LONG ISLAND BIOLOGICAL ASSOCIATION

ANNUAL REPORT
OF
THE BIOLOGICAL LABORATORY

COLD SPRING HARBOR
LONG ISLAND, NEW YORK
1940

LONG ISLAND BIOLOGICAL ASSOCIATION

INCORPORATED 1924

ANNUAL REPORT

OF

THE BIOLOGICAL LABORATORY

FOUNDED 1890

FIFTY-FIRST YEAR

1940

WILTON LLOYD-SMITH

This Report is dedicated to

the memory of

WILTON LLOYD-SMITH

long a member of the Executive Committee. He served the Association loyally by faithful attendance at the meetings of the Board, by his wise counsel on matters brought before it, and by the special interest he took in contributing himself and securing contributions from others toward the work of the Association.

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REPORT OF THE DIRECTOR

To the Officers and Members of the Long Island Biological Association:
Ladies and Gentlemen:

I have the honor to submit my report for the year 1940.

SYMPOSIUM

The subject selected for the Symposium on Quantitative Biology was that of Permeability. This Symposium was the eighth of the series and started on June 18th, continuing until July 17th. The subject, which is a very wide one, was covered in 28 papers presented by 32 authors. The number of people, including visitors, who attended the meetings was much the same as in previous years. Most of the participants were able to be in residence at the Laboratory for considerable periods of time, which gave a very desirable continuity to the Symposium as a whole.

The papers, together with the edited discussions which followed them, will be published as Volume VIII of the Cold Spring Harbor Symposia, with the title "Permeability", and it is expected that the book will be available for distribution this January. The volume will be somewhat smaller in size than the 1939 and 1938 volumes, but the subject is adequately covered by the papers and the discussions.

RESEARCH

As in previous summers, our research space was almost fully occupied from the middle of June until the middle of August. Some of the research workers were among those taking part in the Symposium, and in a number of cases the Laboratory was able to supply facilities and technical assistants out of the portion of the Rockefeller Foundation grant which is given for that purpose. As the reports of the various workers show, the subjects investigated were quite varied, although all were along the lines of experimental biology and general physiology. The following is a brief summary of the kind of work done:

Drs. H. A. Abramson, Manuel Gorin, and L. S. Moyer: A continuation of previous work on the electrophoresis of pollen through the skin; the preparation of a book on the electrophoresis of proteins; and the introduction of estrogenic hormones into the skin by electrophoresis (with Mr. M. J. Kempner).

Dr. Donald H. Barron and Mr. Cecil Gloster: The isolation in the spinal cord of the fibers that mediate the discharges of the respiratory center to the phrenic nucleus.

Drs. L. R. Blinks and R. K. Skow: The continuation of bioelectric studies on Valonia, particularly the effect of acidity and of oxidants.

Drs. R. T. Cox and Walter Rosenblith, and Miss Janice Cutler: A continuation of studies on the electric eel, four electric eels being made available by the New York Aquarium and being kept at the Laboratory during the summer. Detailed measurements of the electromotive force and electrical resistance of the eel were made, and after killing and

sectioning it a comparison was made between its electrical and anatomical characteristics. Observations were also made on the fatigue of the electrical organs and the velocity of passage of the discharge.

Dr. H. Davson: A new method of measuring permeability to non-electrolytes was devised, and studies were made of the effect of fluoride on the potassium permeability of the red cell.

Dr. J. A. de Tomasi: The behavior of cell constituents exposed to the action of vital dyes; the development of a method for clearing whole animal specimens and at the same time using dyes to outline certain body systems.

Dr. R. F. Furchgott: Electrophoresis studies on the mammalian red cell, the results of which indicate that surface injury occurs at low ionic strengths, at low pH levels, in the presence of traces of copper, and in ghosts after certain methods of preparation.

Dr. A. J. Grout: Last September Dr. Grout completed his monumental series of volumes on the "Moss Flora of North America North of Mexico" on which he has worked for the last ten years or more.

Dr. L. H. Kleinholz: The molting and calcium deposition of crustaceans and their relation to removal of the eye-stalks.

Dr. L. S. Moyer: Electrometric titration curves of serum proteins (with Dr. Gorin); the coating of surfaces with mixtures of proteins (with Mrs. Moyer); the mechanism of film formations in mixtures of proteins and surfaces (with Dr. Gorin).

Miss Priscilla M. Porter: The investigation of the agglutinating properties of ghosts prepared from human red cells, the result being that ghosts prepared by means of hemolytic agents such as saponin and chloroform do not show any detectable change in their agglutinative properties; the nature of the increased resistance of red cells to hemolysis on standing which seems to be due to a change in the state of the cell membrane rather than to an inhibitory substance being liberated from the cells.

Dr. Sidney Velick: An investigation into the factors underlying the resistance of red cells to hypotonic saline, and an extension of the work to the red cells of anemic blood.

Dr. C. O. Warren: The study of the metabolic response of rabbit bone marrow in vitro to low oxygen tensions ranging from completely aerobic to completely anaerobic conditions (with the assistance of Miss Jane Dunn).

Complete reports of the individual investigators will be found on pages 18-33.

INSTRUCTION

Last summer the Laboratory offered three courses, one in Experimental Surgery and one in Experimental Endocrinology during the first part of the summer, and one in Marine and Fresh Water Zoology during the second part. Unfortunately the general level of registration was very low, the total number of students in the three courses combined

being only 12. This small registration was probably partly due to unsettled conditions in the country generally, and may have also been due to some of the courses having outlived their usefulness. In any event, the effect upon our income was the same: about $1700 less than what would be expected in a normal year.

The course in experimental surgery was given by Dr. Donald H. Barron of Yale University and the University of Cambridge, England. His report will be found on page 17. As a supplement to the course Dr. Barron gave a course of instruction in neuro-anatomy, which was largely attended by the students at the Laboratory. The course in experimental endocrinology was again instructed by Dr. Hans O. Haterius of Wayne University Medical College, and the course in marine and fresh water zoology by Dr. W. A. Dreyer and Dr. Marvin C. Meyer. For further information about these courses, I refer you to the reports of instructors on pages 17-18. In all, four of the students were graduates, and the remaining eight undergraduates.

OTHER SCIENTIFIC ACTIVITIES

As in previous summers, there was held at the Laboratory a series of evening lectures on Tuesdays. These lectures were on subjects of general interest to our guests and students and to visitors from nearby institutions. The Laboratory is indebted to the eminent scientists who contributed to the lecture program: Dr. S. B. Barker, Cornell Medical School; Dr. L. R. Blinks, University of California; Dr. D. M. Wrinch, Johns Hopkins University; Dr. D. H. Barron, Yale University and The University of Cambridge, England; Mr. E. H. Anthes, Bausch & Lomb Optical Company; Dr. J. S. Nicholas, Yale University; Dr. Walter Rosenblith, New York University; Dr. L. H. Kleinholz, Harvard University; and Dr. G. E. Hutchinson, Yale University.

Friday evenings, from June 21st to July 29th, Dr. Richard T. Cox, Professor of Physics at New York University, gave a series of lectures entitled "Phenomena of atomic nuclei and their application to research in biology". These lectures, of course, were much more specialized than those given on Tuesday evenings, but were well attended by investigators interested in the principles of the relation of radiation to biological problems.

On the 29th of June, 1940, a special meeting of the Association was held in order to celebrate the completion of fifty years activity of the Biological Laboratory. The meeting was held in Blackford Hall, and was attended by over 200 members and their friends. Short addresses were given by Mr. Arthur W. Page, President of the Association; Dr. Robert Cushman Murphy and Dr. Harold C. Urey, members of the Board of Directors. Tea was served by members of the Women's Committee. An exhibit of scientific interest was set up in the John D. Jones Laboratory by Drs. H. A. Abramson, L. R. Blinks, C. W. Coates, R. T. Cox, W. Rosenblith, C. B. Davenport, J. A. de Tomasi; Messrs. M. Halvorsen and M. J. Kempner; and Mrs. Jane D. Harris.

In the evening Mrs. Merle-Smith kindly invited those present at the Laboratory to supper at her home, and to her, as well as to all the others who took part in the celebration, we offer our thanks for bringing about a very pleasant occasion.

In connection with the anniversary Dr. Davenport, the Secretary of the Association, undertook the task of writing a history of the Laboratory from the time of its founding until now. Guests at the meeting received copies of this illustrated booklet, which we hope will make the origin and aims of the Laboratory better known.

LIBRARY

There is little to report about the library situation. We are still carrying as many of the current journals in physical chemistry and physiology as we can afford and have added considerably to the collection of monographs. The bound volumes of the journals now almost fill the room on the second floor of the George Lane Nichols Memorial, which was allotted to them last year, and this year additional space has been made for reprints in the attic. These reprints are now arranged alphabetically in cases and indexed according to author and subject, the whole collection now totaling more than 25,000.

As in previous years, the investigators at the Laboratory have been allowed to use the library of the neighboring Department of Genetics of the Carnegie Institution of Washington, and this, together with the library exchange service and our own collection, seems to have been sufficient for investigators working here during the summer months.

INSTITUTIONS REPRESENTED

The following institutions were represented last summer by students, investigators, or people taking part in the Symposium, who were actually in residence at the Laboratory.

American Museum of Natural History
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Cambridge University, England
College of the Ozarks
College of William and Mary
Columbia University
Columbia University, College of Physicians and Surgeons
Cornell University Medical College
Dalhousie University, Canada
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Duke University Medical School
Elmira College
Grinnell College
Harvard University
Johns Hopkins University
Johns Hopkins University, School of Hygiene and Public Health
Long Island College of Medicine

Magnolia Petroleum Company
McGill University, Canada
Montclair Teachers College
New York State College of Agriculture, Ithaca
New York University, College of Arts and Sciences
New York University, College of Medicine
New York University, Washington Square College
Northwestern University Medical School
Oxford University, England
Princeton University
Rockefeller Institute for Medical Research
Stanford University
Temple University Medical School
Trinity College
University of California
University of Chicago
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University of Illinois
University of Kentucky
University of Minnesota
University of Missouri
University of Pennsylvania
University of Pittsburgh
University of Rochester, School of Medicine and Dentistry
University of Wisconsin, School of Education
Washington University
Wayne University College of Medicine
Yale University
Yeshiva College

LABORATORY BUILDINGS

There is not much to report about the laboratory buildings this year, apart from what falls into the category of general upkeep. No changes have been made; indeed, as I reported last year, most of the buildings are in good condition and little requires to be done to them.

At the beginning of the summer the survey of the equipment of the Walter B. James Laboratory for Biophysics, started during the winter, was completed, and chemicals and glassware were moved to the stockroom in the George Lane Nichols Memorial laboratory. Movable electrical equipment was sorted and tested and more perishable items transferred to the George Lane Nichols Memorial, which is heated throughout the year. The machine shop equipment was rearranged so as to be more accessible and to be safer from damage and deterioration.

As in past years Mrs. Harris has been in charge of work on the houses and grounds, and she gives the following report of what has been done: "During 1940 the House Committee, with Mrs. Percy Jennings as chairman, was active in sending out cards to members stating our

yearly needs and in writing many personal letters by means of which we were able to raise funds for the purchase of 18 good wool blankets, for repairing and upholstering two pieces of valuable furniture, and for replacements of other needed household articles. We also received donations of about 40 pieces of furniture or household articles, 20 women donating money and furniture, and 18 others helping with the 50th Anniversary celebration.

"A list of over one thousand names and addresses was compiled in card catalogue form for the invitations to the anniversary, and a considerable amount of work was done in the John D. Jones Laboratory in preparation for the exhibits. A tea was organized with the help of Mrs. Philip Roosevelt, Mrs. George Franklin, Mrs. Arthur Page, and Mrs. Acosta Nichols presided at the tea. We are also indebted to: Mrs. Willis Wood, Mrs. Percy Jennings, Mrs. Paul Hammond, Mrs. Walter Jennings, Mrs. George Nichols, Mrs. Russell Leffingwell, Mrs. George Louis, Mrs. Henry de Forest, Mrs. Grover O'Neil, Mrs. Fairman Dick, Mrs. Marshall Field, Mrs. Mansfield Ferry, Mrs. Walter Murphy, Mrs. Charles Bleecker, Mrs. Grinnell Martin, Mrs. F. Huntington Babcock, and many of the women of this Laboratory for their donations and help with the anniversary tea. Mrs. Merle-Smith invited the Laboratory members and the Symposium guests, about 85 at that time, for dinner after the exhibits.

"Most of the work on the grounds has been routine upkeep. This fall, however, we have drained and graded an unsightly marshy spot north of the tennis court, and continued construction on a new piece of road."

ACKNOWLEDGMENTS

The work of the Laboratory has been made possible only by the generous support of The Rockefeller Foundation, The Wawepex Society, the officers and members of the Women's Committee, and by the many generous contributors in the neighborhood.

Respectfully submitted,
ERIC PONDER.

REPORTS OF INSTRUCTORS

EXPERIMENTAL SURGERY

The aim of the course in Experimental Surgery was to provide the students with an adequate anatomical knowledge combined with the essential technical knowledge in wound treating so that they could apply the principles to any surgical procedure they might be obliged to attempt. To this end the first half of the course was devoted to intensive drill on the anatomy of the mammal as seen in routine surgical procedures on the rat. During this same period the students were acquainted with anesthetics, volatile and injected. During this period each student worked alone.

In the second half of the course they were divided into teams in which they rotated from surgeon to assistant to anesthetist. At this time the operations carried out earlier on rats were repeated on rabbits, with every precaution for maintaining asepsis. During this period no single animal was infected post-operatively and but one was lost under the anesthetic.

Among the more difficult operations performed in this period were a hemidecortication, semisection of the spinal cord, transplantation of the ureter to the anterior abdominal wall, end to end anastomosis of the small intestine, end to end anastomosis of the intestine and formation of a gastric fistula. All of these animals survived to enjoy a period of post-operation good health.

The high quality of the surgical work carried out by the students reflects especial credit upon them, for they were without previous experience or great technical knowledge in either anatomy or physiology.

DONALD H. BARRON

EXPERIMENTAL ENDOCRINOLOGY

The class program followed the schedule of previous years, i.e., daily lectures and laboratory, the former comprising a survey of the physiology of reproduction and the endocrine organs involved, the physiology of the thyroid and parathyroid glands, together with certain aspects of the endocrine role in water and electrolyte metabolism. The class learned the techniques of the more fundamental experimental procedures, including, for example, the preparation and care of animals, estrual rhythm determination, physiological and histological effects of gland extirpation, gross and microscopic effects of administration of hormone preparations, studies on the adrenalectomized animal, viz., deficiency syndrome, hormone administration, salt therapy, and water balance. Responsibility was shared in the preparation of histological material and, by exchange, each student obtained a representative set of slides of tissues recovered during the experimental procedures.

The following contributed generously of their time, in lectures and demonstrations: Drs. Ponder and Barron, of the Laboratory, Dr. Bates of the Carnegie Institution, and Dr. E. W. Blanchard of the Scientific Laboratories of Schieffelin and Company.

HANS O. HATERIUS

MARINE AND FRESH WATER ZOOLOGY

The course in Marine and Fresh Water Zoology was offered from August 1 to September 7, 1940. The work included sixteen field trips (69 hours), nine lectures (13 hours), laboratory (124 hours) and a written examination (2 hours). Four students were enrolled and granted certificates upon completion of the work. In addition, Professor M. C. Old, Hofstra College, Long Island, was a visitor on the trips and contributed to the study of sponges. The class roll, schedule of trips, and check lists of animals collected, have been filed in the usual manner in the John D. Jones Laboratory.

The work was organized from an ecological point of view, emphasis being placed upon animal communities and the particular habitat of individual animals, such information being recorded on the check lists. In addition to identification of material, the laboratory work included suitable experiments on toleration, choice of bottom and chromatophore activity. Analysis of water samples for temperature, salinity, pH, oxygen and carbonates was carried on. This work was aided greatly by the purchase of necessary glassware which was made a part of the permanent equipment of the course and installed in the John D. Jones Laboratory.

WILLIAM A. DREYER

MARVIN C. MEYER

REPORTS OF INVESTIGATORS

Dr. Harold A. Abramson's Report
College of Physicians and Surgeons, Columbia University, and the Mount Sinai Hospital, New York

The work of the previous years was, in general, continued. Further studies were made on the electrophoretic administration of pollens into the skin. Whereas previously the electrophoretic treatment of hay fever encountered a good deal of difficulty because of the instability of the solutions, an investigation with glycerol showed that this medium was not irritating to the skin and did not prevent the electrophoresis of histamine, dyestuffs, and pollen antigens. A 50 p. c. glycerol medium was finally adopted as a vehicle for electrophoretic therapy of the hay fevers.

With Miss Porter, an investigation of mixed agglutination was continued. Miss Porter had previously found that ghosts of red blood cells could be readily agglutinated by iso-agglutinating serum. Mixtures of group A ghosts and B cells were studied with mixtures of specific sera to ascertain if mixed agglutination occurred under these conditions. As yet the phenomenon has not been observed in this system. Further studies are planned.

With Dr. M. H. Gorin, two papers were prepared for the symposium on permeability. With Drs. L. S. Moyer and M. H. Gorin, a book on the electrophoresis of proteins was begun.

Mr. Kempner attempted to introduce estrogenic hormones into the skin of rats by electrophoresis. In addition, certain experiments were made on the pore patterns of the rat's skin.

Dr. Donald H. Barron's Report
Yale University and University of Cambridge, England

In collaboration with Mr. Cecil Gloster, an attempt was made to isolate in the spinal cord in the cat the fibres that mediate the discharges of the respiratory center to the phrenic nucleus. To this end a technique was developed by which the spinal cord could be approached from the ventral surface after the removal of the odontoid process of the axis and the ventral arch of the atlas. Lesions in the ventral columns of the cord were produced in some 20 animals that survived the operation. These animals were killed 14 to 22 days post-operatively and are now being prepared for histological study.

The manuscript of a lecture given in the regular Tuesday evening series of lectures was prepared and has since been published under the title "Recent Advances on the Physiology of Birth".

In addition a series of lectures or informal talks with lantern slides was given to all who wished to attend on the anatomy of the mammalian central nervous system.

J. H. Bartlett's Report
(Fellow of Rockefeller Foundation)

During the year 1940-41, while on leave from the University of Illinois, I am attempting to acquire a broad knowledge of biophysics. The summer of 1940, spent mainly at Cold Spring Harbor, was devoted to orientation in the fields of nerve physiology and cell permeability. The mechanism of Lillie's iron wire model of the nerve excited my interest, with the result that a detailed investigation is now being made (at Cornell Medical School).

For my purpose of formulating a research program, the atmosphere during the past summer has been ideal. There was no mad rush to get "results", but rather a willingness to sit down and discuss the importance of various lines of work. The prevailing idea has been to take time out to think things over, which really saves much lost motion in the long run. I have been greatly benefited in two ways: a valuable background has been obtained, and a very definite research program has been developed.

Dr. L. R. Blinks and Dr. R. K. Skow's Report
Stanford University

Dr. Blinks came to the Laboratory in early May, and was able to work here on material brought from Florida. Bioelectric studies on Valonia were continued, the effects of acidity and of oxidants being the chief problems studied. Although earlier work had shown that the potential of these cells was not greatly influenced by pH in the more normal physiological range (pH 6 to 9.5), it was found that lower pH values, especially when well buffered, gave large changes of P. D., which were quite reversible. These may be attributed partly to a resultant decrease of response to other ionic gradients, notably of potassium ions, but also to the direct effects of the H ion itself. This opens up interesting possibilities of metabolic control, and helps to explain certain effects of light, temperature, and respiratory stimulants.

Oxidants were found to be of particular interest, ferricyanide acting much like a strong acid, and probably operating via the increased acidity resulting from its reduction at the cell surface. Permanganate, on the other hand, had just the opposite effect upon the P. D., resembling CO_2 and other penetrating weak acids. Perhaps it penetrates the cell more readily than does ferricyanide. On the other hand, it may form weak acids (as it is well known to do from various organic substances) which in turn penetrate the cell. In agreement with this is the inhibition of the permanganate effects with ammonia, a penetrating base.

Increased pH was found to stabilize and increase the effects of KCl on the P. D. of Valonia, tending to abolish the "cusps" which characterize these effects, and create a smooth topped "plateau".

Dr. R. T. Cox, Dr. W. A. Rosenblith, and Miss Janice Cutler's Report
New York University

At the beginning of the summer, the survey of the equipment of the Walter B. James Laboratory, initiated during the winter, was carried forward. Chemical material and glassware were transferred to the stockroom in the George Lane Nichols Memorial Laboratory. Movable electrical equipment was sorted and tested and put in stock in the Walter B. James Laboratory. The shop equipment was rearranged to be more accessible, in the absence of a machinist, to those working at the Laboratory, and to be safer from certain kinds of damage and deterioration.

One of the larger rooms of the Laboratory was cleared and used for research in respiration. This research was carried on by Dr. Charles Warren and Miss Jane Dunn, and is described elsewhere in this Report.

Another of the larger rooms was prepared for research on the electric eel, Electrophorus electricus (Linnaeus). Other rooms were made available for occasional use in other research.

The research on the electric eel continued studies made in recent years at the New York Aquarium, at New York University in the Department of Physics, and at the Museu Goeldi of Para, Brazil. It was conducted by Dr. Cox, Dr. Rosenblith, and Miss Cutler, with the assistance from time to time of Mr. C. W. Coates and Mr. Robert S. Mathews of the New York Aquarium, Dr. M. Vertner Brown of the College of the City of New York, Mr. Herman Yagoda, and others.

By the courtesy of the New York Aquarium, four electric eels were made available for the research and were kept at the Biological Laboratory during much of the summer. One of these was made the subject of detailed measurements of its electromotive force and electrical resistance. It was afterwards killed and sectioned, and a comparison was made between its electrical and anatomical characteristics. The results will appear in Zoologica, Vol. XXV, Part 4, Dec., 1940. Specimens of the tissue of this electric eel were furnished to Mr. Herman Yagoda for chemical analysis and to Dr. Donald H. Barron of the University of Missouri, Dr. T. L. Smith of the College of the Ozarks, and Dr. Ralph G. Meader of Yale University for anatomical study.

One of the specimens studied had the peculiarity of producing almost exclusively one of the types of discharge characteristic of the species, the minor discharge, produced by the small electric organs known as the bundles of Sachs. This peculiarity made it possible to study the minor discharge without the masking effect of the major discharge. The velocity with which the minor discharge travels along the organ was especially studied by Dr. Rosenblith and Miss Cutler. This study, which supplements a similar one previously made on the major discharge, will, it is hoped, soon be ready for publication.

Some observations were made, in collaboration with Dr. Brown, on the fatigue of the electric organs, and some were also made on the response of the electric eel to an electric current in the water surrounding

it. It is planned to continue these observations at the New York Aquarium.

Dr. Hugh Davson's Report
Dalhousie University

1. A New Method of Measuring Permeability to Non-Electrolytes.

If erythrocytes are placed in a Ringer solution containing 0.33 M non-electrolyte until diffusion equilibrium is achieved and then centrifuged down and placed in a Ringer solution of concentration x, the degree of haemolysis observed may be used as a measure of the permeability constant of the non-electrolyte if the permeability constant to water is known. Thus:

$$dV/dt = k_1 A \left[\frac{21 - S}{V} - x \right]$$

$$dS/dt = k_2 A \left[\frac{I - S}{V} \right]$$

Whence $$dV/dS = \frac{k_1}{k_2} \left[\frac{21 - S - xV}{I - S} \right]$$

which on integration gives:

$$V = \left[\frac{a(I - S)}{(1 - ax)} \right] + \frac{I}{x} + c(I - S)^{ax}$$

Where V is the volume of the cells; S is the amount of non-electrolyte in them; I is the isotonic concentration. k_1 is the permeability constant for water and k_2 that for the penetrating substance. c is a constant of integration, and a equals k_1/k_2.

Preliminary experiments indicated the feasibility of the method and the more exact work is being continued.

2. The effect of fluoride on the erythrocyte's potassium permeability.

Fluoride causes a marked escape of potassium from the rabbit erythrocyte; that this is not due to a direct poisoning of a metabolic activity responsible for the maintenance of the concentration gradient but is probably due to the accumulation of intermediate products of metabolism at the cell surface thereby causing it to leak potassium through a direct action on the membrane is made apparent by the following facts.

(a) The curve of potassium loss with time shows an initial incubation period in which the escape of potassium is very small.

(b) This incubation period is considerably lengthened by washing the cells in isotonic NaCl.

(c) The incubation period can be brought back to its original length by addition of serum, an ultra-filtrate of the same, or Ringer containing bicarbonate, phosphate, magnesium, calcium, potassium, and glucose; each of these constituents is necessary.

(d) High concentrations of fluoride, e.g., 0.165 M, have very little effect on the potassium permeability.

3. An interesting observation which may have some bearing on the mechanism of catastoichic haemolysis was that addition of an excess of calcium to cells which had been treated with fluoride caused haemolysis. Addition of calcium fluoride had no effect and since the amount of calcium added was unimportant so long as it was sufficient to precipitate all the fluoride, it would appear that the removal of the fluoride from the cell membrane caused sufficient damage to the latter that haemolysis ensued. The haemolytic effect of plasma when added to cells previously treated with taurocholate may thus find its explanation in the removal of the taurocholate from the cell membrane by the plasma.

Dr. J. A. de Tomasi's Report
(John D. Jones Scholar)
The Biological Laboratory

1. Development of a new method whereby photomicrographical technique, in black and white as well as in color, is considerably simplified. Such a method is not only easy and rapid, but is intended to meet the needs of many laboratories and operators limited to a very small budget. A paper covering the subject is being prepared for publication early in 1941.

2. Work has been initiated on the behavior of cell constituents exposed to the action of vital dyes. A good many cells from various marine and fresh water and land organisms have been subjected to preliminary tests. There are indications that valuable further information on the permeability of the protoplast may be secured in this way. Fertilization of germ cells appears to be an interesting factor affecting in some unknown way the reactions to supra-vitam and intra-vitam stains.

3. With the assistance of Mr. Robert Lee Austin, work has been initiated toward development of a method permitting clearing of whole animal specimens and at the same time the outlining of certain body systems by the use of special dyes, either injected or otherwise applied. It has so far been established that instead of alkalies, certain oils may be preferred for clearing tissues under the conditions required, and that the concurrent differential staining of the skeletal and circulatory systems is achievable in at least a number of instances.

Dr. Robert F. Furchgott's Report
Cornell University Medical College

Electrophoresis studies were made to obtain more information about the surface of the red blood cell. The Abramson micro-electrophoresis cell with slight modifications was used. First of all the electrophoretic mobility of unhemolysed human red cells was determined as a function of ionic strength at approximately constant pH (7.2) in isotonic mixtures of glucose solution and saline-phosphate buffer solution. It was found that above an ionic strength of about 0.02 the mobility is a linear function the reciprocal of the Debye function of the ionic strength.

In the light of Gorin's recent equations this indicates that the red cell surface is a smooth surface of large radius of curvature. Below an ionic strength of about 0.02 the mobility is no longer a linear function. This probably means that below this ionic strength the cell surface undergoes a decrease of charge density, and possibly indicates injury of the surface.

The mobility as a function of pH at an ionic strength of 0.172 was also determined for human red cells, for the lipid extract of the cells, and for the stroma protein of the cells. The pH-mobility curve obtained for intact cells extended from pH 10.3 to pH 1.7. The isoelectric points of the cells, lipid, and protein were found to be 1.7, 2.6, and 4.7 respectively. The pH-mobility data lead to the conclusion that the human red cell surface is composed largely of lipid and is dominated by strong acid groups, possibly the phosphoric acid groups of cephalin molecules.

Changes of mobilities of human red cells and ghosts under certain experimental conditions were also studied. Among these were the changes with time at low pH levels, changes with time at low ionic strengths, changes in the presence of traces of cupric ions, and changes (in ghosts) after certain methods of preparation.

Mr. Cecil Gloster's Report
New York University College of Medicine

Since comparatively little is known about the physiology of the ventral columns of the spinal cord, together with the possibility that some important tracts may be located here, we made a study of this region. It soon became evident that possibly the present knowledge of these columns is limited since no adequate surgical procedure exists which permits access to this region of the spinal cord. Our first problem was that of working out a method of approach, making the ventral cords readily accessible for experimental work. Before long it became apparent that we had to devise an approach whereby a lesion could be made in the ventral columns, without sacrificing the animal, a problem which taxed our ingenuity to its fullest extent.

Injection anaesthesia consisting of sodium amytal (1.0 milligram per 7 grams body weight in males, or 1.0 milligram per 10 grams is used in females) is used. When the animal has reached a state of surgical anaesthesia, it is tied down on a standard rat board, with its dorsal surface resting on the board. The ventral surface is thus left exposed, giving an easily accessible anterior approach to the spinal cord. The hair in the cervical region is removed with an electric razor.

An incision is made which runs from the manubrium strenum to a point ¼" caudad to the symphysis mandibuli. By means of blunt dissection the subcutaneous fascia and connective tissue is separated. The incised skin is reflected to the sides by retractors placed on the edges. Blunt dissection is continued deep through the superficial cervical portion of the platysma, the neighboring supra and infrahyoid

muscles and isthmus of the thyroid gland.

Since the sternohyoid muscle covers the trachea, a longitudinal incision is made through this muscle to make the trachea accessible. A thread loop is placed around the trachea and the carotid sheath of the opposite side. These structures are then drawn laterally away from the incision in the sternohyoid muscle.

In the region of the atlas and axis, the deep muscles of the back are severed from their bony attachments. By means of a curette the vertebrae are cleaned of muscle fragments, and with a pair of curved, fine-toothed rongeurs the ventral portions of the atlas and axis are removed. This requires considerable care or a fatal hemorrhage may result. It has been found that in general as long as the operator remains near to the midline there is little danger of encountering a severe hemorrhage.

The dura mater is incised with iridectomy scissors. The cerebro-spinal fluid which oozes from beneath the dura is removed by means of sponges. With a fine cataract knife or iridectomy scissors an incision is made into the animal's spinal cord, just medial to the anterior columns. Any blood which is present after the lesion has been made is removed with a pledget of cotton wool. No attempt is made to repair the dura. The superficial muscles are sutured by means of a continuous suture, while the skin is repaired with a discontinuous suture.

Post-operative observations indicate that the pyramidal tract has not been encountered, which is in accord with the level of the lesion which is well below the decussation of the pyramids.

Since the technique was only perfected toward the end of the summer, little time was left to observe any changes in the behavior pattern of the animals with lesions. Further studies now being conducted will possibly give an indication of the function of these tracts. This technique permits a pure ventral column lesion, without encroaching upon any other part of the spinal cord.

Dr. A. J. Grout's Report

The Summer School of Bryology, Newfane, Vt.

On last September thirtieth the final part, volume 2, part 4, of the Moss Flora of North America North of Mexico was issued. From numerous prominent botanists came congratulations on the completion of a "monumental" work.

In April I made a visit of nearly two weeks to Puerto Rico at the invitation of Dr. William C. Steere, exchange Professor at the University of Puerto Rico from the University of Michigan. With Dr. Steere I travelled over 1000 miles by automobile and collected over 125 species of mosses and hepatics.

Last October-November I spent nearly three weeks at the New York Botanical Garden studying the mosses of the West Indies and Mexico.

The Garden under the supervision of Dr. H. A. Gleason plans

to continue the publication of the North American Flora begun by Dr. N. L. Britton and others. This will include the completion of Volume 15 on mosses of which two parts were published more than twenty-five years ago. The succeeding parts will be illustrated and will include comparative notes and descriptions of well marked varieties. I have undertaken to write the first portion of Part three of Volume fifteen on the genus Fissedens. This involves a great deal of labor as the nomenclature is badly confused and type specimens and original descriptions are widely scattered and difficult to consult on account of war conditions.

The Summer School of Bryology at Newfane was attended by four graduate students. Mrs. Haring, associated with Vassar College, spent three weeks studying her collections and making an index for Volume two of the Moss Flora.

Mr. James R. Line, a teacher in the Hamilton, Ohio, High School, spent about two weeks studying his Alaskan collections and the local moss flora about Newfane.

Miss Irma Schnooberger of the Alma High School, Michigan, and Miss Frances Wynne, student assistant at the University of Michigan, spent the entire six weeks as a part of their work for a Ph. D., Miss Schnooberger working on a manual of the Sphagnaceae of Michigan, and Miss Wynne working on the variations and intergradations among the species and genera of the aquatic Amblystegieae.

Miss Mildred Wickes, a former student, continued her exploration of Labrador for another season. Her numerous and valuable collections of Labrador mosses were determined at Newfane.

Besides the identification of Miss Wickes' collections, nearly 200 mosses from the region around Rimouski, Quebec, collected by the Rev. Ernest Lepage have been identified, also some hundreds of specimens from other collectors and institutions.

Numbers 376-400 of my North American Musci Perfecti were issued in July.

Lastly, an annoted List of the Hepaticae of Florida, by J. B. McFarlin, has been edited for publication in the Proceedings of the Florida Academy of Sciences.

Mr. M. J. Kempner's Report
Long Island College of Medicine

Electrophoretic methods were used to study the possibility of increasing the absorption of certain estrogenic substances through the skin. The vaginal smear in castrated rats was used as a means of assay, while stilbestrol and theelin were the estrogens used.

Before the application of stilbestrol, the skin was closely clipped, shaven, or a depilatory used. The skin over the chosen area was carefully cleansed with ether and alcohol, and after the designated period of time of application of the estrogen the skin was again carefully cleansed with ether and alcohol.

A current of 1 ma., running through a 2 sq. cm. lead electrode was used in all experiments. The stilbestrol was used at the positive pole. It was found that when 1 c. c. of a solution containing 0.2 p. c. of stilbestrol was used and the current applied for 10 minutes, the animal showed complete estrus within 36 hours and remained in estrus for 7-8 days. Control animals in which the same technique was applied, except for the application of current, remained in estrus for approximately four days.

When stilbestrol was applied to the skin for 10 minutes without any current, and in a concentration of 0.1 p. c., or one-half the strength previously used, it was found that the animals showed full estrus changes within 36 hours. They consistently remained in estrus for 50-70 hours, while the animals in which electrophoresis was used remained in estrus for periods ranging from 50-80 hours. These changes could not be considered significant, as in several series of animals the control animal remained in estrus for periods ranging from 8-13 hours longer than the experimental animal.

In several series of animals, currents of 1 ma. were applied with the 0.1 p. c. stilbestrol solution for periods of 2.5 and 5 minutes. In these animals, many control groups remained in estrus for longer periods of time than animals in which the current was used.

Theelin in oil was also used, but the factors of continuous transmission of an electric current through an oily solution, the complete removal of this type of solution from the skin of the animal after its application, and other factors, are problems that must be studied before the result we obtained can be reported.

Over forty series (3-4 animals in each) of these experiments have been completed at the present time, but it is felt that no definite conclusion can be drawn concerning the increased absorption of stilbestrol until we have repeated the use of 0.2 p. c. stilbestrol with a current of 1 ma. applied for periods of from 10-20 minutes.

The work has been conducted with the aid and cooperation of Dr. H. O. Haterius and Dr. H. A. Abramson.

Dr. L. H. Kleinholz's Report
(John D. Jones Scholar)
Harvard University

Results of eye-stalk removal, to which endocrine effects on viability and molting have been ascribed by some authors, were studied in Uca pugilator. Of 64 eye-stalkless individuals, 22 p. c. died at the end of 40 days; 10 p. c. of the normal controls died during the same interval. No hormonal effect on viability is indicated by these figures. Of the same 64 individuals, all but 4, which died before ecdysis, molted once during the period of observation; 4 molted a second time; none of the controls molted. These results confirm reports that stalk removal shortens the intermolt period in crustaceans. There is no rigorous evidence proving mediation of this effect by either nervous or humoral agents.

Deposition of calcium in the exoskeleton of newly molted crabs was studied. Normal crabs contain 16.5 p. c. Ca; animals which were killed within 5 minutes after completing ecdysis contained less than 1 p. c.; thus, there is no significant storage of Ca as an internal reserve before molting. Ca deposition proceeds normally in newly molted, starved individuals; sea water is therefore the source of this Ca. Thirty days after molting Ca in the exoskeleton constitutes 58 p. c. of the normal amount; at this time eye-stalkless individuals begin to molt a second time. In delaying this second molt by inanition, Ca rises to 86 p. c. of the normal level. The Ca in the exoskeleton depends on the duration of the intermolt period, within the limits of normal concentration.

Dr. Laurence S. Moyer's Report
University of Minnesota

(1) In collaboration with Drs. Abramson and Gorin, a large part of the summer was spent in writing a book on the electrophoresis of proteins. It is expected, in this book, to survey the whole of this rapidly advancing field. A considerable portion of the original publications to be reviewed have originated from the Biological Laboratory.

(2) With Dr. Gorin, electrometric titration curves of several serum proteins were determined on highly purified preparations. Advances in the theoretical aspects of electrophoresis made it highly desirable to obtain more accurate data on such systems than were available in the literature. Consequently, horse serum was fractionated and the resulting protein preparations were titrated with the glass electrode. The results give a clearer picture of the way blood proteins react with acids and bases.

(3) As a conclusion to the preliminary account of the work on synthetic mixtures of proteins described in the Report for 1938, additional results have been obtained. Although, as reported before, egg albumin coated on surfaces becomes covered by gelatin but is incapable of covering gelatin-coated surfaces, the system casein-gelatin is quite different. With Mrs. Moyer it was found that casein and gelatin do not interact appreciably when either is coated on a surface and exposed to solutions of the other, if the pH is higher than about 6. If, however, the pH is brought below this value, casein becomes more and more insoluble and under these conditions there is a limited zone in the neighborhood of the casein isoelectric point within which it adsorbs gelatin. The results were obtained by following the electrophoresis of the casein particles in the presence of gelatin and comparing their behavior with casein and gelatin alone. These results indicate what may be expected to occur in certain natural mixtures of proteins as they exist in biological systems.

(4) As a continuation of the preceding work we have been able to show that particles of quartz and collodion adsorb different protein constituents from horse serum. Quartz becomes coated with one of the albumins while collodion takes on a film of globulin. Rabbit and human serum gave nearly the same result. These findings indicate that

the film stabilizing the fat droplets in the blood is probably of protein nature. This work was also done with Mrs. Moyer.

(5) Investigations on the mechanism of film formation in mixtures have been carried out with Dr. Gorin. The electrophoretic mobilities of quartz and collodion particles were determined after exposure to mixtures of serum proteins or after they had been coated with one protein and then exposed to another or to serum. The results indicate that there is little tendency for the various constituents used to adsorb on each other, although one protein may replace another at a surface. The result is usually a film of one of the protein components rather than a mosaic. The nature of the underlying surface influences the adsorption, with the more hydrophilic proteins being adsorbed more readily by the more hydrophilic surface, and vice versa. In certain cases the results are complicated by irreversible adsorption.

Dr. Eric Ponder's Report
The Biological Laboratory

1. Properties of ghosts. This year my investigation of the properties of red cell ghosts was completed, and the results are given in a paper which will appear in the February issue of the Journal of Experimental Biology. To summarize these results: When rabbit red cells are hemolysed by water they swell to a critical volume and then lose their pigment, thereafter returning very rapidly to their original volume and discoidal form, even although the fluid surrounding them is hypotonic. If NaCl is added to restore the tonicity, the ghosts undergo a preliminary shrinking, but again after a few minutes return to their original volume and shape. These observations suggest that there is some structure in the interior or on the surface of the red cell which, in the absence of osmotic forces, determines its volume and special shape.

After hemolysis the red cell retains more hemoglobin than would be expected from an equilibrium. It can be shown that this hemoglobin is probably adsorbed, and the amount is so great that it is likely that the adsorption takes place on structures in the cell interior and not on its surface only.

Cells hemolysed by water, although they regain their original shape, are incapable of being turned into spheres between slide and coverslip or by the addition of lecithin or rose bengal. The lysis is accordingly accompanied by some permanent change in surface properties.

2. Dr. Cox and I constructed a new form of diffractometer for measuring red cell diameter and red cell volume by the diffraction method. In the new instrument the monochromatic light of the green line of the mercury arc is rendered parallel and passed through a film of the cells; the resulting diffraction patterns are then photographed on specially sensitive plates. The positions of the various maxima and minima are found by means of a microphotometer.

This diffraction method has several advantages over those now in use. The readings are entirely objective. The plates can be kept

for future reference, and there is no doubt about the constants in the diffraction equation. A description of the method will appear shortly in the Journal of General Physiology.

3. Through the kindness of Dr. Franz C. Schmelkes I have been able to test some of the C-10 alcohols as accelerators of hemolysis. As might be expected, n-decanol is a more powerful accelerator than n-nonyl alcohol, but tetrahydro-decanol, geraniol, and citronellol, three C-10 alcohols with a branched instead of a straight chain, have a much lower accelerating effect; indeed, geraniol, which has two double bonds, scarcely accelerates at all. These investigations will have to be continued with other members of the C-10 alcohols (if they are available), or with available isomers, etc. With some of the lower alcohols the difficulty is that the substances are soluble only in very small quantities and that solution takes at least several days.

4. The remainder of the work for the year is contained in a paper with Hugh Davson (see his report), a paper with Robert F. Furchgott shortly to be published in the Journal of General Physiology (see his report), and two papers read at the Symposium on permeability. The first of these with Harold A. Abramson and Manuel Gorin is concerned with certain properties of the red cell surface in relation to electrophoresis. The second is on the subject of the red cell as an osmometer. Here it is shown that the rabbit red cell sometimes behaves as a perfect osmometer and sometimes as an imperfect one, and that neither of the two main hypotheses put forward to account for this behaviour (salt loss, and the presence of bound water) is satisfactory. This being so, I have suggested that under certain circumstances the red cell gelates so that forces which resist deformation come into play and prevent the cell swelling as a perfect osmometer. This effect can be imitated by deliberately fixing cells with formol, and a consideration of the elastic constants at least does not lead one to the conclusion that the necessary resistance to deformation is impossible. I realize that it is unconventional to invoke elastic forces as modifying osmotic phenomena, but the elastic forces ought to be investigated and known before their possible effects can be dismissed.

It is a pleasure to acknowledge a special contribution by Princess Gagarin, which provided for assistance and some of the material used in these investigations.

Miss Priscilla M. Porter's Report
Bennington College

My work at the Laboratory this summer consisted of two main parts: (1) the investigation of the agglutinating properties of ghosts of human red cells prepared in several different ways, and (2) some experiments concerning the inhibition and acceleration of hemolysis.

(1) It was found that ghost cells prepared with such hemolytic agents as saponin and chloroform do not change their agglutinative properties, showing that the surface of the red cell concerned with such

a reaction is not appreciably damaged.

(2) (a) The nature of the increased resistance of red cells to hemolysis on standing was investigated. It was found that no inhibitory substance was given off, but, as the change in resistance was large, the state of the cell membrane was probably altered. A short note on these results has been published in the Proceedings of the Society for Experimental Biology and Medicine.

(b) The method by which indol acts on red cells to accelerate hemolysis was also investigated. It was found that the indol collects on the surface of the cells, thereby sensitizing them to the action of the hemolysin.

Dr. Sidney Velick's Report
Johns Hopkins University

The experiments undertaken this summer with the technical assistance of Miss Murdina MacFarquhar were designed to determine whether the existing hypotheses concerning the essential factors involved in osmotic hemolysis were able to account quantitatively for the observed phenomena, and if not, to determine the nature of the other variables. The approach was a new one in that it considered not only the properties of the average red blood cell from different animals but also the differences between individual red blood cells in a single animal.

The role of the geometry of the cells in these processes has been pointed out in papers by Castle and Daland and by Ponder. As originally stated, the hypothesis is that a red cell hemolyses in a hypotonic medium when the absorption of water has caused its conversion from a disc to a sphere with the same area as the original disc. Such a shape transformation permits an increase in volume of the order of magnitude of 80 per cent without stretching the membrane. Actually the present experiments show that hemolysis occurs in the major fraction of the red cell population when the amount of swelling is about 85 per cent of the maximum permitted by the theory. This fact was not brought out in the earlier work, in one case because of a systematic experimental error, and in the other because the calculations applied only to the small most resistant fraction of the red cell population.

An equation was developed showing the percentage increase in volume of a cell in the shape transformation from disc to sphere, as a function of the original ratio of diameter to thickness, and a second equation showing the percentage increase in volume of cells of any shape as a function of the tonicity of the medium. A method was developed for determining the percentage hemolysis as a function of tonicity. The three simultaneous equations were solved graphically and it was thus possible to calculate the frequency distribution of diameter thickness ratio in a single red cell population. The diameter distribution was measured directly and it was thus possible, with certain assumptions, to calculate a thickness distribution which is not directly accessible experimentally. The same thickness distribution was arrived at inde-

pendently by calculating the thickness distribution that must be correlated with the measured diameter distribution to give a calculated volume distribution equal to that obtained by diameter measurement of cells rendered spherical (without volume change) by the addition of lecithin. The thickness distribution arrived at is such that cells of greater than average diameter have less than average thickness and cells of less than average diameter have greater than average thickness. In spite of this inverse correlation there is a direct correlation between cell diameter and cell volume.

When the experiments were extended to anemic blood a new and interesting variable entered the picture, namely the frequency distribution of cellular water concentration. That the average cell hemoglobin concentration varies widely in the anemias has been known for a long time, and staining methods have indicated that individual cells in a single sample may be widely variant in hemoglobin concentration. Taking advantage of the relation between cell density and cell water concentration, it was possible by differential centrifugation to isolate from a single sample of blood fractions differing by as much as fifteen per cent in cell water content. By repeated fractionation an approximate frequency distribution of cell water concentration was arrived at and its relation to the percentage hemolysis curve demonstrated. Those cells of less than average water content are usually less resistant to osmotic hemolysis than the others in spite of the fact that they swell less in hypotonic media. This can sometimes be accounted for by shape compensation, but other factors also seem to be involved. The large young cells of low water content frequently do not give an optically clear hemolysate in distilled water. The membrane therefore has different physical and probably chemical properties. In view of the number of variables many more experiments by other approaches are required to settle the question.

Dr. Charles O. Warren's Report
Cornell University Medical College

The experiments were part of a program for the study of the physiology of bone marrow, carried out under the tenureship of a Lewis Cass Ledyard, Jr. Fellowship from New York Hospital, and with the technical assistance of Miss Jane Dunn. We studied the metabolic response of rabbit bone marrow in vitro to low oxygen tensions. Simultaneous measurements were made of the respiration and glycolysis of marrow slices suspended in serum under five different oxygen tensions, ranging from completely aerobic to completely anaerobic conditions. As respiration decreased at the low oxygen tensions, glycolysis was found to increase in a reciprocal fashion. We never found any significant increase in glycolysis without a corresponding decrease in respiration. Since the energy lost to the marrow by the decrease in respiration was much greater than that gained by the increase in glycolysis, the net change at low oxygen tensions was a decrease in energy available to the

marrow. Accordingly, we have no evidence from these experiments that low oxygen tension acts as a direct metabolic stimulant to bone marrow, although we know that if the whole animal is exposed to low oxygen tension the marrow responds by an increased growth and multiplication of young red blood cells. We are therefore continuing the studies at the present time by investigating the metabolic response of bone marrow when the whole animal is exposed to low oxygen tensions and hope to be able to report on the completed project at an early date.

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