formed.* The oil is very soluble in all of the well-known fat solvents. It contains some free fatty acid, but consists chiefly of glycerides of caprylic, lauric, myristic and palmitic acids.† Glycerides of caproic and capric acids are present in appreciable quantity; also a trace of stearin and some olein.‡ The fat dissolves readily at a comparatively low temperature in an equal quantity of glacial acetic acid. Such a solution becomes turbid at 40° C.§ By reason of its content of lower fatty acid radicles cocoa-oil has a high saponification value. Cocoa-oil is particularly resistent to the hydrating effect of superheated steam.‖

The following data were obtained for the percentage fat-content in the fresh endosperm. The method of determination used was Dormeyer's:¶

	1	2	3	4	5	6	7	Gen'l Average.
Fresh endosperm, *a*.	38.27	40.01	36.71	35.10	34.60	38.90	38.60	
b.	36.14	40.54	35.02	34.90	34.10	40.70	38.40	
Average,	37.20	40 28	35.87	35.00	34.35	39.80	38.50	37.29

The ether extracts containing the oil were free from lecithin

* Schaedler: Technologie der Fette und Oele des Pflanzen- und Thierrichs, 843. 1892.

† The presence of palmitin (tri) is doubted by Ulzer: Chemisches Centralblatt, II: 1143. 1899.

‡ The so-called "cocinic acid" or "cocostearic acid" derivable from "cocin" or "cocinin" is, like the latter, a mixture. The former is a mixture of some of the above fatty acids; the latter of their glycerides. See Oudemans: Chemisches Centralblatt, 192. 1861.

§ Valenta. Quoted by Vaubel: Physikalischen und chemischen Methoden quantitativen Bestimmung organischer Verbindungen, 1: 162. 1902.

‖ Klimont: Journal of the Society of Chemical Industry, 21: 126. 1902.

¶ Dormeyer: Jahresbericht über die Fortschritte der Thier-Chemie, 26: 42. 1896. The fresh tissue was finely divided and weighed, then dried to constant weight at 100–105° C., and all of it extracted with anhydrous sulphuric ether. The usual amounts of tissue were used.

and could be almost entirely saponified. Hammerbacher * in the saponification of 25 grams of the pure oil obtained the following results:

	Grams.	Per Cent
Fatty acids convertible into *insoluble* lead salts,	15.1488	60.595
Fatty acids convertible into *soluble* lead salts,	9.5282	38.113
Glycerin,	0.5596	2.238
Total,	25.2366	100.946

The excess in weight of products is explained by the addition of hydroxyl groups in the cleavage of the triglycerides. König had previously found the glycerin content of cocoa-fat to be 2.08 per cent. Hammerbacher therefore concludes: "It follows from these results that this vegetable fat consists in greatest part of *free* fatty acid."

That there is some error in this conclusion, however, is evident from the results of later work. Benedikt † reports the glycerin content of cocoa-oil to be 13.3–14.5 per cent. Crossley and Le Suer found that the content of *free* fatty acid in terms of oleic acid varied between 2.50 and 8.86 per cent.‡

Stellwaag || studied the fat extracted from cocoa cakes. This oil was rancid, of course. He found the quantity of *free* fatty acid to be only 9.84 per cent. The fat from the ether extract melted at 23° C. The saponification figure was 244.4. The extract contained 81.14 per cent. of neutral fat. The amount of unsaponifiable matter was 0.51 per cent. The molecular weight of the fatty acids was given as 207.¶

Studied through the oleo refractometer of Amagat and Jean, cocoa-oil is found to rotate to the left like an animal fat.**

The composition of cocoa-oil as determined by König †† is:

C.	H.	O.
74.15 per cent.	11.73 per cent.	14.12 per cent.

* Hammerbacher: Landwirtschaftlichen Versuchs-Stationen, etc., 18: 472. 1875.

† Benedikt und Zsigmondy: Chemiker Zeitung, 9: 975. 1885.

‡ Crossley and Le Suer. Quoted by Hopkins: Oil-Chemists' Handbook, 38, table iv. 1900.

|| Stellwaag: Landwirtschaftlichen Versuchs-Stationen, etc., 37: 135. 1890.

¶ See also König, Menschlichen Nahrungs- und Genussmittel, etc., 2: 389. 1893.

** Blyth: Foods, Their Composition and Analysis, 359. 1896.

†† König: *loc. cit.*, 2: 385. See also, Brandes, Pharmaceutisches Centralblatt, 751. 1838.

The following facts regarding cocoa-oil have been compiled from various sources. They may be compared with similar data for other fats and oils given in the standard works of König, Staedeler, Lewkowitsch and others:

A. The heat of combustion of cocoa-oil is 9,066 small calories per gram.* It is as low as that of any other fat; slightly lower than butter. This is due to the fact that it contains a large proportion of fatty acids of low molecular weight.

B. Melting point is at 24° C. Congealing temperature is 22–23° C. Fatty acids from it melt at 24.6° C. They congeal at 19° C.†

C. Saponification value = 257.3–268.4‡

D. Iodine number = 9.0–9.5; same for its fatty acids = 8.5–9.0.§

E. Specific gravity = 0.9115 at 40° C.‖

F. Acid value = 9.95–35.21.

G. Reichert-Meissl figure = 7.4; Hehner = 88.6–90.5.

H. Barium figure (König-Hart) = 117–120.

I. Molecular weight of the mixed fatty acids = 196–211.

The use of cocoa-fat and other cheap vegetable oils as a substitute for butter among the poorer classes has been increasing. Cocoa-fat is better adapted for cooking than for table use. It is frequently employed as an adulterant of ordinary butter. Prepared cocoa-fat makes a fairly good substitute for common butter. The fresh material becomes rancid after a time, because of its accumulating content of free fatty acid resulting from bacterial agency. Volatile acids are formed. Its tendency to rancidity is not as great, however, as that of animal fats. The fatty acid present in the fat to begin with can easily be removed with insoluble basic compounds, such as magnesia. By this means

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* Merrill. Quoted by Sherman and Snell: Journal of the American Chemical Society, 23: 166. 1901.

† König: Menschlichen Nahrungs- und Genussmittel, etc., 2: 322. 1893.

‡ König: *Ibid.*

§ Benedikt. Quoted by Vaubel: Physikalischen und chemischen Methoden quantitativen Bestimmung organischer Verbindungen, 2: 235. 1902.

‖ Values given after E–I inclusive are quoted by Hopkins: Oil-Chemists' Handbook, 38, table iv. 1900. See also Lane: Journal of the Society of Chemical Industry, 20: 1033 1901.

a "butter" is made from this oil which has the merit of enduring hot climates without becoming rancid. This product has been recommended for military and naval uses.*

Among the prominent commercial products is the cocoa-butter made in Mannheim, Germany.† König‡ found this product to have the following percentage composition :

Water.	Solids.	Organic Matter.	Inorganic Matter.	Fat.	Fatty Acid.	Nitrogenous Substance.
0.15	99.85	99.848	0.002	99.848	trace.	trace.

It has been stated that cocoa-butter is not very easily digested and that it does not agree with sick people.§ The recent researches of Bourot and Jean,‖ however, show that a specially prepared cocoa-butter melting at 31° C. and containing only a trace of free fatty acid, is quite as easily and completely digested as ordinary butter.¶

We have already alluded to some of the commercial uses to which cocoa-fat is put. Soaps made from it combine with or hold an unusual amount of water while still retaining special hardness, one pound of the oil yielding three pounds of soap.** It is thus well adapted for the preparation of toilet soaps. The soaps made from cocoa-oil are characterized by great solubility in salt solution and can be precipitated from such fluid only by the addition of a very large excess of salt. The so-called "marine" or "salt water soap" has the property of dissolving as well in salt water as in fresh water and is made of cocoa-oil and soda.††

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* Rusby : Reference Handbook of the Medical Sciences, 3 : 164. 1901.

† See Leffman and Beam : Select Methods in Food Analysis, 182. 1901.

‡ König : Menschlichen Nahrungs- und Genussmittel, etc., 2 : 309. 1893. See also Schaedler, Technologie der Fette und Oele des Pflanzen- und Thierreichs, 1340. 1892.

§ Liebreich : Encyklopaedie der Therapie, 1 : 744. 1896.

‖ Bourot und Jean : Jahresbericht uber die Fortschritte der Their-Chemie, 26 : 58. 1896. See also v. Knieriem, Chemisches Centralblatt, 2 : 672. 1898.

¶ "Cocoanut cream," a dietary product much used in the tropics, is made by grating the endosperm and squeezing through cloth the fluid from the finely divided material. In a warm climate the resultant mixture contains much oil and is a very delicious accessory food. Besides the oil, the "cream" contains chiefly carbohydrate and proteid. See page 332 for references to similar fluid obtained from the endosperm by pressure in our own experiments.

** Ebermayer : Physiologische Chemie der Pflanzen, 344. 1882. See also Joss, Pharmaceutisches Centralblatt, 449. 1834.

†† See Schaedler, Technologie der Fette und Oele des Pflanzen- und Therreichs, 1178–1188, 1892, where may be found the results for percentage composition of the sodium soap, given at the bottom of the next page :

The harder fats of the oil make excellent candles. They are used also as constituents for suppositories and related therapeutic products. Medicinally the oil is employed repeatedly as a substitute for lard, olive oil and cod-liver oil. It is also made the chief substance by bulk in various salves and in cold cream, pomade and similar cosmetic preparations. In ointments and cerates it is especially valuable because of its ready absorption when rubbed on the surface of the body; further, it takes up an unusual amount of water—a useful quality when it is desired to apply saline solutions externally. It shows little tendency to produce chemical changes in substances with which it may be associated.

Crude Fiber and Carbohydrates.—Cellulose is a prominent constituent of the endosperm. Associated with the fibrous elements is a polysaccharide, present in comparatively large quantity. This substance is only slightly soluble in water, is insoluble in alcohol, but readily soluble in salt solution. It is precipitated along with globulin when saline extracts of the kernel are dialyzed (page 341). The gum is readily transformed into sugar by the action of diastase or ptyalin.

The fluid pressed from the finely divided endosperm contains a slight amount of reducing sugar—dextrose. Galactose appears to have been identified also.* Cane-sugar is also present.

The following results were obtained in our determinations of the percentage content of crude fiber in the fresh tissue: †

	1	2	3	4	5	General Average.
Fresh endosperm, *a*	3.96	3.20	2.98	3.40	2.78	
b	4.21	3.80	3.12	3.52	2.98	
Average,	4.08	3.50	3.05	3.46	2.88	3.39

Proteids.—That the meat of the cocoanut contains at most only a very small amount of proteid matter is seen at a glance from the following percentage results for content of nitrogen.‡

Water.	Fatty Acid.	Sodium Oxide (combined).	Sodium Oxide (free).	Other Salts.	Residue.
58.74	32.82	4.26	1.50	2.26	0.42

See also the Dispensatory of the United States of America, 1899: 1619, for references to objectionable chemical qualities of some cocoa-soaps.

* Green: Soluble Ferments and Fermentation, 100. 1899.

† Determinations were made, after the fresh weighed material had been dried and thoroughly extracted with ether, by the method adopted by the Association of Official Agricultural Chemists: Bulletin, Division of Chemistry, U. S. Department of Agriculture, 46: 26.

‡ In these determinations the Kjeldahl method was employed.

	1	2	3	4	5	6	7	General Average.
Fresh endosperm, *a*	0.657	0.734	0.806	0.738	0.766	0.776	0.701	
b					0.740	0.781	0.756	
Average,	0.657	0.734	0.806	0.738	0.753	0.778	0.729	0.742

The fresh endosperm contains an average of 0.74 per cent. of nitrogen which, multiplied by the usual factor, 6.25, would indicate 4.63 per cent. of "albuminoid." Some of this nitrogen, however, is undoubtedly closely associated with the fibrous elements. Much of it probably is in the form of nitrogenous extractive.* Some of the nitrogenous substance is soluble in 95 per cent. alcohol.

The proteid present in the endosperm appears to consist chiefly if not exclusively of globulin and proteose (globulose ?), the globulin predominating in quantity.† We have made several samples of cocoa globulin by the method used by Osborne for the preparation of edestin—in general as follows :‡ The kernel was run through a hashing machine and the finely minced substance freed from fat by repeated extraction in ether for several days. The ether adherent to the tissue was evaporated at room temperature and the ether-free tissue then extracted in 10-per-cent. salt solution for 24–48 hours. The saline extract was then filtered off and globulin thrown from its solution either by the dilution process, by dialyzing for several days in running water, or by treatment with ammonium sulphate to complete or half-saturation. The deposit of globulin resulting thereby always contained an appreciable amount of gummy carbohydrate. The carbohydrate admixture was eliminated by subjecting the deposit to the action of diastase or ptyalin for 24–48 hours, in the presence of thymol at 45° C. in neutral fluid, during which time it was transformed into soluble reducing sugar.§ The globulin residue left behind after this treatment was

* The factor 6.25 is here too large, also, because the proteids present contain about 18 per cent. of nitrogen. See pages 343 and 344. Stutzer found that, of the total nitrogen of cocoa-cakes, from 1.8 to 6.9 per cent. was contained in non-proteid substance. Quoted by Dietrich and König : Zusammensetzung und Verdaulichkeit der Futtermittel ; 2 : 987, 1380. 1891.

† The amount of nucleoproteid must be very slight.

‡ Osborne : See various papers in the Journal of the American Chemical Society since 1894.

§ Similar difficulty was experienced by Osborne, who got rid of the gum by repeated dialysis and precipitation with ammonium sulphate. Journal of the American Chemical Society, 17 : 429, 539. 1895.

further purified by re-solution and re-precipitation. For quantitative analysis some of the final product was washed in water, alcohol and ether, and dried at 100°–105° C. to constant weight.

Sometimes the globulin prepared in this way was both crystalline and amorphous. At other times it was entirely crystalline. Triangular, hexagonal and rhombohedral forms were frequently seen, although octahedra predominated.* The crystals so closely

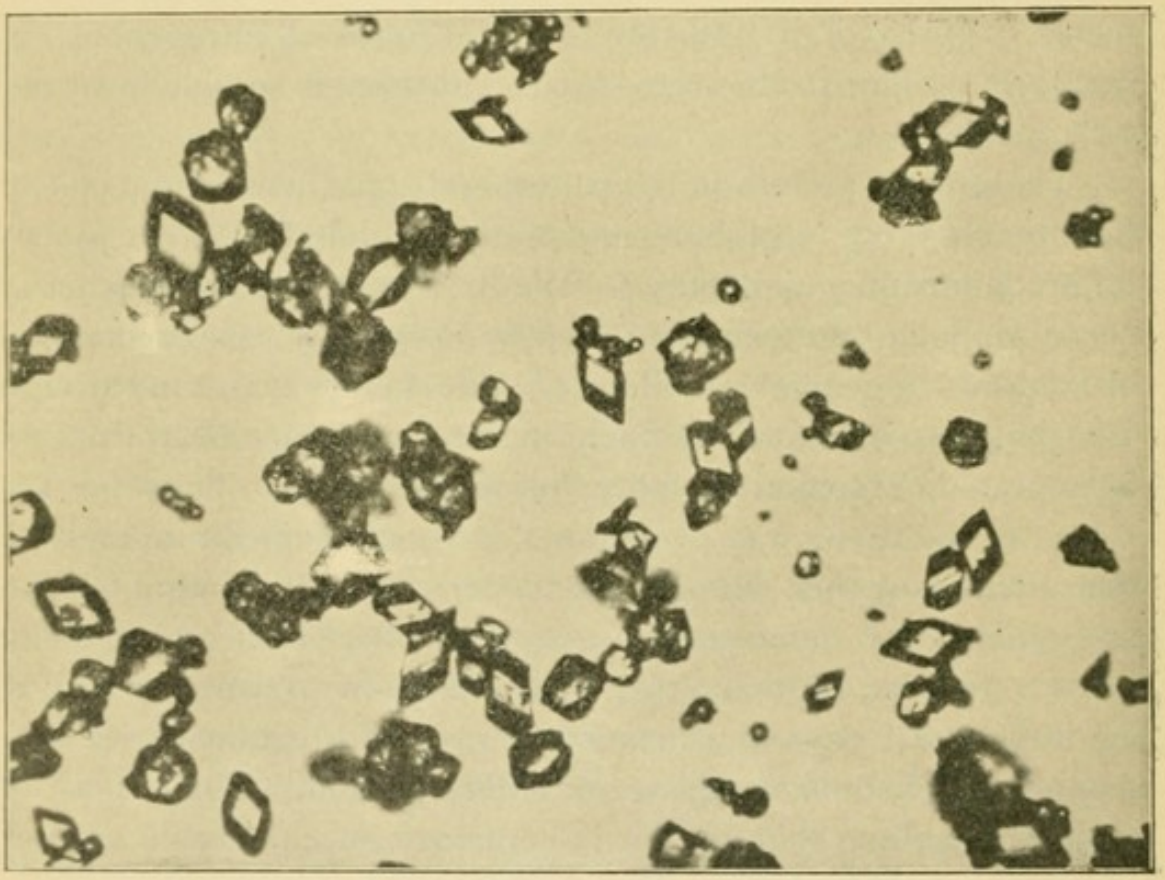


FIG. 4. Crystals of cocoa edestin.

resemble those we have repeatedly made from hempseed and linseed by the same method, and are so like those given by Osborne for edestin,† that we felt satisfied from the beginning our globulin would prove to be of the edestin type. Careful study of the reactions of the substance convinced us of this fact, for it gives all of those attributed to edestin by Osborne.

* The large proportion of gum extracted by the saline solution made it difficult not only to prepare the proteid in pure form but to obtain it quantitatively. Besides, the edestin passed in part into an insoluble modification during the manipulations. An appreciable loss resulted, therefore, in each preparation. We obtained as much as 25 grams of the purified product from the kernels of twelve nuts.

† Osborne: Journal of the American Chemical Society. See also his paper on crystalline vegetable proteids in the American Chemical Journal, 14 : 28. 1893.

On the opposite page we give a microphotographic view of edestin crystals from our second preparation. Although not the purest, we have selected this preparation for this purpose because its crystals are mostly rhombohedra. These forms rarely occur in abundance in edestin precipitates, octahedra being more commonly obtained. Most of the larger masses among the crystals shown here are "rounded" octahedra; not in perfect focus because they are thicker than the rhombohedra. The smaller particles consist of globular matter and crystal pieces.

The crystals given in *Fig. 4* were photographed for us by the writer's colleague, Dr. Edward Leaming, who cordially gave us the benefit of his large experience. We wish here again to extend to Dr. Leaming our sincere thanks for his valuable assistance.

That the substance under discussion is edestin is further shown by the results of analysis. We append our results for nitrogen content, as determined by the Kjeldahl method, calculated for ash-free substance:

PERCENTAGE OF NITROGEN IN COCOA EDESTIN

Preparation.	1	2	3	4	5
Analytic results.	17.87	17.85	17.66	18.14	18.23
	17.77	17.96	17.69	18.21	18.20
	17.79	17.91	17.78	18.18	18.28
Average.	17.81	17.91	17.71	18.18	18.24
Ash.	0.41	0.13	1.12	1.90	1.84

Preparations 1, 2 and 3 contained amorphous material, possibly some of the gummy matter referred to on page 342, in spite of our efforts to completely remove it. Preparations 4 and 5 were obtained from 1 and 3 by further treatment with diastase and by recrystallization by dialysis from 10-per-cent. salt solution. They were practically wholly crystalline.

The above results show that the globulin separated from the cocoanut by the methods here employed is edestin.*

This same proteid of the cocoanut was examined by Ritthausen, † who termed it conglutin without really identifying it with that substance. His analyses gave it a content of nitrogen of 17.87–17.91 per cent. Chittenden and Setchell ‡ referred to it by

* The edestin from barley contains 18.10 per cent. N. That from maize 18.12 per cent.; from rye, 18.19 per cent.; wheat, 18.39 per cent. Osborne: Journal of the American Chemical Society, 17: 547. 1895.

† Ritthausen: Jahresbericht über die Fortschritte der Thier-Chemie, 10: 18. 1880.

‡ Chittenden and Setchell: Quoted by Chittenden, Digestive Proteolysis, 32. 1895.

the name of phytovitellin. The composition they gave for it is in general accord with that of edestin (nitrogen content = 18.40 per cent.), and as they obtained it partly crystallized in octahedra, Osborne * has lately suggested that the substance is edestin. The results we have obtained confirm Osborne's deduction.

The proteose to which we have already alluded was obtained from the globulin filtrate. The latter was freed from traces of globulin by the coagulation method, the hot filtrate evaporated to a small bulk on the water-bath and the proteose precipitated and purified by the usual method. † About four grams were obtainable from fifteen nuts. The product contained both proto and deutero forms. Some heteroproteose was also detected in the products formed on dialysis and a trace of dysprotose was obtained.

The following results for nitrogen content in the ash-free substance were obtained by the Kjeldahl method:

PERCENTAGE OF NITROGEN IN COCOA PROTEOSE

Preparation.	1	2	3	General Average.
Analytic results.	18.67 18.50 18.58	18.48 18.46 18.40	18.57 18.61 18.54	
Average.	18.58	18.45	18.57	18.53
Ash.	1.71	1.08	1.21	1.33

These results differ only slightly from those reported by Chittenden and Setchell.‡ This difference may be explained by the fact that mixtures of proteoses have been analyzed in each case by Chittenden and Setchell, and by us. Their preparation of proteose contained 18.25 per cent. of nitrogen.

In his volume entitled Digestive Proteolysis, Chittenden gives the analytic results for eleven different proteids and the proteoses derived from them (page 67). For seven of these the nitrogen of the corresponding proteose is somewhat higher than that of the original proteid. Analysis of our own preparations has shown the percentage of nitrogen to be greater in the proteose than in the globulin, a result in accord with the majority rule just noted.

* Osborne: Journal of the American Chemical Society, 18: 13. 1896.

† MacDougal: Practical Text-book of Plant Physiology, 164. 1901.

‡ Chittenden and Setchell: Quoted by Chittenden, Digestive Proteolysis, 32. 1895.

There appeared to be only a very slight amount of an albumin in our extracts—a coagulable substance which was not precipitated from its neutral solution when the latter was half-saturated with ammonium sulphate.*

Osborne's methods of extracting glutenin and gliadin † in dilute alkali and acid, and in dilute alcohol, after the removal of globulin, proteose and albumin as above described, gave mere traces of proteid substances in solution, derivatives, doubtless, of the proteids already referred to, which perhaps had not been completely removed from the residual tissue ; or possible nucleoproteid.

Peptone could not be detected in any of our extracts.‡

Ash.—Composition is referred to on pages 331 and 335. Qualitatively our results were the same as those there given.

Enzymes.—Water, salt solution and glycerin each failed to extract appreciable quantities of either proteolytic or adipolytic enzymes from the endosperm of the fresh, ungerminated nut, although an active amylolytic ferment was extracted by all of these fluids. The large quantities of fat and fatty acid in the endosperm suggest that an emulsifying ferment may be present. This, however, may be localized in the germ, increasing to physiological quantity and activity only in the process of germination (see page 358). The proteoses present in the endosperm seem to imply the presence of a proteolytic ferment. Possibly, however, the proteoses represent a residue from which the globulin was derived by reverse process.§

We have already referred to the fact that oxidase has been detected in the milk. Traces of it are also contained in the endosperm.

Average Composition.—The average results of our analyses of the endosperm are summarized in the following table, which presents the data obtained for the composition of the fresh tissue and the dry solid matter derived from it (constant weight at 100–105° C.).

* Cohnheim : Chemie der Eiweisskörper, 150. 1900.

† Osborne and Campbell : American Chemical Journal, 15 : 392. 1893.

‡ Small quantities of non-proteid nitrogenous substances were detected by Ritthausen : Chemisches Centralblatt, 230. 1880. Compare, also, with recent results respecting proteoses obtained by Bokorny : Chemisches Centralblatt, 1 : 1167. 1902.

§ See recent papers in the Zeitschrift für physiologische Chemie by Schulze and Kutscher and their associates.

PERCENTAGE COMPOSITION OF THE ENDOSPERM

Constituents.	Fresh Endosperm.	Dry Endosperm.*
Water.	46.31	
Solids.	53.69	
Inorganic matter.	1.03	1.93
Organic matter.	52.66	98.07
Fat (substance soluble in ether).	37.29	69.45
Crude fiber (cellulose).	3.39	6.31
Proteid (N × 5.5)†	4.08	7.60
Soluble carbohydrate, non-nitrogenous substance, extractive, etc. (by difference).	7.90	14.71
Nitrogen.	0.742	1.382

The previous results obtained by Hammerbacher‡ for the fresh endosperm from nuts of eastern origin were as follows:

Water.	Solids.	Inorganic Matter.	Organic Matter.	Fat.	Crude Fiber.	Proteid.	Non-nitrogenous Extractive.
46.64	53.36	0.97	52.39	35.93	2.91	5.49	8.06

COMPOSITION OF SHELL AND HUSK.—We have already alluded to some of the uses to which the shell and husk of the cocoanut are put by reason of the chemical and physical qualities they possess. Some facts regarding their chemical composition were given in the table on page 335.

The following percentage results of elementary analysis of the powdered shell were obtained by Baumhauer;§ all samples having finally been thoroughly extracted in alcohol and ether, and then dried at 120°–150° C.:

	Cocos nucifera.				*Cocos lapidea.*	
	1. Extracted in boiling water.	2. Extracted in boiling water, dilute alkali and acetic acid.	3. Extracted in boiling water, concentrated alkali and acetic acid.	4. Extracted in alkali and in chlorine water.	5. Same treatment as 1.	6. Same treatment as 4.
C.	52.99	47.19	46.27	43.72	52.20	44.20
H.	5.88	6.09	5.81	6.11	5.80	6.24
Ash.	1.43	——	1.00	——	0.22	0.55

*According to Dietrich and König (König, Menschlichen Nahrungs- und Genussmittel, etc., 1: 612. 1893) the *air-dried* substance contains the following in percentage of the total dry weight:

Total Substance Soluble in Water.	Proteids Soluble in Water.	Sugar (Sugar-Yielding Substance).
15.16	2.27	9.25

† The factor 5.5 is used because the proteids of the endosperm contain 18 per cent. of nitrogen. See references in this connection on page 341, footnote.

‡ Hammerbacher: Landwirtschaftlichen Versuchs-Stationen, etc., 18: 472. 1875.

§ Baumhauer: Pharmaceutisches Centralblatt, 601. 1844.

Nitrogen was detected in small amount in the powders which had not been treated with alkali. The alkaline extracts contained substance, precipitable by acetic acid, with the following percentage composition: From *Cocos nucifera*, C = 50.04, H = 5.81, ash = 4.45; from *Cocos lapidea*, C = 52.15, H = 5.93, Ash = 1.00.

Tromp de Haas and Tollens * were able to show the presence of a large amount of pentosane (xylan) in the endocarp, the powdered material yielding an abundance of xylose on hydration in 4 per cent. sulphuric acid. Mannose was absent from the acid solution from which the xylose had been crystallized. After xylan had been completely removed from the shell-powder by the above method, dextrose was derived from the residue on treatment with sulphuric acid in the usual manner.

In his very complete histological studies of the cocoanut, Winton † recently called attention to the fact that both the husk and shell contain a brown substance which is quickly changed to a reddish color by caustic potash, but is unaffected by alcohol, ether or any of the specific reagents for proteids, fats or resins. He also states that no immediate effect is produced by ferric chloride solution, but on long standing the color is changed to olive green. Winton has pointed out the presence of minute silicious bodies among the fibers of the husk.

Winton, Ogden and Mitchell ‡ give the following percentage data for the composition of the shell:

Water,	7.36	Alcohol extract,	1.12
Solids,	92.64	Reducing matters calculated as	
Organic matter,	99 46	starch.	20.88
Inorganic matter,	0.54	Starch,	0.73
Soluble in water,	0.50	Crude fiber,	56.19
Insoluble in HCl,	0.00	Nitrogen,	0.18
Ether extract,	0.25	Albuminoid (N × 6 25),	1.13
Non-volatile,	0.25	Quercitannic acid,	1.82
Volatile,	0.00		

During germination the shell remains unaltered. The husk soon begins to decay. See page 351.

* Tromp de Haas and Tollens: Chemisches Centralblatt, 2: 359. 1895.

† Winton: American Journal of Science, IV. 12: 265. 1901. Facts are also given regarding the use of powdered cocoa-shell and the husk as adulterants of ground spices.

‡ Winton, Ogden and Mitchell: Report of the Connecticut Agricultural Experiment Station, 2: 210. 1898.

COCOANUT PEARLS.—Within the nut there is occasionally found a small stony substance of a bluish white color, a kind of vegetable bezoar, called in India *calappa*, which is eagerly purchased by the Chinese, who ascribe great virtues to it as a sort of amulet to preserve them from diseases. The cause of its formation in the nut is unknown.

According to Harley and Harley * these pearls, like those of molluscan origin, appear to consist almost entirely of calcium carbonate, with water and organic matter in smaller proportion. Riedel, quoted by Harley and Harley, states that in 1886, while in North Celebes, he found a pearl in the endosperm of the cocoanut. One such a pearl was pear-shaped in form and 28 mm. long.

We are greatly indebted to Dr. D. Morris, Imperial Commissioner of Agriculture for the West Indies, for the following very interesting quotation from a letter to Dr. MacDougal:

"More than two hundred years ago Rumph, an eminent botanist in the East, sent as a present to the Grand Duke of Tuscany a ring in which a cocoanut pearl had been set. Further, Rumph himself described cocoanut pearls in his great work with considerable minuteness and gave illustrations of two of them. One was perfectly round, the other was oval or egg-shaped. * * * Travelers in the Philippine Islands have heard of cocoanut pearls, but seldom or ever have seen them. The natives, it is said, keep "cocoanut stones" as charms against disease and evil spirits. The rajahs, we were told, highly prized them and wore them as precious stones. It was only a few years ago that real cocoanut pearls were at last brought to England. One is now at the Museum at the Royal Gardens at Kew, brought by Dr. Hickson. It is almost egg-shaped, perfectly white, and composed almost entirely of carbonate of lime. It has, in fact, a somewhat similar composition to the pearl of the oyster, and yet there is little doubt it is a purely vegetable product." †

* Harley and Harley: Proceedings of the Royal Society of London, 43: 464. 1887–88.

† "Besides these cocoanut pearls," quoting further from Dr. Morris' letter to Dr. MacDougal, "Rumph describes what he calls 'Melate' pearls taken from the flowers of a Jasmine; and a 'Champake' pearl taken from the flower of a Michelia. If we had not already seen the pearl of the cocoanut it would have been impossible to believe that there were such things as Jasmine and Michelia pearls * * * Of their composition, mode of occurrence and true nature we have yet to learn." See the article by Harley and Harley referred to above.

See also, on the subject of cocoanut pearls, the Proceedings of the Boston Society of Natural History, 1861 and 1862; The Tropical Agriculturalist, 1887; Nature, 1887.

III. Changes in the Cocoanut during Germination

The nuts for our studies of the changes occurring during germination were obtained fresh, fully developed and with their husks still on them, directly from Jamaica. Immediately after their arrival at the New York Botanical Garden they were imbedded in earth until they were nearly covered. The earth was kept saturated with water and a tropical temperature was maintained. These conditions closely approximated those attending normal germination.

Morphological Changes.—Nearly four months elapsed before the shoots began to appear through the husks, the fibers of the husk having been pressed aside in their upward progress. At this stage the stem of the shoot was an inch or more in diameter at the "root-crown," sharply tapering toward the point of penetration at the surface of the husk. As the growth proceeded it seemed to gradually become more and more rapid, and by the end of a year the plants had attained the height of two or three feet, with a stem about an inch in diameter throughout most of its length. By this time the part of the husk under the earth had decayed considerally; it became softer and more porous, and several stout roots had developed through it and penetrated the soil to the depth of a foot or more.*

The appearance of the nuts and their plants at this period of their growth is shown in the cut on page 350. Unless otherwise stated, the chemical analyses reported farther on were made of the parts at this stage of their development.

It may not be amiss, in describing the morphological changes induced in the nut during the process of germination, to also briefly review, at the same time, the more important facts regarding structure of the nut as it exists in the ungerminated condition.

The entire fruit is, strictly speaking, intermediate between a nut and a drupe — a "drupaceous nut." The outer covering,

* For facts regarding germination and cultivation see Bailey: Cyclopedia of American Horticulture, 1: 341–343. 1900. Also, Wittmack, L.: Die Keimung der Cocosnuss. Ber. d. deut. bot. Ges. 14: 145. 1896.



FIG. 5. Germinating cocoanut at the end of a year, showing plumule and roots, with husk little altered except where it was in contact with the earth.

usually removed before the nut appears upon the market, is a thick fibrous layer comprising the exocarp, the epicarp consisting of a smooth, thin, tough coat of a brownish or grayish color. (See pp. 323 and 324). The endocarp, or what is commonly known as the shell of the nut, is composed of three carpels whose lines of fusion are always apparent. The nut lies in the husk with the end containing the "eyes" toward the pedicel. Each carpel contains an "eye," so-called, and under one of these three eyes, the

softest, is the germ imbedded in the endosperm. The fertile carpel may be recognized from the fact that it has the greatest degree of divergence between the longitudinal fusion lines of the carpels. The true integuments of the ovule are reduced to a thin brown coat closely adhering to the abundant endosperm.

The embryo is a cylindrical body about 8 mm. in length lying below one of the natural openings of the endocarp and in a line perpendicular to the exterior surface of the endosperm. When germination begins the embryo elongates and, having pushed through its thin coverings, begins to enlarge at both ends. From the outer end arises the plumule and the roots; the inner end is an extension of the true cotyledon and is developed into a special absorbing organ. See *pl. 19*.

The absorbing organ is of a soft spongy texture and all through it are the ramifications of vascular strands which converge to the narrow "neck," which connects the absorbing tissue with the stem. The cotyledon, and by this term, hereafter, we shall mean the part of that structure specialized for absorption, can attack only the part of the endosperm to which it is contiguous. In the earlier stages of growth this absorption is confined to the part nearest the young shoot, which we may hereafter refer to as the proximal end of the nut. Finally, however, the cotyledon fills the entire cavity of the nut and somewhat thins the endosperm distally, also.

The milk may persist in the nut until the cotyledon has almost filled the cavity. After germination has proceeded for some time the milk becomes insipid to the taste, and contains fragments of cellulose and large drops of floating fat. In nuts in which germination has continued for a year the cotyledon has entirely filled the cavity, but usually there is still left a third to a half of the endosperm undigested. This residual portion in normal cases is little affected, except that it is softened superficially, and to the taste suggests nothing different from the meat of the ordinary ungerminated nut.

In its natural development the roots of the plant soon take firm hold of the soil and, long before the endosperm is completely absorbed, junction between the shoot and the absorbing organ is broken, the husk decays and the plant enters an inde-

pendent career. Neither the husk nor the shell appears to serve any other than passive mechanical function, and only the constituents of the endosperm and milk, so far as the nut is concerned, nourish the young plant before it finds in the soil the elements provided there in abundance for its growth to maturity.*

In our microscopic studies, particularly of the cotyledon, pieces of the fresh part were "fixed" in a mixture of glacial acetic acid (⅓) and 70% alcohol (⅔). After remaining in this fluid for a few hours the pieces were transferred to 70% alcohol and later to 85% alcohol, in which they were kept. Sections were cut with a razor and mounted in glycerin. Treated with iodine, such sections of the cotyledon showed an abundance of starch in all cells except those of the outermost layer or epidermis. This outer layer stained yellow with iodine. That it contained an abundance of oil was shown by its deep black reaction with osmic acid. Large globules of oil are, however, distinctly visible in the epidermis under the microscope without the aid of osmic acid. Oil may also be found in the subepidermal layers, but it rapidly diminishes in quantity as the distance from the outside increases and as the starchy deposit accumulates. Needle-like crystals may be very readily found in the epidermal cells. These resemble crystals of tripalmitin, but the fact that so much oil appears in globules, and that the breaking down of fats must occur to a large extent in these cells, would suggest that they are palmitic acid rather than the fat itself.

The above facts make it appear that the starch is formed indirectly at least from the oil.† See references under enzymes, pages 345 and 358.

See *pl. 19* for drawings of parts mentioned above.

CHEMICAL CHANGES.—The following summary gives briefly the effects of germination on the individual nuts examined:

I. *Not Analyzed.*—A. Development had proceeded for nearly six months. The plumule protruded six inches above the husk. Roots had developed through the husk—two were about a quarter of an inch in diameter. The stem was very thick at the "root-

* Note references on page 357 to the functions of the husk in holding water and possibly furnishing nutrient matter in its decay.

† Ebermayer: Physiologische Chemie der Pflanzen, 347. 1882.

crown" ; sharply tapered to the point of surface penetration. The fibers about the stem were tightly pressed together. The neck of the absorbing organ was very woody and fibrous in character. Absorption of the endosperm at the proximal end was quite marked; distal portion undiminished. Milk cavity largely filled by the absorbing organ. A small space at the distal end remained, containing viscid white material full of large oil globules; quantity less than 10 c.c.—doubtless concentrated milk. It was strongly acid in reaction from acid phosphate, reduced Fehling's solution, gave only a faint biuret reaction and was free from starch. The inner surface of the endosperm in the distal portion was soft, having the consistency of lard.

The weight of the whole shoot, minus the roots, in the fresh condition was 28.1 grams. Dry, the weight was 4.25 grams or 15.1 per cent. of the fresh substance, indicating a presence of 84.9 per cent. of water in the original plant.

B. This nut, although germinating for the same period of time, was not quite as far advanced as the previous one, having shoots that were just emerging from the husk. The stem was thicker, however. In most respects its internal condition was exactly the same as that of the first. The fluid in the distal cavity was less in quantity, not as turbid, contained less oil—otherwise was the same as that of the previous nut.

The weight of the entire plumule was 38.8 grams. Dried, it weighed 5.75 grams. Thus it contained 14.8 per cent. of solid matter and 85.2 per cent. of water.

II. *Analyzed.*—1. Growth continued for eight months. The cotyledon entirely filled the cavity. About half of the endosperm was absorbed; practically all of that proximally except a thin layer. The distal residue of endosperm appeared to be normal in taste and appearance except on the surface, where the soft layer previously referred to—one fourth the entire thickness—could again be seen. The outer surface of the cotyledon, that part in contact with the endosperm, was much corrugated; the whole organ, solid but spongy, sweet and agreeable to the taste, pyriform. In the tables on page 354 and 355 the results of our analyses for this nut are indicated by the numeral 1.

GENERAL COMPOSITION OF THE PARTS OF THE GERMINATED COCOANUT

Parts of the Nut and its Plant.	Percentage of fresh Tissue.				Percentage of Solids.	
	Water.	Solid Matter.			Organic Matter.	Inorganic Matter.
		Total.	Organic.	Inorganic.		
I. *Cotyledon.*						
A. Central, vascular portion: *a*—1	89.10	10.90	99.14	0.86	92.07	7.93
" " " *b*—1	87.71	12.29	99.10	0.90	92.25	7.75
" " " *c*—2	91.62	8.38	99.00	1.00	88.10	11.90
" " " *d*—2	91.41	8.59	99.11	0.89	89.71	10.29
" " " *e*—3	88.99	11.01	99.20	0.80	92.77	7.23
Central, between center and surface: *f*—3	86.07	13.93	98.94	1.06	92.36	7.64
Average.	**89.15**	**10.85**	**99.08**	**0.92**	**91.21**	**8.79**
B. Outer, corrugated portion: *a*—1	84.95	15.05	98.69	1.31	91.30	8.70
" " " *b*—2	82.79	17.21	98.93	1.07	93.85	6.15
" " " *c*—3	80.83	19.17	98.26	1.74	90.93	9.07
Outer portion—neck: *d*—3	78.98	21.02	98.58	1.42	93.24	6.76
Average.	**81.89**	**18.11**	**98.62**	**1.38**	**92.33**	**7.67**
II. *Residual endosperm.*						
C. Proximal portions after much absorption: *a*—2	19.09	80.91	99.13	0.87	98.92	1.08
b—3	23.42	76.58	99.16	0.84	98.90	1.10
Average.	**21.25**	**78.75**	**99.15**	**0.85**	**98.91**	**1.09**
D. Medial portions: *a*—1	31.65	68.35	99.03	0.97	98.58	1.42
b—1	30.36	69.64	99.06	0.94	98.65	1.35
c—2	28.68	71.32	99.25	0.75	98.95	1.05
d—3	25.77	74.23	99.23	0.77	98.96	1.04
Average.	**29.12**	**70.88**	**99.14**	**0.86**	**98.78**	**1.22**
E. Distal, normal portion: *a*—3	46.08	53.92	99.02	0.98	98.12	1.82
III. *Stem of the plant.*						
F. Base, "root crown," with petioles at lowest parts: *a*—1	86.21	13.79	98.95	1.05	92.37	7.63
b—1	86.51	13.49	98.70	1.30	90.38	9.62
c—2	85.15	14.85	98.84	1.16	92.06	7.94
d—3	84.31	15.69	98.68	1.32	91.60	8.40
Average.	**85.55**	**14.45**	**98.79**	**1.21**	**91.60**	**8.40**
G. Parts above the base, with more petioles: *a*—2	82.47	17.53	98.80	1.20	93.20	6.08
b—3	79.87	20.13				
Average.	**81.17**	**18.83**				
IV. *Petioles.*						
H. Alone, or with young leaves: *a*—1	83.63	16.37	98.57	1.43	91.27	8.73
b—1	82.55	17.45	98.63	1.37	92.15	7.85
c—3	82.13	17.87	98.75	1.25	93.01	6.99
d—3	82.17	17.83	98.63	1.37	92.34	7.66
Average.	**82.62**	**17.38**	**98.65**	**1.35**	**92.19**	**7.81**

GENERAL COMPOSITION OF THE PARTS OF THE GERMINATED COCOANUT.—*Continued*

Parts of the Nut and its Plant.		Percentage of fresh Tissue.				Percentage of Solids.	
		Water.	Solid Matter.			Organic Matter.	Inorganic Matter.
			Total.	Organic.	Inorganic.		
V. *Leaves.*							
I. Mature or nearly so :	*a*—1	74.66	25.34	98.35	1.65	93.49	6.51
	b—1	71.99	28.01	98.10	1.90	93.20	6.80
	c—2	72.60	27.40	98.34	1.66	93.93	6.07
	d—2	72.51	27.49	98.41	1.59	94.19	5.81
	e—3	68.45	31.55	97.96	2.04	93.52	6.48
	f—3	70.65	29.35	98.39	1.61	94.52	5.48
Average.		71.81	28.19	98.26	1.74	93.81	6.19
J. Very youngest :	*a*—3	87.22	12.78	98.50	1.50	86.67	13.33
VI. *Roots.*							
K. Short, not developed outside of husk, with soft tips :	*a*—1	87.08	12.92	98.46	1.54	88.09	11.91
	b—2	89 89	10.11	98.67	1.33	86.89	13.11
	c—2	86.41	13.59	98.43	1.57	88.44	11.56
	d—3	87.46	12.54	98.83	1.17	90.70	9.30
Average.		87.71	12.29	98.60	1.40	88.53	11.47
L. Parts of longer roots, taken near the stem :	*a*—1	77.92	22.08	98.80	1.20	94.59	5.41
	b—2	82.65	17.35	98.85	1.15	93.34	6.66
	c—3	81.09	18.91	98.50	1.50	92.09	7.91
	d—3	79.47	20.53	98.77	1.23	94.00	6.00
Average.		80.28	19.72	98.73	1.27	93.51	6.49
M. Parts of longer roots, taken outside the husk :	*a*—1	81.70	18.30	97.94	2.06	88.76	11.24
	b—1	84.64	15.36	98.47	1.53	90.05	9.95
	c—2	82.79	17.21	98.40	1.60	90.74	9.26
Average.		83.04	6.96	98.27	1.73	89.85	10.15

2. This nut represented germination after ten months and was essentially like the preceding in all respects. The roots were thicker and a number of good sized ones had not yet proceeded through the husk. Their ends were soft and watery, rounded and blunt. Analyses of this nut are referred to in the tables on pages 354–355 by the numeral 2.

3. Essentially the same as No. 2, both in stage of development and conditions of parts, although the time of germination was about two months longer. Analyses of the parts of this nut are referred to in the tables on pages 354–355 by the numeral 3.

4. This nut had germinated for just about a year. The follow-

ing weights of the fresh parts were very carefully taken; the data for nitrogen were determined by the Kjeldahl method.*

	Weight in Grams.	Percentage of Total Weight.	Percentage of Nitrogen.
Cotyledon,	196	28.1	
Central part,			0.14
Cortical layer,			0.31
Endosperm,	155	22.2	
Distal portion,			0.65
Proximal portion,			0.93
Shell,	161	23.0	
Stem,	16	2.3	
Lower part,			0.70
"Root crown,"			0.53
Roots,	41	5.9	
Inside of husk,			0.27
Outside of husk,			0.54
Petioles and young leaves,	58	8.3	0.29
Petioles,			0.39
Old leaves and petioles,	71	10.2	0 45
TOTAL WEIGHT,	698		
Shell and contents,	512	73.4	
Whole plant,	186	26.6	
Cotyledon and endosperm,	351	50.3	

The tables on pages 354–355 give all our results for general composition of the parts of the germinated nut.† Numerous deductions may be drawn from these results, as to growth and metabolism.

The central part of the cotyledon, with its vascular network, contains more water and less solid matter than any other part of the germinated nut. The proportion of solid substance in it increases toward the corrugated epithelium, being greatest in the "neck," where the structure is fibrous and woody.

That the absorbing organ completely takes up the milk is very evident from the way it fills the cavity and from its own composition, but it is likewise apparent from our results that water is also with-

* The husk was not weighed because it was decayed underneath and water-logged. Nitrogen was not determined in the shell because its substance remains unaltered during germination.

† The methods of determination were the same as those used previously. The roots, outside of the husk, which had been in the wet soil, were hurriedly rinsed with water to remove inorganic matter, then wiped dry with a towel and at once cut into thin cross sections for analysis.

drawn from the residual endosperm, this absorption being greatest at the proximal end of the nut, where absorption was begun in the first place, and least at the distal end, where it had hardly commenced. The roots also are seen to have absorbed considerable moisture.

The lowest part of the stem contains almost as little solid matter and is nearly as watery as the cotyledon. The percentage of water in the stem diminishes as the distance away from the "root crown" increases. The watery condition of the lower part of the stem is increased, doubtless, by the fact that the surrounding husk is impregnated with water, thus favoring direct absorption by osmosis and at the same time preventing evaporation from the surface of the growing tissue.

The amount of solid matter in the petioles is also comparatively slight, little more than in the lower part of the stem. In the leaves the water is greatest in the youngest, as would be expected; least in the oldest — those most exposed to the air.

The roots at the tips are soft and watery, but the older they become the more solid matter they develop and the more woody material they accumulate.

In the distribution of the inorganic matter in the fresh parts it is noticeable that the proportion of saline substance increases with a decrease of water and *vice versa*, as in the cotyledon, in the residual endosperm and throughout the plumule. This condition is such as might be expected. The relation of the inorganic to the organic matter in each part, however, is variable. The substance of the cotyledon and the stem contains a greater proportion of salts than that of the endosperm and the leaves, the roots likewise holding a fairly large amount of saline matter. The substance of the endosperm contains least of all, from which fact it is quite clear that the inorganic matter of the plumule has been absorbed, not only by the cotyledon from the milk, but also by the roots from the fluid in the husk and the surrounding earth.

At the beginning of germination the inorganic matter and water of the milk are doubtless sufficient for the changes that occur, the organic matter coming chiefly from the endosperm. Some time before the cotyledon fills the milk cavity and completely absorbs the milk, the roots have begun to take water and inorganic

matter from the fluid in the husk—possibly also organic substance from the disintegrating husk fibers—and thus they absorb new nourishment from a large supply. Growth of the plumule is consequently favored. The plumule soon reaches such a height and development as to enable it to make increasing contributions to the plant metabolism from the gaseous products the air affords. By this time the whole growth has become practically independent of the reserve material of the seed.

ENZYMES.—We made only a few preliminary studies of enzyme distribution. Extracts were made in water, dilute salt solution and glycerin. The indicators used in nearly all the experiments were prepared from the materials in the nut itself.

The extracts of the cotyledon were acid to litmus (phosphates), though, as indicated by lacmoid, they contained no free acid. Diastatic ferment was found to be distributed in abundance in all parts of the cotyledon. Oxidase was also present. Only the very slightest proteolytic action was manifested by the cotyledon extracts, even when they were obtained in particularly concentrated form. In some experiments the results were entirely negative, however. Cellulose-dissolving and fat-splitting enzymes were not detected in either the cotyledon or the residual endosperm, although we cannot be sure that in our few experiments they have not escaped us.* Germination progresses so slowly that possibly some of the enzymes are present in only very minute quantity at any one time—in such amount, perhaps, as to be undiscoverable by the methods commonly employed for ferment detection. We did not examine the parts of the plumule in this connection.

At this point, before we were able to come to any very definite conclusions as to the enzymes present and before we could determine the distribution of proteids, fats, carbohydrates, etc., in the parts of the plant, we were obliged to discontinue our work. The writer hopes to extend these experiments on the germinated cocoanut to a consideration of related problems of nutrition.

* See our references to enzymes on page 345. Lipase seems to have been found in the germinating cocoanut by Lumia: Jahresbericht über die Fortschritte der Thier-Chemie, 28: 724. 1898.

Explanation of Plate 19

FIG. 1. An end view of the cocoanut, without its husk, showing the three carpels and the "eyes." The fertile carpel is the one included in the largest angle.

FIG. 2. A sectional view through the end of an ungerminated nut, with its husk removed, showing the form and location of the germ imbedded in the endosperm (under the micropyle). The shell is indicated by the heavy outline.

FIG. 3. Another sectional view similar to that of Fig. 2 showing development of the absorbing organ after germination had proceeded for a few weeks. The incipient stem and roots are to be seen. The cotyledon has enlarged within and without the shell.

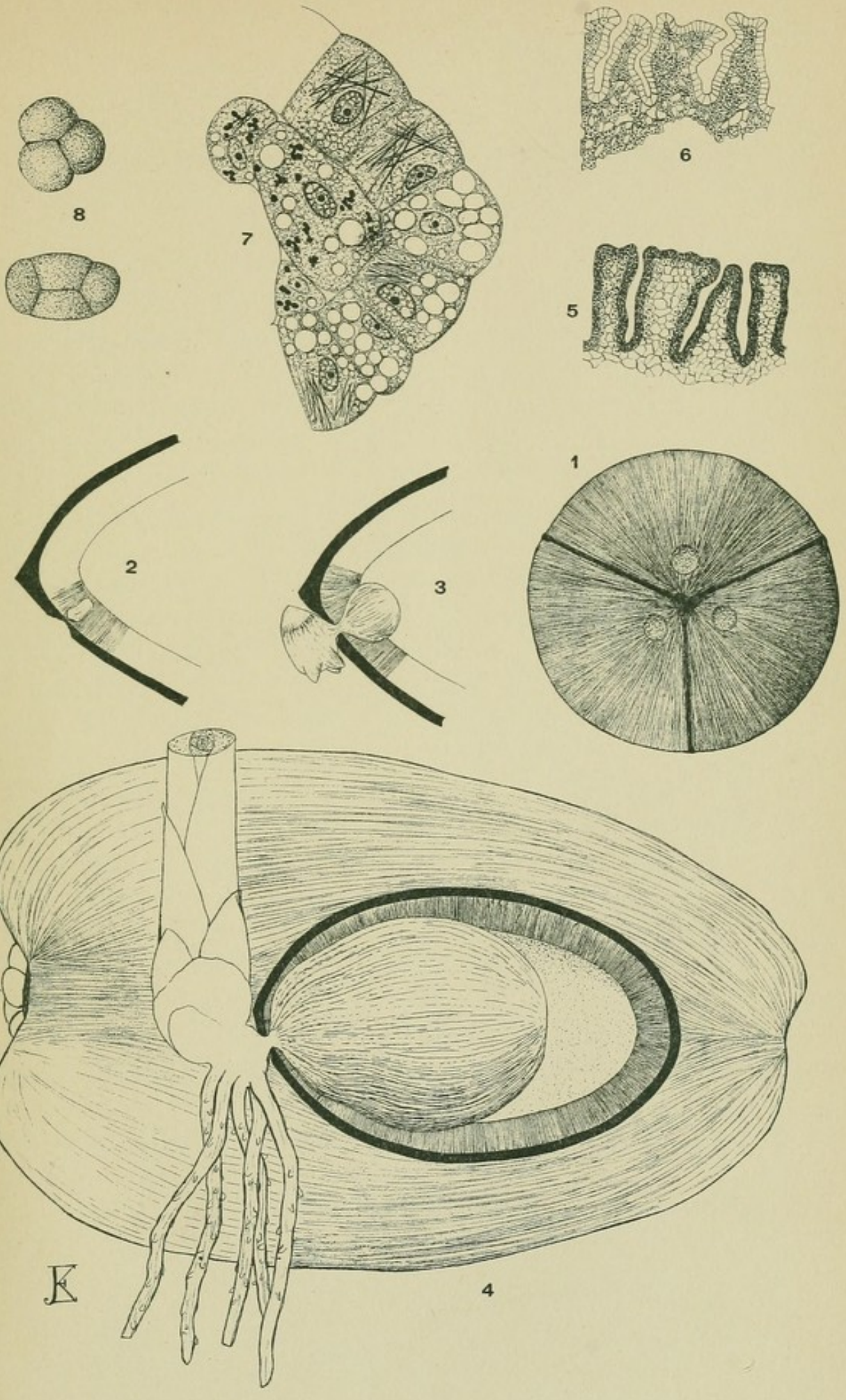
FIG. 4. A longitudinal section through the whole nut somewhat to the side of the median line. It shows the nut imbedded in its fibrous husk and the conditions found after germination had gone on for about five months. The absorbing organ has filled about two thirds of the cavity of the nut but has absorbed comparatively little of the endosperm. The stem has proceeded upward through the husk, the roots downward through the husk into the soil. The leaves have been cut off above the husk. (See page 350 for appearance of full plumule.)

FIG. 5. A section through the layer of the cotyledon normally in contact with endosperm, showing the corrugated, villiform arrangement of the absorbing epithelium. This section having been treated with osmic acid shows the localization of the fat globules.

FIG. 6. A section through that part of the cotyledon given in Fig. 5. This section, treated with iodine, shows the localization of starch.

FIG. 7. A few cells from the absorbing epithelium of the cotyledon after enlargement. This figure shows the large clear oil globules and the darkly staining starch grains in the subepidermal cells. The crystals seen in most of the cells appear to consist of fatty acid, possibly palmitic.

FIG. 8. Enlarged starch granules from subepidermal cells.



COCOS NUCIFERA.

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On the Physiological Action of some of the Heavy Metals in Mixed Solutions*

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It has been shown by several investigators that, in mixed solutions containing the lighter metals, the physiological action of the electro-positive elements may in a degree antagonize each other, and a mixture of several toxic solutions of these compounds may be much less harmful than any one of the constituents taken singly. Researches concerning the physiology of sea-water have shown this to be conspicuously the case. Work on soil-solutions by Kearney and Cameron † has developed a similar situation.

The neutralizing action of the various ions on those of the heavy metals has been less studied and the present paper presents, in a preliminary way, results gained from a series of experiments performed during the summer of 1902 in the Plant Physiological Laboratory at Wood's Hole, Massachusetts.

As a test object, the primary radicle of seedlings of *Lupinus albus* was used. The method of procedure consisted essentially in suspending the seedlings on glass rods for at least 24 hours in each experiment, in such a manner as to immerse the radicles in the solution under study. In most cases the seedlings were under observation for 48 hours. At least four seedlings were used in each experiment of a series, and our deductions were drawn from the figures for average growth. With only a very few exceptions each member of the quartette manifested the same tendency. The solutions were carefully prepared from pure chemicals and were believed to be very accurate. The experiments were made in beakers.

In order to get a basis for comparison, we made a number of solutions of different compounds of each of the heavy metals used,

* Read by title at the meeting of the Botanical Society of America, held at Washington, D. C., December, 1902.

† Kearney, T. H., and Cameron, F. K. Some Mutual Relations between Alkali Soils and Vegetation. U. S. Dep. Agric. Rep. No. 71. 1902.

and determined the strongest concentration in which the plants were able to make appreciable growth. This point of undoubted growth furnished a point of departure in making comparisons. The

TABLE I

SIMPLE SOLUTIONS.* HEAVY METALS. AVERAGE GROWTH-RATES IN MILLIMETERS†

Conc. Gm. Mol.	$CuSO_4$	$CuCl_2$	$Cu(CH_3CO_2)_2$	$AgNO_3$	$HgCl_2$	$ZnSO_4$
m/8192					2.0	6.0
m/16384	0.5	1.0	1.0		9.0	9.0
m/32768	1.0	1.0	2.0		13.0	18.0
m/65536	3.5	3.0	3.0	2.0	16.0	18.0
m/131072	11.0	10.5	11.0	6.0		16.0
m/262144		16.0	15.0	13.5		
m/524288				22.0		
Check.	14.0	14.0	12.0	12.5	15.0	15.5

* All control experiments in this and subsequent series were made in water which had been very carefully distilled several times. All of the solutions were prepared from distilled water obtained under constant conditions.

† The growth-rates recorded in these tables are those for the first 24 hours.

accompanying tables give the average growth-rates at the end of twenty-four hours.

Table I summarizes the growth-rates obtained in a series of solutions of salts of copper, silver, mercury and zinc. In order

TABLE II

SIMPLE SOLUTIONS. LIGHT METALS AND UREA. AVERAGE GROWTH-RATES IN MILLIMETERS

Conc. Gm. Mol.	NaCl	Na_2SO_4	KCl	KNO_3	$Ca(NO_3)_2$	$CaCl_2$	$CaSO_4$	$MgCl_2$	Urea
m/8						1.5			0.5
m/16	2.5					9.6			1.0
m/32	3.5	1.3	0.7	3.4	20.0	16.0			3.5
m/64	7.5	1.0	2.0	4.0	20.5		35.0		8.0
m/128	13.5	2.8	6.6	8.0	21.0				
m/256		5.8	7.0	9.0	21.0				
m/512								5.5	
m/1024								6.2	
m/2048								9.4	
m/4096								12.5	
Check.	15.5	10.0	10.0	14.0	10.0	12.5	13.0	10.0	15.2

to test the possible antagonizing influence of compounds with other bases, a number of salts of sodium, potassium, calcium and magnesium were used.

A similar point indicating the maximum concentration per-

mitting growth was obtained for each of the salts of the lighter metals taken. This was necessary in order to get some idea of the permissible concentrations in which the latter might be used. Table II presents in brief the growth-rates made by the lupine roots in solutions of the salts of the second group of metals (and of urea).

SIMPLE MIXTURES

Knowing now the effect, on the growth-rate, of the heavy metals under study, also that of the salts of the lighter metals which were made use of, we have a basis for ascertaining the action of these compounds when their solutions are mixed. The method of procedure in this connection consisted briefly in mixing, with a series of graded solutions of the heavy metals, a definite quantity of the salts of the lighter metals. Two general classes of salts could be chosen for this purpose; first, those in which a common anion occurs in combination with the different cations made use of; second, salts in which also the anions differed. By making up solutions molecularly, as was done in every case, a comparison of results obtained from the same cations, combined in the two ways indicated, would enable us to judge of the action of the anions.

Table III summarizes growth-results obtained by growing lupine radicles in a series of solutions of copper salts mixed with salts of one lighter base in varying degrees of concentration. The growth-rates obtained are directly comparable to those resulting from the action of the simple solutions of the heavy metals. It will be noted that when copper sulphate is mixed with calcium sulphate, the latter containing $m/144$ grams per liter, a corresponding growth-rate is found in the case of the simple solution (Table II) at a concentration of copper sulphate indicated by $m/65536$, and in the case of the mixed solution (Table III) at a concentration of $m/16384$. These facts indicate that the presence of the given amount of calcium sulphate enables the plant to withstand four times as much copper as it was able to withstand when the latter occurred in simple solution. A further inspection of the tables shows that when calcium sulphate is present in weaker solutions, the antagonizing action is still strong, even when calcium sulphate is present in a concentration of $m/512$.

When to copper chloride, magnesium chloride is added in a

series of graded concentrations, an inspection of the tables shows that in general little, if any, diminution in the toxic action of the copper follows. This seems to indicate that magnesium is not able to diminish the poisonous action of the copper under the conditions present.

When to copper chloride, calcium chloride is added in the proportion of *m*/128, a decided decrease in the killing power of the copper is seen. When to copper chloride, sodium chloride, *m*/16, is added, a strikingly reversed situation appears. Not only is the harmful action of the copper not diminished, but the mixture seems to be slightly more poisonous than the simple copper solution or

TABLE III

MIXED SOLUTIONS. HEAVY METAL AND LIGHT METAL. COMMON ANIONS. AVERAGE GROWTH-RATES IN MILLIMETERS

Concent. of Solution of Heavy Metal	$CuSO_4 + CaSO_4$			$CuCl_2 + MgCl_2$			$CuCl_2$ + $CaCl_2$	$CuCl_2$ + NaCl	$AgNO_3$ + KNO_3	$HgCl_2$ + $CaCl_2$	$ZnSO_4$ + $CaSO_4$
	$CaSO_4$ *m*/144	$CaSO_4$ *m*/256	$CaSO_4$ *m*/512	$MgCl_2$ *m*/128	$MgCl_2$ *m*/256	$MgCl_2$ *m*/512	*m*/128	*m*/16	*m*/256		*m*/256
m/2048		0.0	0.0								33.5
m/4096	0.0	0.0	0.0							0.0	39.0
m/8192	0.0	0.0	1.0					0.0		0.0	37.5
m/16384	3.0	3.0	1.5	1.0	2.0	2.0	2.0	0.0		1.0	37.0
m/32768	8.5	7.0	3.5	1.0	3.0	1.0	6.0	0.0	1.0	2.0	
m/65536	14.0	17.0	14.0	4.0	4.0	3.0	10.5	0.5	3.0	8.0	
m/131072		19.5				5.0	15.0	1.0	5.0		
Check in water	12.0	11.0	11.0	10.0	14.0	12.0	14.5	12.0	11.5	12.0	13.0
Check in Solution of Light Metal	29.0	26.5	26.0	4.0	9.0	4.5	20.0	1.0		24.5	35.0

the simple sodium solution. This would seem to indicate that to the poisonous action of the copper that of the sodium chloride itself is added.

When to a solution of silver nitrate a solution of potassium nitrate, *m*/256, is added, no very marked change in the action of the heavy metal is noticeable, the growth-rate coinciding approximately with that seen in the solution of the pure silver salt. What difference there is seems to be in the direction of greater toxicity.

When to mercuric chloride, calcium chloride, *m*/256, is added

no diminution in the poisonous action of the corrosive sublimate is seen. Indeed, the mixture is markedly more poisonous than the solution of the simple salt. Zinc sulphate gives a very different result when calcium sulphate, *m*/256, is added. Whereas growth is much retarded in a *m*/8192 solution of zinc sulphate, in the mixture at *m*/2048 growth is more than twice as rapid as in the control grown in water. We have here a very marked stimulation in the growth-rate, resulting from the addition of the lighter metal to the zinc.

When to salts of the heavy metals compounds of the lighter

TABLE IV

MIXED SOLUTIONS. HEAVY METAL AND LIGHT METAL. DIFFERENT ANIONS. AVERAGE GROWTH-RATES IN MILLIMETERS

Concent. of Solution of Heavy Metal	$CuSO_4$ + $CaCl_2$ *m*/128	$CuSO_4$ + $CaCl_2$ *m*/128(dup.)	$Cu(CH_3CO_2)_2$ + $Ca(NO_3)_2$ *m*/32	$HgCl_2$ + $Ca(NO_3)_2$ *m*/32	$CuSO_4$ + Urea *m*/64
m/4096				0.0	
m/8192			2.0	1.0	
m/16384	1.5	3.0	5.0	6.0	
m/32768	3.0	4.0	9.5	16.0	0.0
m/65536	7.0	10.0			0.5
m/131072		16.5			4.5
Check	12.0	15.5	13.0	14.0	11.5

metals are added, in the form of salts in which the anion differs from that in the copper compound, a condition of things is found which is not essentially different from that just cited. In Table IV results bearing on this point are presented.

It will be noted that when calcium chloride, *m*/128, is added

TABLE V

MIXED SOLUTION. COPPER SULPHATE WITH CANE SUGAR. AVERAGE GROWTH-RATES IN MILLIMETERS

Concent. of Copper Solution	Cane Sugar 2*m*	Cane Sugar *m*	Cane Sugar *m*/2	Cane Sugar *m*/4	Cane Sugar *m*/8	Cane Sugar *m*/16	Check in Water
m/65536	0.0	2.7	4.0	7.5	5.5	6.5	15.5

to copper sulphate, a marked diminution in the poisonous action of the copper compound takes place to a degree equal to that seen when the anions are similar. When to copper acetate cal-

cium nitrate, $m/164$, is added, a similar situation results. In the case of solutions of mercuric chloride to which calcium nitrate has been added, we find no amelioration of the poisonous action of the corrosive sublimate, the growth being, if anything, less in the mixed solution than in that of the mercuric chloride alone.

In view of what has been said, the question naturally arises as to the effect of non-electrolytes in solution with the heavy metals. In this connection but two compounds were studied: cane sugar and urea. Tables IV and V present the results obtained. It will be seen that in a solution of copper sulphate, $m/65536$, to which cane sugar in concentrations varying from m to $m/16$ has been added, the growth-rate in general increases as the concentration of cane sugar diminishes. The growth-rate is markedly greater in the solution of copper sulphate containing cane sugar varying in concentration from $m/4$ to $m/16$ than in the copper solution alone. This growth-rate was not a persistent feature, however, since in all the mixtures except that containing cane sugar at the least concentration, $m/16$, no growth took place in the second twenty-four hours. It appears, therefore, that when cane sugar is added in proper proportions, as in these experiments, the poisonous action of copper is somewhat diminished. This is probably due to the formation of copper saccharate and a consequent lessening of the number of Cu ions.*

As regards the effect of the addition of urea, $m/64$, it appears that the inhibiting action of the mixed solution is greater than that of the simple copper salt, the addition of the urea seeming to increase the total poisonous action.

COMPLEX MIXTURES

In order further to test the effect of additions of lighter metals to salts of the more poisonous elements, more complicated syntheses were made. These were of two classes: one mixed solution in which all of the salts present had a common anion; a second mixed solution in which the anion of the salt of the heavy metal did not appear in any of the compounds of the lighter

* See Loeb, J., and Gies, W. J. Weitere Untersuchungen über die entgiftenden Ionenwirkungen und die Rolle der Werthigkeit der Kationen bei diesen Vorgängen. Archiv für die ges. Physiologie, 93: 261. 1902.

metals. Mixtures were made in which, in addition to a copper salt, salts of sodium, magnesium, calcium and potassium, successively, were added. In every case, Ca excepted, the concentration in which each compound was present was that which, while distinctly retarding growth, still permitted it. We have, therefore, in every case, a salt entering into the combination in a concentration sufficiently great to be a distinctly toxic agent. The concentrations and other data in this connection appear in Table VI.

It will be noted in each case that the copper salt permits a slight growth. When the sodium salt is added, the mixture be-

TABLE VI

COMPLEX MIXTURES OF ONE HEAVY METAL AND AN INCREASING NUMBER OF LIGHTER METALS. AVERAGE GROWTH-RATES IN MILLIMETERS

With common anions									With different anions		
(*a*) Contents of Solutions		Av. Growth 24 hrs.	(*b*) Contents of Solutions		Av. Growth 24 hrs	(*c*) Contents of Solutions		Av. Growth 24 hrs.	Contents of Solutions		Av. Growth 24 hrs.
	mol			mol			mol			mol	
$CuCl_2$	65536	5.5	$CuCl_2$	32768	1.0	$CuCl_2$	65536	2.5	$Cu(CH_3.CO_2)_2$	65536	8.0
$CuCl_2$	65536		$CuCl_2$	32768		$CuCl_2$	65536		$Cu(CH_3.CO_2)_2$	65536	
NaCl	64	2.5	NaCl	64	1.5	NaCl	128	1.0	Na_2SO_4	128	3.0
$CuCl_2$	65536		$CuCl_2$	32768		$CuCl_2$	65536		$Cu(CH_3.CO_2)_2$	65536	
NaCl	64		NaCl	64		NaCl	128		Na_2SO_4	128	
$MgCl_2$	512	7.0	$MgCl_2$	512	2.5	$MgCl_2$	1024	6.0	$MgCl_2$	512	8.0
$CuCl_2$	65536		$CuCl_2$	32768		$CuCl_2$	65536		$Cu(CH_3.CO_2)_2$	65536	
NaCl	64		NaCl	64		NaCl	128		Na_2SO_4	128	
$MgCl_2$	512		$MgCl_2$	512		$MgCl_2$	1024		$MgCl_2$	512	
$CaCl_2$	32	14.5	$CaCl_2$	32	7.0	$CaCl_2$	64	9.0	$CaCl_2$	32	17.0
$CuCl_2$	65536		$CuCl_2$	32768		$CuCl_2$	65536		$Cu(CH_3.CO_2)_2$	65536	
NaCl	64		NaCl	64		NaCl	128		Na_2SO_4	128	
$MgCl_2$	512		$MgCl_2$	512		$MgCl_2$	1024		$MgCl_2$	512	
$CaCl_2$	32		$CaCl_2$	32		$CaCl_2$	64		$CaCl_2$	32	
KCl	128	19.0	KCl	128	5.5	KCl	256	10.0	KNO_3	128	22.0
Check		13.5			10.5			10.5			13.0

comes somewhat more harmful than the copper salt alone. The addition of magnesium to the mixture raises the growth-rate to a point beyond that reached in the copper solution, indicating a slightly beneficial antagonistic effect. When to these the calcium salt is added, the growth-rate immediately assumes practically normal proportions. This neutralizing or antitoxic effect of the calcium is very marked. When to the combination just referred

to the potassium salt is added, the growth-rate is still further increased. In the last mixture we have five salts, each, with the exception of the calcium compound, in a concentration strong enough to interfere distinctly with growth. As a result of their presence together, not only is there no addition of poisonous effects, but a neutralization of toxicity to such degree as to permit in the mixed solution a growth-rate equal to or greater than that seen in the check culture.

When the concentration of copper solution was doubled and the concentration of the other salts left as before, we found that the action of the copper was more slowly overcome, and even in the most complex mixtures studied, the growth-rate was still below that of the check. Apparently, the poisonous activity of the copper in these cases was greater than such as could be neutralized by the quantities of other salts added to it. When, on the other hand, the concentration of the copper solution was kept as in the first instance and the concentration of the lighter salts added was diminished by half, the neutralizing action of the latter was markedly less. In the most complex mixtures under these latter conditions the observed growth-rate only equaled that of the control. Apparently this fact was due to the unneutralized copper action, since each of the other salts present were below a harmful concentration.

Returning, now, to mixtures in which the anion of the copper salt is not duplicated in any of the other salts present, we see a result essentially like that just noted. When to copper acetate, for example, salts of the metals used before are added in quantities equal to those indicated in Table VI, a similar result is seen. The growth-rate in the pure copper salt in this case is somewhat greater, since the $CH_3.CO_2$ anion is slightly less poisonous than the Cl or the SO_4 anion. The addition of the sodium salt again increases the toxicity of the mixture. The further addition of the magnesium salt diminishes the harmful action somewhat, the activity of the mixture being, roughly, the same as that of copper acetate alone. The entrance of the calcium salt, as before, produces a marked acceleration of growth, the rate jumping to a point considerably above the control. The final addition of the potassium salt still further increases this stimulation. As a result of this ex-

periment it appears that it is immaterial here, as before, whether the lighter metals enter the solution in compounds containing an anion common to that of the heavy metal, or whether the anions be different.

DISCUSSION OF RESULTS

From the evidence at hand in these experiments it appears that, in solutions of salts, the conspicuously effective component of the molecule is the cation or the metal. This presumption, raised by the similar physiological effects produced by the cation of various salts of the heavy metals in equimolecular quantities, is strengthened by the action of mixtures containing a salt of the heavy metal with salts of lighter metals.

In case several salts having the same cation are mixed in solution the same lack of conspicuous influence on growth on the part of the anion is to be seen.* It is clear that the effect exerted upon the lupine roots by the salts of the heavy metals tested, differed according to the concentration of the salts. When sufficiently diluted, solutions containing copper, silver, mercury or zinc ions exerted a more or less clearly marked *stimulating* effect on growth. At a greater concentration, perhaps double that causing stimulation, a retarding influence was usually seen, and in a concentration approximately doubling this, growth was much interfered with; and on again doubling the concentration, little or no growth took place.

The effect of adding solutions containing Ca, Mg or Na ions was seen to vary with the character of the cation introduced. In mixtures containing but two salts (Tables III and VI) sodium seemed to show an increased poisonous action as though that of the sodium were added to that of the cation of the heavy metal. When to a solution of copper, a salt of magnesium was added, the mixture seemed to act with nearly the same intensity as the simple solution containing the copper in like quantity, exerting, therefore, little influence on the poisonous activity of the copper. When calcium was added, a marked reduction of the poisonous activity of copper ions was observed, a result seen even more strikingly in

* The physiological action of every dissociated salt in solution is doubtless an expression of the resultant biological effect of its component cations and anions. In these experiments the influence of the cations was predominant.

the case of zinc. Investigations by Swingle,* Clark,† Rumm and others on the action of Bordeaux mixture, although concerning very different proportions from those here involved, all testify to this power of calcium to neutralize the poisonous action of copper. Whereas the presence of calcium reduced the harmful effects of the copper to about one fourth of that seen in the simple copper solution, the antagonizing action of the calcium reduced the toxic action of the zinc to, at most, one sixteenth of that of the simple zinc solution. In the case of silver, the addition of calcium seemed to exert no ameliorating action. As far as the evidence at hand goes, it appears that such ameliorating action as was observed and would be expected stands in an inverse relation to the poisonous activity of the heavy metal.

From the above, as well as from the work of others, it appears conclusive that certain cations in mixed solutions exert a physiological action antagonistic to that exerted by other cations. The question next arises as to the nature of this modification and its seat. Does a mixed solution of calcium sulphate and copper sulphate or copper chloride, for example, produce the change (which brings about this physiological result) by affecting the condition of the copper in the solution outside of the cell, or does it bring about modifications within the cell itself? Is this antagonism an extracellular chemical change or an intracellular physiological change?

We have two sorts of cases to deal with. In the one case the salts have a common anion and in the other case the anions differ. We may set aside any such changes as the formation of double salts or the setting back of molecular ionization in the former case, since it has been shown that like results are seen when the mixed salts have common anions. This would seem to be a probable situation from *a priori* reasoning also, since in most cases the solutions of the salts of the heavy metals were so dilute that practically complete ionization took place. In that event, no matter what its associated anion was, the metal acted as free ions.

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* Swingle, W. T. Bordeaux Mixture. U. S. Dep. Agric. Div. Veg. Path. and Physiol. Bull. No. 9. 1896.

† Clark, J. F. The Toxic Properties of some Copper Compounds with special reference to Bordeaux Mixture. Bot. Gaz. 33 : 26. 1902.

In the cases of our mixtures of salts having different anions, chemical reactions might be regarded as possible, with a consequent change in the forms of molecules. Here again, however, the great dilution of the salt of the heavy metal in our most important mixtures produced complete or nearly complete ionization, the heavy metal acting practically as free ions. We can then hardly regard changes of an ordinary chemical nature as being responsible for the differences in the physiological results. We think that interior physiological modifications are responsible for the observed differences in growth rate. This belief implies that the simple salt of the heavy metal and the mixture of this salt with that of a lighter metal, after penetration into the cell, affect the processes there being carried on in such a way as to bring about different results on cellular growth. In studying the effect on growth of simple solutions of copper and calcium salts, for example, we see that at the concentrations employed copper retards growth whereas the calcium salts greatly stimulate it. With each we have, in all probability, to do with antagonistic phases of physiological action. When we examine the results in cases like the above, it seems highly probable that the so-called antitoxic action of ions is due to different interior physiological modifications, and that the growth-rate observed in such experiments as these represents the physiological sum of oppositely acting stimuli, or of antagonistic protoplasmic changes.*

It has been shown that when salts of heavy metals are sufficiently dilute they exert a *stimulating* effect on growth, and when solutions of calcium and similar salts are concentrated enough, they hinder or entirely prevent growth, and may, in the case of the more soluble chloride and nitrate, prove fatal. Coupin † has shown that at different dilutions compounds exhibit *three distinct phases of physiological action.* When the solution is sufficiently dilute it seems too attenuated to produce any effect on growth. As the concentration increases, a *stimulating* phase is seen, which, on further concentration, passes over into the *retardation* phase—pronounced in proportion to the concentration.

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* Loeb and Gies, *l. c.*, 267.

† Coupin, H. Sur la toxicité du chlorure de sodium et de l'eau de mer e l'égard des végétaux. Rev. Gén. Bot. 10: 177. 1898.

In the mixtures of copper and calcium employed in our experiments, we may have had concentrations of each salt in different phases of action due to the degree of concentration. In $CuCl_2$, $m/65536$, we see that the Cu concentration is in the phase hindering growth, the resulting elongation of the root being about 3 mm. When $CaCl_2$, $m/128$, was tested, it was found to be in a concentration markedly stimulating when referred to the control in water, growing 20 mm. in the former case, against 14.5 mm. in the check. These opposite tendencies were brought together in the mixed solution with the result that the concentration (in terms of the copper salt permitting the growth-rate seen in the simple copper solution) moved up to approximately four times that observed in the simple copper solution. The stimulating action of the calcium seems to have operated against the retarding action of the copper, and the result is a marked diminution in the poisonous action of the copper.

The opposite result is seen in the mixture containing $CuCl_2$ and NaCl. The latter is in its growth-retarding phase until more dilute than $m/128$. Hence at $m/16$ it is in its growth-retarding phase, and when added to $CuCl_2$ at $m/65536$, likewise in this phase, the result is a sum of toxicity and an increased depression of growth-rate follows the combined action of the two. This also applies to the mixtures containing magnesium.

In considering the more complex mixtures of salts, indicated in Table VI, the chemical nature and influences of the resulting solutions are not readily determined. Much more concentrated solutions result in such mixtures with consequent decrease in dissociation. The probability that we are dealing with various kinds of non-ionized molecules, as well as with an indefinite number of ions, makes it impossible to speak definitely with confidence of the significance of our results in this connection. In general one may say that here, as in sea water, another complicated mixture of molecules and ions, the entrance of the calcium salt into the mixture is the stage in the synthesis at which the growth-rate approaches that seen in the check, and the final addition of the potassium salt seems further to increase the growth-rate. Or, in other words, all the salt solutions except the calcium entered the mixture in a concentration at which singly they would cause a

retardation of the growth-rate without bringing it to a standstill. Of the compounds present, therefore, the calcium salt only entered in a concentration representing the stimulus phase. The marked effect following the entrance of the calcium and the potassium may, in part, be due in these experiments to the cumulative increase of concentration of the solution, with the corresponding decrease in the rate of ionization and the diminished number of active ions. The fact that the potassium salt, although added in a concentration hindering growth when taken singly, increased the growth-rate when added to the mixture as its last member, seems to strengthen this supposition. Of course, changes of this nature represent changes in the solution itself and lie outside of the cell, and should not be confused with the mutually antagonistic intracellular action of ions in the case of very dilute solutions.

Reprinted from the Journal of the New York Botanical Garden, 1902, iii, p. 169.

ON THE NUTRITIVE VALUE AND SOME OF THE ECONOMIC USES OF THE COCOANUT.

By William J. Gies.

Few if any vegetable products furnish so many useful articles as the cocoanut. It forms the chief food of the inhabitants of most tropical coasts and islands, where the kernel is not only eaten in the ripe and unripe conditions, but is also prepared and served in various ways. It forms an accessory part of the diet, and is found in many of the confections of civilized man all over the globe. The milk is considered an agreeable cooling beverage in the tropics, although it is diuretic in its effect, and causes irritation of the mucous membrane of the bladder and urethra when taken too freely. Immoderate use of the fruit is said to cause rheumatic and other diseases.

Experiments recently published in the Bulletin of the Torrey Botanical Club by Professor Kirkwood and the writer (see Garden Contribution No. 26), conducted in part in this Garden and with the coöperation of Dr. MacDougal, indicate that the nutritive value of the endosperm of the cocoanut resides mainly in its high content of oil and moderate amount of carbohydrate. Of the former, the fresh endosperm contains 35–40 per cent.; of the latter, approximately 10 per cent. The amount of proteid is very slight, being little more than 3 per cent. The quantity of inorganic matter is 1 per cent. The water amounts to nearly 50 per cent. The chief constituent of the milk, aside from water (95 per cent.), is sugar, nearly all of the solid matter being thus composed, as the very sweet taste amply testifies. Various alcoholic beverages have been made from fermented cocoanut milk.

The endosperm is very agreeable to the taste, and, with the exception of the cellulose (3 per cent.), is readily digestible. Domestic animals eat it eagerly, and the cocoanut crab feeds on it almost exclusively. The residue left over after the fat has been expressed from the "copra" is widely used in Europe as food for cattle; also as fertilizer.

The use of cocoa fat as a substitute for butter among the poorer classes has been increasing, and it is frequently employed as a butter adulterant. The tendency of cocoa fat to rancidity is not as great as that of animal fats, and for this reason "butters" made from it keep well, and have been recommended especially for military and naval uses. Recent researches show that "cocoa butter" is quite as agreeable to the taste, and as easily and completely digested, as ordinary butter. Its heat of combustion is 9.066 small calories per gram.

"Cocoanut cream," a dietary product much used in the tropics, is made by grating the endosperm and squeezing through cloth the fluid from the finely divided material. In a warm climate the resultant mixture contains much oil and is a very delicious accessory food. Besides the oil, the "cream" contains chiefly carbohydrate and proteid.

Soaps made from cocoa oil combine with, or hold an unusual amount of water, while retaining special hardness, and are characterized by great solubility in salt solution. The so-called "marine" or "salt-water" soap has the property of dissolving as well in salt water as in fresh water. The harder fats of the oil make excellent candles. Cocoanut oil and resin melted together yield a mixture capable of being used with success in filling up the seams of boats and ships, and in tropical countries for covering the corks of bottles as a protection against the depredations of the white ant.

The fibrous husk (coir) is widely used for the construction of ropes, brushes, bags, matting, etc. The hard shell is easily polished and lends itself to the formation of various utensils and ornaments. It also has a high fuel value. The powdered shell and husk are occasionally used as adulterants of ground spices.

The milk of the nut, as has already been pointed out, is strongly diuretic. The endosperm shares with the milk the property of a *taenicide*, and has been used as a vermifuge in India for many years, where it is regarded as an excellent means of expelling the flat worm. The harder fats of the oil are used as constituents for suppositories and related therapeutic products. Medicinally the oil is employed repeatedly as a substitute for lard, olive oil and cod-liver oil. It is also made the chief substance by

bulk in various salves and cold cream, pomade and similar cosmetic preparations. In ointments and cerates it is especially valuable because of its ready absorption when rubbed on the surface of the body, and on account of its ability to hold an unusual amount of water or saline fluid. It shows little tendency to produce chemical changes in substances with which it may be associated.

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Reprinted from the Yale Scientific Monthly, 1898, iv, p. 204.

ON THE DECOMPOSITION AND SYNTHESIS OF PROTEIDS IN LIVING PLANTS.*

By William J. Gies.

Few substances are so widely distributed in nature as proteids and certainly none are of more consequence from a biological point of view. The tissues of all plants and animals contain these substances in large proportion, and of the invariable organic constituents of every living, functionally active cell the albuminous are undoubtedly the most important. That proteids are probably the most complex compounds with which the chemist has to deal, and therefore, also, the most elusive in chemical research, are deductions to which the experiences of all investigators seem to point conclusively. In spite of the fact, however, that they have long been the subjects of persistent and carefully conducted chemical investigation, our knowledge of their molecular arrangement still remains decidedly indefinite and all attempts thus far completely to unravel the constitution of the proteid molecule have resulted negatively. Each of the various theories which have been proposed in regard to structural formulae depends entirely upon the products obtained in proteid decompositions, since all of the numerous attempts to prepare albuminous material artificially have invariably resulted in failure. Since the decomposition products of proteid matter are so multitudinous and, under different conditions so various, it is not at all difficult to comprehend why the biological chemist is so much in the dark as to the real configuration of the albumin molecule and why, in the total absence of data afforded by artificial synthesis, he is able to form only hypotheses as to the manner in which these analytic residues are held in the undecomposed substance.

Although proteid matter has never been prepared in the laboratory from any of its decomposition products, its synthesis is con-

* Being an abstract of a paper upon this subject recently presented before the Chemical Club of Yale University.

stantly taking place in plants and, to a certain extent, in animals as well. The ultimate origin of proteids may be traced to the vegetable kingdom, however, for plants are constantly transforming inorganic into albuminous matter, as a part of the process of their development, whereas in animal metabolism, albuminous syntheses are wholly dependent upon proteid residues assimilated after digestion of the food. Consequently, plants are preëminently the producers in the biological economy, whereas animals are essentially the consumers, and in view of the power of the vegetable cells to build up the simple elements into the organic matter upon which animal life is either directly or indirectly dependent, the transformations taking place in plants must ever be matters of prime interest to all chemists and biologists.

In recent years considerable attention has been given to quantitative determinations of the chemical changes taking place in various species of living plants, with results adding very materially to our knowledge in this relation. Preëminent among those who have attempted to solve the problems of the proteid transformations in growing plants is Professor E. Schulze of Zürich, who for nearly thirty years has been steadily at work upon various phases of the physiological chemistry of vegetation. Most of the experiments conducted by him to ascertain directly the facts in regard to proteid decompositions and syntheses were carried out upon carefully developed seedlings. In these, of course, the transformations of the reserve material of the seeds could be accurately followed and the facts learned by a very simple as well as accurate method — one, also, which obviates the great difficulties encountered in an investigation of the changes occurring in larger vegetable forms and which, besides, does away with the complications arising from the extraneous influences attending the development of plants under normal conditions and in the usual environment. Consequently, the results in regard to proteid transformations obtained by this method must necessarily be referred to the matter originally stored up in the seeds, since the utilization of all other nitrogenous substances is carefully precluded, and thus a more satisfactory idea of the steps of cleavage and construction is afforded than could possibly be derived from a study of ordinary plants under perfectly normal conditions.

Of the most important nitrogenous compounds, aside from the proteids, commonly found in plants, glutamin, arginin, phenylalanin and vernin were discovered by Schulze and his pupils.* Tyrosin, amido-valerianic acid, allantoin and guanidin, though long known to be constituents of the animal body as products of proteid catabolism, were first found in the vegetable kingdom by Schulze † and those working under him, and were separated from seedlings in which considerable transformation of albuminous material had taken place. Leucin was first isolated from plants by Gorup-Besanez ‡ and Borodin § long since demonstrated that asparagin is widely distributed as a plant constituent. The latter substance occurs most abundantly ; the etiolated seedlings from one species of lupin seeds, for example, having been found by Schulze to contain as much as 28 per cent. of the crystalline compound, calculated in terms of the dry plant substance. Glutamin and arginin are sometimes the predominating crystalline constituents in seedlings, though they rarely mount higher in quantity than from 2 to 3 per cent. of the dry tissue. The amido acids may be separated from practically all seedlings, although as a rule in only comparatively small quantities.

That these important nitrogenous constituents of growing plants bear a very close relation to those formed by hydrolytic cleavage of proteids will be seen at a glance. Thus glutamin and asparagin are the amides of glutamic and aspartic acids. Both of the latter may be readily formed from proteids by hydrolysis. Tyrosin, leucin, amido-valerianic acid and arginin may be prepared without any special difficulty in artificial digestion of proteids. By the action of mineral acids vernin readily decomposes into guanin, a substance which is found in the animal boyd and is closely related to uric acid. Arginin may be broken down into urea in the laboratory and guanidin is the imide of urea, the chief end product of proteid catabolism is the human organism. Allantoin breaks down into urea and may be formed from uric acid, both of which bodies are common products of proteid catabolism in the animal system. When albuminous substances are

* Schulze. Zeitschr. f. physiol. Chem., 1897, xxiv, p. 18 et seq.

† Schulze. *Ibid.*

‡ Gorup-Besanez. Berichte der Deutsch. chem. Gesellschaft, vol. vii, p. 146.

§ Borodin. Botan. Zeitschr., Nos. 51 and 52.

decomposed with nitric acid, phenylalanin is among the resultant products.

It should not be inferred, however, from the comparisons just drawn, that the plant constituents named above are to be looked upon as decomposition products only. Whilst there is abundant evidence that they ensue as a result of cleavage of the plant porteids, and, as has just been shown, afford a suggestive analogy with the products formed in the hydrolysis of albuminous substances, both in the animal organism and in the chemical laboratory, some of them appear to play important parts in constructive processes as well. The similarity just noted certainly offers direct evidence in favor of the view to which modern research has been steadily leading us, that, with reference to general chemical and nutritional processes, there is not so much difference between plants and animals as has been supposed. Not only are there lower plants, entirely free from chlorophyll, which, so far as chemical processes are concerned, represent intermediate steps between higher plants and animals, but the differences existing between the higher plants and animals themselves are more of a quantitative than a qualitative kind. In the animal organisms the processes of oxidation and cleavage prevail, whilst in the plant those of reduction and synthesis predominate.

This apparent similarity in the general nutritional processes of animals and plants has indeed been the guiding influence in recent investigations of the physiological chemistry of vegetation and perhaps much of our information in this connection is partly based upon deductions drawn almost wholly from such analogy. Thus Prof. Schulze, who has long been an acknowledged authority in this particular domain of biochemical science, assumes that the preliminary metabolic changes taking place in plants are dependent in large part upon the action of unorganized ferments. This theory, for the reason just suggested, seems so thoroughly plausible that it has come to be generally regarded as an expression of absolute fact and is readily accepted in explanation of the transformations of proteids, for example, because the chief nitrogenous plant constituents, exclusive of the albuminous compounds, are closely related to, and in part are identical with, those formed by the animal enzymes during proteolysis in the alimentary

canal. Although this view seems very probable, and consequently may be accepted provisionally, it must not be forgotten, nevertheless, that exact knowledge of the occurrence and distribution of enzymes in plants is decidedly limited and that whilst such ferments as diastase, myrosin, papain, bromelin and others are known to occur — all of them being comparable in their activities to the various enzymes of the alimentary tract — the results of experiment and research have afforded little insight into their real relation to the changes going on within plants.

As has just been indicated, Schulze's conclusions rest upon the assumption that seeds and plants contain enzymes capable of transforming the vegetable proteids into crystalline nitrogenous products, and occurring in sufficient quantity, besides to make them permanent as well as important agents in the plant nutritional processes in general. The hydrolytic action of these agents is considered to account only for the catabolic products, however, so that they are the essential factors in proteid decomposition. According, then, to Schulze's view of the proteid transformations in growing plants, the decompositions are practically the same in character and manner as those taking place in the animal organism. In the animal, proteids, as is well known, are broken down by hydrolysis in the alimentary canal into proteoses and peptones, which products may subsequently be converted into amido acids of the fatty and aromatic series, organic bases, ammonia, hydrogen sulphide and other bodies. Of the organic bases formed, lysin and arginin are among the more important. Plants also contain, as constant products derived in presumably the same general manner, amido acids of both the fatty and aromatic series and also arginin, and in them, likewise, the initial products of hydrolysis appear to be proteoses and peptones. In both plants and animals the sulphur of the decomposed proteid is eventually found for the most part in salts of sulphuric acid.

Schulze has found that asparagin, glutamin and arginin accumulate in seedlings in consequence of proteid transformations and that they unquestionably result partly from albuminous decomposition on the one hand, partly as products of synthesis on the other. His data indicate that this is true also of tyrosin, leucin and the other amido acids already mentioned, and that the proc-

esses of decomposition and synthesis in relation to plant proteids in general go hand in hand. In all of these instances enzyme action is held responsible for the cleavages.

This investigator has carried out many experiments to determine the relative occurence, in the different parts of germinating plants, of the various nitrogenous extractives and has obtained very interesting results. His work indicates a very unequal distribution of the metabolic products. Thus, experimenting with etiolated seedlings rich in asparagin, such as those from lupin seeds, he observed that in the cotyledons, in which of course the decomposing reserve proteid was contained, the proportion of asparagin to the other non-proteid nitrogenous compounds was much less than in the hypocotyl, and he looks upon this accumulation of asparagin in the developing stem as part of the evidence in favor of the view he entertains that asparagin is built up from nitrogenous residues resulting from proteid decomposition and that it is directly concerned in synthetic operations. In another series of experiments of essentially the same character, the figures obtained in quantitative estimations also tend to confirm the view that asparagin is built up at the expense of non-proteid nitrogenous substances, for in these instances, whilst the proportion of proteid matter was diminished very little, the percentage of non-proteid nitrogenous matter aside from asparagin was greatly reduced, whereas the latter substance was relatively greatly increased. Similar results were obtained in connection with glutamin.

Quantitative experimental results with reference to the distribution of the different substances throughout the seedlings suggest, further, that the crystalline nitrogenous bodies such as asparagin and tyrosin are in part secondary products of the proteid cleavages and that the primary are proteoses and peptones. The same data indicate, also, that both the primary and secondary products arising during germination are, or may be in part at least, broken down eventually into very simple substances, and that the resultant nitrogenous residues, probably ammonium salts or perhaps ammonia itself, together with others, are built up, first into asparagin, glutamin and related compounds, and then into proteids. It seems entirely probable, further, that the proteid regenerated in this way is soon subjected to the usual decomposition

and that this cycle of analysis and synthesis proceeds continually as the plant develops. According to this theory, then, the observed accumulation of both asparagin and glutamin is due in great part, if not entirely, to a synthesis of the ultimate decomposition products of the plant proteids.

Kinoshita and Suzuki * have found that in plant metabolism asparagin may be synthesized from ammonia and non-nitrogenous organic substances. Schulze has separated ammonium salts from fresh germinating plants and, as has just been pointed out, is convinced that ammonia is an important factor in proteid synthesis. It is well known that ammonia appears here and there in different animal tissues and fluids, and considerable significance has recently been attached to this fact. Ammonia under these circumstances must have been formed as a result of proteid catabolism. Moreover, physiological investigation has clearly shown that bodies such as urea are frequently the results of the synthesis of simpler decomposition products. Thus in the formation of urea from ammonium carbonate in the body — a process well known to occur — we have a type of this same synthetic decomposition, once more obtaining proof of the similarity of the qualitative chemical reactions in plants and animals. Here again analogy with events in the animal system may be taken to strengthen deductions with reference to facts in vegetable chemistry.

Of what use to the plant, it may be asked, is this transformation of simple nitrogenous substances into asparagin and glutamin? Hansteen's † recent work suggests the answer. This observer found that, when plants of the duckweed species were given glucose together with asparagin, an abundant formation of proteid resulted in them. The close relation of glutamin to asparagin leads Schulze to include it in the general assumption, therefore, that the synthesis of some of the products of proteid decomposition into asparagin and glutamin is really a process which may be looked upon as a stage of proteid regeneration and which, for that reason, is of great consequence to the plant.

As to the relation of carbohydrates to proteid transformations in seedlings, Schulze offers numerous experimental results to

* Kinoshita and Suzuki. Zeitschr. f. physiol. Chem., 1897, xxiv, p. 73.

† Hansteen. Berichte der Deutsch. botan. Gesellschaft, Vol. xiv, p. 362.

strengthen his conclusions. It is well known that a diet rich in carbohydrates has the effect of sparing proteid in animal metabolism and that it generally results in increasing the quantity of albuminous matter contained in the system. Quite analogous with this effect, Schulze finds that, in seedlings rich in proteid, the accumulation of amides is greatest as a rule in those poorest in non-nitrogenous material, and conversely, that, in seedlings containing the most abundant non-nitrogenous reserve material in proportion to the albuminous matter, the proteid decomposition is least. This circumstance is in harmony, also, with the conclusions to be drawn from the work of Kinoshita, Hansteen and others, viz., that certain of the simple nitrogenous substances resulting from albuminous decomposition in plants are regenerated into proteid by becoming united with non-nitrogenous substances such as glucose.

With reference to the proteid transformations going on in full grown and normally developed plants little is to be said because little has been done to determine the facts. The difficulties in the way of such experimental work are obvious. While our knowledge in this regard is decidedly limited, no facts can be advanced in opposition to the assumption that in ordinary green plants these transformations are practically identical in quality with those known to occur in seedlings.

Schulze's experiments with tubers and upon the changes ocurring in roots afford confirmatory testimony to all that has been concluded in regard to proteid transformations in seedlings. It seems quite probable that in the synthetic processes taking place in roots, crystalline nitrogenous substances are formed which are essentially the same in character as those formed in seedlings, and that the nitrogen taken from the soil in inorganic combination, as he assumes, is transformed in the roots, for the most part, into asparagin and glutamin. Tubers show much the same results as those obtained with etiolated seedlings.

Whatever uncertainty, in regard to the experimental data adduced by Schulze and others, exists as a result of the weaknesses of this greatly abbreviated review of them, may perhaps be cleared up by the following summary of the results of Schulze's experiments and the conclusions to which they have led him: During

the development of seedlings, and probably also of plants under normal conditions, the proteids are in part decomposed by hydrolysis, presumably through the intermediation of enzymes. The preliminary products of this hydrolytic cleavage are proteoses and peptones, and among the constant products resulting from the continued hydration of these, occur amido acids of both the fatty and aromatic series and nitrogenous bases such as arginin. Whether in this process asparagin and glutamin are formed directly, or whether aspartic and glutamic acids first result, and these in turn are formed into the amides, Schulze does not pretend to say. The greater part of these nitrogenous products are further decomposed in the plant catabolic processes, from which less complex nitrogenous residues result — probably some simple compound of ammonia or perhaps even ammonia itself. These ultimate nitrogenous decomposition products, then, become the important factors in the building up of asparagin and glutamin, and possibly other related compounds, which synthesis is necessary in order to transform the simpler bodies into substances better adapted for utilization in the construction of new proteid matter. In the alterations of the non-nitrogenous reserve materials such insoluble substances as starch and fat are transformed into soluble bodies. A part of the latter are made " functionally active," to use Schulze's phrase, by being converted into glucose or related carbohydrate. Finally, according to this investigator's view, the amides eventually unite with the carbohydrate radicals to form the albuminous compounds characteristic of the plants, completing in this way the cycle of proteid decomposition and regeneration.

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CHEMICAL STUDIES OF THE PITCHER PLANT, SARRACENIA PURPUREA.

By William J. Gies.

At the suggestion of Dr. MacDougal, a few weeks ago, I began an investigation of the digestive powers of the pitcher plant, *Sarracenia purpurea.* Two previous references to this matter will serve to indicate the desirability of such a study.

A few years ago Vines, in referring to the mode of secretion of the digestive juices of insectivorous plants, had the following to say regarding *Sarracenia :*

"In . . . *Sarracenia* there are, according to Batalin, no specialized glands, but the effect of the contact of organic matter (insects, meat, etc.) with the cells of the lower part of the pitcher is to cause the excretion of some substance (*probably the digestive secretion*) between the cuticular and the deeper layers of the cell-wall of the cells which have been touched, and this is followed by the rupture of the cuticular layer. This rupture has the effect not only of bringing the excretion into relation with the introduced organic matter, but also of enabling the cells which have thus lost their cuticle to absorb the organic matter." (Lectures on the Physiology of Plants, 1886, p. 247.)

More recently Green has written of the pitcher plants as follows :

"Insects attracted to the plants are enticed into entering the pitcher and are drowned in the liquid they contain. Some of these plants, particularly *Sarracenia . . . have nothing but water in the pitchers* and the insects drowned therein undergo ordinary putrefaction, the products of which are absorbed by the plant." (The Soluble Ferments and Fermentation, 1899, p. 210.)

Thus far my experiments in this connection have been directed to the detection of an enzyme or zymogen in the tissue of the pitcher. Plants now growing in the Garden will be utilized later for a study of the properties of the pitcher fluid.

Two quantities of *Sarracenia* from different localities have thus far been placed at my disposal by Dr. MacDougal. Glycerin extracts of the thoroughly macerated tissue of one set of plants showed moderate though distinct digestive action on fibrin at 38° C. in the presence of slight amounts of hydrochloric or oxalic acids, the control experiments giving negative results. All of the extracts of the second set of plants, however, were entirely without digestive action.

In view of the negative results in the second series it is impossible at present to draw a satisfactory conclusion in this connection. It may be that the positive results in the first case were due to a bacterium specially favored by the medium furnished by the constituents of the glycerin extract, or to enzyme in unobserved diseased portions of the plants. Again, the negative results may have been due to a less favorable degree of acidity, or the secreting cells of the pitchers may have been in a "resting condition," without either enzyme or zymogen. Further experiments, with these matters controlled and on pitchers gathered at a more favorable season, will surely settle these questions.

The growing plants in the Garden will also be used for direct determinations of the influence of putrefactive products introduced into the pitcher fluid.

A NEW PIGMENT.

In the course of the digestive experiments I had occasion to try the activity of the extracts under neutral, acid and alkaline conditions. Observing that the diluted neutral extract was practically colorless, the acid mixture crimson and the alkaline fluid green, I made a few tests to determine the significance of the colorific effects.

These tests resulted in showing that *Sarracenia purpurea* contains a pigment which in concentrated glycerin extract has a reddish color, but which when diluted is practically colorless. At such dilution, when scarcely any color is to be seen, a drop of dilute acid produces a bright pink throughout the whole fluid; alkali in minute amounts turns it green. The pink is converted to green by alkali, *vice versa* by acid. Even in crude glycerin

extract the pigment appears to be very sensitive and may be used to advantage in titrimetric work.

I have named the pigment *alkaverdin*, because of the beautiful green produced on treatment with alkali, preferring to reserve the term sarracin for any digestive enzyme which later may be found to exist in the pitcher.

Excellent "test papers" have been made with the pigment in glycerin extract. Ordinary filter paper dipped into the red, concentrated extract is colorless, wet or dry. The dry paper turns a bright pink when dipped into acid, a deep green is produced when in contact with alkali.

The pigment of *Sarracenia* bears superficial resemblance to the coloring matters in red cabbage, the purple flowers of mallow, buckthorn berries, elderberry, dahlia and alkanet root, but various tests, thus far, indicate that it is unlike each in fundamental qualities. Preliminary observations indicate that its solutions are without special influence on the spectrum. A chemical study of the qualities of alkaverdin is now in progress.

The aqueous and saline extracts of *Sarracenia* contain an abundance of dextrorotary, reducing and fermentable substances, the characters of which, together with other bodies as yet undetermined, I hope to report in due time.

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* In this volume the terms mucin and mucoid are used synonymously when connective tissue glucoproteids are described. See footnote No. 3 on the opening page of reprint No. 5.

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