LONG ISLAND BIOLOGICAL ASSOCIATION

ANNUAL REPORT
OF
THE BIOLOGICAL LABORATORY

COLD SPRING HARBOR
LONG ISLAND, NEW YORK
1937

LONG ISLAND BIOLOGICAL ASSOCIATION

INCORPORATED 1924

ANNUAL REPORT

OF

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REPORT OF THE DIRECTOR

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

I have the honor to submit my report for the year 1937.

RESEARCH

The research work of the Laboratory has proceeded along familiar lines during the past year, the problems studied being principally fundamental problems of cell physiology, chemistry and physics. It cannot be repeated too often that such investigations are, in the proper hands, the most profitable and productive in the long run. It is true that years may be spent in perfecting technique and in studies which at the time appear highly academic, but when the essentials of the problem are finally worked out, one is apt to have information of a very important kind for no other reason than because it is so fundamental, while from the seemingly academic investigations an entirely new field of research may grow.

So far as the physiological research at the Laboratory is concerned, we have been principally occupied in closing old chapters and in opening new ones. Last year the bulk of my report was concerned with investigations into the structure of red cell membranes and with how these surface layers, the integrity of which is essential to life, have an "ultrastructure", the molecules being arranged in a characteristic design. At that time there seemed to be a discrepancy between the results obtained by Dr. Fricke's conductivity methods, the polarized light methods upon which we were relying, and chemical analysis; this discrepancy has now been largely resolved, and at last we are able to speak with comparative confidence about the structure of the surface of the cell.

Since the integrity of this complex surface membrane is essential to life, physiologists and biophysicists have long been interested in processes which bring about its destruction, with subsequent death and disintegration, or cytolysis. In the case of the red cell, cytolysis is known by the specialized term hemolysis, a process which results when certain drug-like substances (hemolysins) attack the red cell membrane. Obviously an understanding of the structure which is being attacked is necessary for an understanding of the process, and so we are now attempting to interpret hemolysis in terms of the ultrastructure of the cell surface. With Dr. Hugh Davson, of University College, London, and Dr. Hans Neurath, the Baker Research Scholar of Cornell University, considerable progress has been made along these lines. I would not like to say that the chapter on the structure of the red cell membrane is closed, but those of us who have been working on the problem feel that it has reached the stage where it can be allowed to rest for a while.

If the structure of the cell surface upon which the hemolysins act is important, the structure, in a chemical sense, of the hemolysins themselves is important too. Unfortunately most hemolysins are complex substances whose chemistry is not well understood; it happens, however, that there are a large number of chemical substances which increase the efficiency

of hemolysins, and which are technically known as "accelerators". Many of these have a simple chemical structure, and so an attempt has been made to relate this structure to the efficacy of each substance in rendering the cell membrane vulnerable. An excellent example is benzene, which is not hemolytic in itself but which nevertheless exerts a powerful action on the cell surface, rendering it readily attacked by hemolysins. The results of this investigation have been very encouraging, for there appears to be a beautiful relation between the chemical structure of various derivatives of benzene and their ability to increase the efficiency of hemolysins. A detailed description of these results, which formed the subjects of a Schiff Foundation Lecture at Cornell University (November 2, 1937), will be found on page 37 of this report.

This certainly is a case in which the investigation of a physiological problem is not an academic one, for poisoning by benzene and its derivatives results in a considerable number of deaths each year. Persons exposed to some of the benzene derivatives encountered in industry may develop various blood disorders, in some of which the production of blood cells in the bone marrow stops, resulting in a fatal anemia, and in others of which the bone marrow shows an abnormal form of activity, resulting in conditions not unlike leukemia. For this reason, while the more fundamental actions of benzene and substances allied to it have been under investigation for their own sake, the work has proceeded in close relation to the investigations of Dr. D. R. Climenko and Dr. E. J. Robinson on the effect of the inhalation of benzene on the living animal. Rabbits may be made to inhale the benzene derivatives without any great harm to themselves, and the effects on their red cells, white cells, bone marrow, etc., can be studied under conditions which are not unlike those which obtain in the case of industrial poisoning. As in the test tube, so in the living animal, the benzene derivatives produce a destruction of red cells followed by an activity of the bone marrow; they also effect the white cells of the blood and the white cell-producing power of the marrow, as has been shown in a study by Dr. Climenko and Mr. MacLeod. The whole field of the destruction of red cells in the body of living animals, as opposed to their destruction in the test tube, has been much neglected, but has now become so important in connection with the various investigations in progress at the Laboratory that it will probably make up the major portion of next year's work. The American Association for the Advancement of Science has made available a grant for the study of the subject.

One other benzene derivative is of particular interest, and it looks as if the preliminary work which has been done may lead to important conclusions. At the Symposium on Internal Secretions in July, Dr. C. P. Rhoads of the Rockefeller Institute read a paper in which he showed that the disease known as pernicious anemia is the result of a hemolytic process occurring in the blood stream, and that the efficiency of this process is increased by one of the "accelerators" which we had been studying; in fact, it was suggested that this substance was indol, a comparatively simple derivative of benzene, and known to be a constituent of the blood. These observations led us to undertake a detailed examination of the red

cells in pernicious anemia, using the special techniques established in this Laboratory and comparing the results with the clinical condition of the subjects, kept under Dr. Rhoads' care at the Rockefeller Hospital. So far we have not been able to obtain results on a large number of subjects, but there appears to be no doubt that the red cells of persons with pernicious anemia are entirely different from the cells of the normal person as regards the way in which they are destroyed by hemolysins. Furthermore, even though the patient is cured, so far as his symptoms are concerned, by feeding with liver, the fundamentally abnormal make-up of his red cells persists with but slight modification.

Continuing work under a grant supplied by the Ella Sachs Plotz Foundation, a general study of the reactions of the rabbit bone marrow in producing white cells when stimulated in various ways, and of the metabolism of the cells which are produced, has been completed. The results show that the younger the cell the higher its metabolism, but what is perhaps more important is that the investigations have supplied a technique whereby large numbers of white cells can be collected at almost any stage of their development.

While considering the response of the marrow to various forms of stimulation, the question arose as to whether the activity of the bone marrow is constant or changeable. Two years ago Mr. MacLeod showed by a study of blood films from different parts of the world that variations in marrow activity occur from place to place, and the question now arose as to whether they vary from season to season. He has accordingly completed an examination of blood films taken at suitable intervals from people working in the Laboratory and in the Carnegie Institution throughout the year, and has demonstrated a very significant seasonal variation. Studies of this sort are exceedingly important, although they are exceedingly tedious, for only in this way can reliable standards be laid down for other workers to refer to. A considerable part of the research of the Laboratory consists in laying down such standards, the utility of which is not often apparent until years after the work is completed.

Dr. Fricke's investigations have been of two kinds: first, the chemical effect of X-rays on solutions of a great variety of organic compounds, and particularly on certain proteins, and second, a continuation of work on dielectric constant and conductance measurements, principally on amino-acids and proteins. One of the principal effects of irradiating proteins seems to be that the proteins are "denatured", the chemical structure of the molecules being slightly, but effectively, changed. The methods developed in Dr. Fricke's laboratory allow this denaturation to be followed quantitatively, as if it were a simple reaction. Denaturation of the proteins of living cells would, of course, be very injurious to them, and might account for some of the destructive effects of X-rays in high dosage; so an attempt has been made to throw light on this point by studying the metabolism of white blood cells after irradiation. Large doses of X-rays decrease the metabolism of the cells, and otherwise injure them so that they stick together; these effects might well be due to protein denaturation. During the last year the laboratory's X-ray equipment has been used by a number of investigators, principally in connection with clinical dosimetry.

The descriptions of the researches of individual workers will be found on pages 23 to 40 of this report, and while the subjects studied cover a wide range, they are all in the broadest sense fundamental studies in quantitative biology. Dr. L. S. Moyer, holding the John D. Jones Scholarship, and working with Dr. H. A. Abramson, Dr. A. Sookne, Mrs. E. Z. Moyer, and Mr. J. C. Abels, carried out no less than eight important studies on the electrical properties of proteins and amino-acids. In one of these studies the behavior of ragweed protein was studied for the first time. Mr. Charles Lloyd, holding the Temple Prime Scholarship, carried out an investigation on the degenerative changes in the uterus produced by the withdrawal of hormones. Dr. S. R. M. Reynolds and Mr. John MacLeod completed a preliminary investigation on uterine metabolism and the influence of hormones upon it, the study being made by the same type of respirometer technique as is used for the study of the metabolism of blood cells. Dr. H. A. Abramson and Mr. Irving Ochs have developed a method for the assay of histamine, and with Dr. V. Lubkin have used the method to assay the histamine content of wheals. Dr. N. S. R. Maloeuf completed two papers, one on the rhythmic flashing of the firefly, and one on the water and electrolyte change in the hermit crab.

Mr. Abels with Mr. Ochs developed a method for the determination of organic chloride in biological fluids, and with Mrs. E. Z. Moyer attempted to quantitatively estimate chloroform in blood and urine, and Dr. Cunningham, with Miss Fishburn and Miss Huene, continued their study on water absorption of turtle eggs, on the endocrines of the turtle pituitary, and on replacement therapy in thyroidectomized rats. In addition to his work in conducting the class in Plant Sociology, Dr. Stanley A. Cain reports the publication of three papers during last year, and Miss Lora Bond, holding the Dorothy Frances Rice Scholarship, has continued some of this work in her study of the Hempstead Plains.

SYMPOSIUM ON QUANTITATIVE BIOLOGY

The fifth of the Cold Spring Harbor Symposia on Quantitative Biology was held from June 22nd to July 24th. The subject for the year was that of the Internal Secretions regarded in their more quantitative aspects, and as originally planned the subject fell into three subdivisions: I. Pituitary and Gonad Hormone Chemistry, II. Pituitary and Gonad Relations, III. Hormones and Metabolism.

Forty-five papers were read, and the number of participants, including those who took part in the discussions, was 79; at the same time the meetings were largely attended by visitors, so that the audience would usually number between 50 and 80 people. It ought to be pointed out, however, that it is not part of our policy to increase the size of the Symposium from year to year; in fact, the Symposium this year was, if anything, too large.

The papers have been published in Volume V of the Cold Spring Harbor Symposia with the title "Internal Secretions". This volume is larger than any of those preceding, there being 419 pages exclusive of the index. The increased size of the Volume is of course reflected in its

cost, principally because of the increase of half-tone reproductions, and also in its being off the press nearly a month later than had been hoped. On the other hand, the sale of the Symposia volumes is very satisfactory, the receipts being about $2,500 during 1937. Nearly the whole printing of Volume I is sold, and in view of the demand it may become necessary to print more than 1,000 copies in the future.

The subject of the Symposium to be held in 1938 is Protein Chemistry, which will include a consideration of the constitution and organic chemistry of proteins, the physical chemistry of proteins, enzymes, virus and phage, the properties of the protein hormones, the serum proteins and other related subjects. The subject of Protein Chemistry is a rapidly advancing one which can be approached from the physical, chemical, and biological point of view, and which thus answers to the specifications on the basis of which the Laboratory selects its Symposia subjects. The meetings will begin about June 21st and last until about July 23rd, and programs will be available for distribution about the middle of May.

INSTRUCTION

The number of students taking courses at the Laboratory last summer was 42, which is a considerable increase over the number last year. Of these, 33 were graduate students or Ph. D.'s.

The course in Surgical Methods in Experimental Biology was again under the direction of Dr. George W. Corner of the University of Rochester School of Medicine and Dentistry. The content of the course was essentially the same as it has been for several years past, and each year it is a pleasure to comment on its excellence and the wide extent to which it is becoming known. It is now essentially a graduate class, giving special work to those who expect to go into research. Last spring an animal room, capable of containing all the animals used in the Surgery and Endocrinology courses, was added in the basement of the Davenport Laboratory, properly equipped with cages, hot and cold water, and in charge of a whole-time caretaker. This addition has proved a great convenience. It by no means meets our requirements for animal space, however, as I shall mention later on.

Last summer for the first time in many years it was decided to give a lecture and laboratory course in Experimental Endocrinology, a subject which is not generally taught in universities, or at least not taught by means of laboratory work. The expectation that the course would be a success was fulfilled, for it was almost fully registered (10 students) with a high type of student similar to that registered in Experimental Surgery. The plan of having the lectures in Experimental Surgery and in Experimental Endocrinology in common turned out to be very satisfactory, the students in each course being able to integrate their work with that of the students in the other. The course was under the direction of Dr. Hans O. Haterius of Ohio State University and Dr. Robert Gaunt of New York University, and since the Symposium subject for 1937 happened to be that of Internal Secretions, a number of guest lecturers addressed the class from time to time. As regards the repetition of the course in 1938 and its continuance in other years, I said in my last report that the situation

was one in which one would have to be guided by events. The experience of last summer makes it clear that Experimental Endocrinology is in demand, and that it appeals to the type of student which we wish to attract to the Laboratory. Excellent although the start has been, I believe that it will be possible to build up the course further within the next few years, and particularly to make it so attractive that the students will stay on to do research during the latter part of the summer.

The course in Marine and Fresh Water Zoology was again instructed by Dr. Herman T. Spieth of the College of the City of New York and Dr. William A. Castle of Brown University. Here again it is a pleasure to report that the course was almost fully registered (12 students) and that the quality of work done was very high. This course is becoming stronger year by year, and through it Cold Spring Harbor is becoming more widely known as a place where there is plentiful material for the study of marine and fresh water fauna. Part of the success of the course must be put down to the improved conditions under which it was held, the John D. Jones Laboratory having been suitably divided up by internal partitions, and a considerable sum of money having been spent on providing both large and individual aquaria, and much other apparatus which was badly needed.

The course in Plant Sociology was again given by Dr. Stanley A. Cain of the University of Tennessee, four students being registered. It is true that Plant Sociology is a highly specialized subject which is not ordinarily taught in universities, but the question has arisen as to whether, by a change in content, we could not attract a larger number of students, leaving Plant Sociology to be taken by sufficiently advanced students as a form of research. Dr. Cain has given this question much consideration, and has put forward proposals for a reorganization in 1938. The principal proposal is that the course should be expanded so as to include field exercises in the investigation of "habitat factors", so that not only the composition and structure of a plant community would be studied, but the atmospheric and soil factors would be investigated too. This would necessitate limiting the number of plant communities which could be studied, but the intensive work could be done on three communities; one terrestrial community, one fresh water community, and one salt water community. The investigation of each community would of course include identification of local species and other taxonomic work, but this would be combined with ecological work, i. e., the investigation of environmental factors. Further, some of the field work would be followed up in the laboratory so as to be put on an experimental basis, such as the consideration of the response of different species to a single factor, or of single species to different factors. In this way the course in Plant Sociology would become much more like the course in Marine and Fresh Water Zoology, in which field work and laboratory work are about equally divided, and it is possible that the work of the two classes might be to some extent integrated. The name Plant Sociology, indeed, may have been an unfortunate selection, not because it is inapt but because it is unfamiliar. The title Plant Ecology, or the more general term Plant Communities, would probably describe the new course better.

While this plan would have more appeal for the student in botany, it would be unfortunate if the Laboratory were to retire from the field of Plant Sociology in the stricter sense of the words. Plant Sociology is a subject which is strictly quantitative and which certainly comes into the category of Quantitative Biology, which it is our policy to support. Dr. Cain has therefore suggested that a service would be rendered to the subject if during the second half of the summer the Laboratory would sponsor a short conference lasting about a week, at which time leaders in the field could define their position and bring about a much needed integration of their views. It is possible to arrange for the publication of their papers as a group in a suitable Journal, and such a conference would not only have the effect of making the Laboratory and its work better known among botanists and plant sociologists, but would also do much to prevent the sudden "drop in intellectual potential" which occurs when the Symposium is finished. We ought not to be satisfied until the numbers of people making use of the Laboratory are more equally divided between the first and second parts of the summer, although it may take some years to bring this about. The conference would also be of great interest to those associated with the course of Marine and Fresh Water Zoology, because the consideration of plant communities and that of animal communities are obviously closely related.

In past years the work of all the classes has been hampered by a lack of good microscopes; students are supposed to bring their own, but they rarely do. This year an arrangement has been made with the Bausch and Lomb Optical Company for the rental of an adequate number of microscopes (6 monoculars and 4 binoculars) with the understanding that after ten years rental the instruments become the Laboratory's property. We could not afford to purchase this number of instruments in any one budget year, but as the microscopes are returned to Rochester at the end of the summer for overhaul and safekeeping, they ought to last practically indefinitely, since modern microscopes undergo very little deterioration. The instruments are rented by the Laboratory to the students at the rental price which we pay to Bausch and Lomb, and in this way the arrangement pays for itself.

EVENING LECTURES

June 22nd. Dr. W. T. Astbury, The University, Leeds, England—"The Structure of Proteins As Revealed by X-ray Diffraction Methods".

June 29th. Dr. K. C. Blanchard, Washington Square College, New York University—"Metabolic Water".

July 6th. Dr. George W. Corner, University of Rochester School of Medicine and Dentistry—"Surgery in the 12th Century".

July 13th. Dr. C. B. Davenport, Carnegie Institution of Washington—"The Later Development of the Human Outer Nose".

July 20th. Dr. L. S. Moyer, University of Minnesota—"Latex Particles as Indicators of Plant Relationship".

July 27th. Dr. M. L. Crossley, Calco Chemical Company—"Color and Chemical Configuration".

Aug. 3rd. Dr. Eric Ponder, The Biological Laboratory—"Recent Advances in Our Knowledge Regarding the Actions of Lysins".

Aug. 10th. Dr. Harold Mestre, Bard College—"The Submarine Day".

Aug. 17th. Dr. S. A. Cain, University of Tennessee—"The Transcontinental Drift".

Aug. 24th. Mr. E. H. Anthes, Bausch and Lomb Optical Company—"The Development of the Modern Microscope".

Dr. Harold A. Abramson gave a series of lectures on "Allergy and Its Mechanisms" on the five Fridays between June 25th and July 23rd.

LIBRARY

Again we have not had much money to spend on the library, the small sum available having been largely expended on subscriptions to current journals in physiology, physics and physical chemistry. Some important additions have been made, principally in the form of standard reference books required for the courses of Plant Sociology and Marine and Fresh Water Zoology. The library situation, however, is not nearly as bad as it appears, because between our own collection and that of the Carnegie Institution we have quite a good working library of current periodicals. The difficulty comes when back numbers of the journals are required, but we have an excellent exchange service with the great libraries of the city which, when all is said and done, can be reached within an hour. For some years back it has been the hope of the Scientific Advisory Committee that special funds could be obtained to house the library in another building and to buy back numbers, but until this happens we must be content to go on as we are.

INSTITUTIONS REPRESENTED

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

Alfred University, American Museum of Natural History, Barnard College, Boston City Hospital, Brooklyn College, Brown University, Bryn Mawr College, Calco Chemical Company, Carnegie Institution of Washington—Department of Embryology, Carnegie Institution of Washington—Station for Experimental Evolution, College of Charleston, College of the City of New York, College of the Ozarks, Columbia University, Columbia University—College of Physicians and Surgeons, De Pauw University, Duke University, E. R. Squibb and Sons, Hahnemann Medical College, Harvard University, Haverford College, Hollins College, Indiana University Medical College, John Wyeth and Son, Inc., Johns Hopkins University School of Medicine, Long Island College of Medicine, Mayo Clinic, McGill University, New York University—College of Medicine, New York University—Washington Square College, Northwestern University Medical College, Ohio State University, Pennsylvania State College of Optometry, Princeton University, Radcliffe College, Rockefeller

Institute for Medical Research, Schering Corporation, State University of Iowa, Temple University, Tufts College, U. S. Department of Agriculture —Bureau of Animal Industry, U. S. Department of Agriculture—Division of Pharmacology, University of California Medical School, University of Chicago, University of Illinois, University of Leeds (England), University of Maryland, University of Michigan, University of Michigan Hospital, University of Minnesota, University of Pittsburgh, University of Rochester, University of Rochester School of Medicine, University of Tennessee, University of Toronto, University of Virginia Medical College, University of Wyoming, Wabash College, Wayne University College of Medicine, Western Reserve University, Wheaton College, Yale University, Yale University School of Medicine.

EXHIBITS

In September of this year the third annual exhibit of the work of the Laboratory was given in conjunction with the annual meeting of the Long Island Biological Association. The meeting took place in Blackford Hall, and the exhibit was preceeded by a short business meeting and a meeting of the Board of Directors. President Page gave a short account of the history of the Laboratory, and the Director gave an account of the work done during the past year. Dr. Harold Urey then spoke briefly about the value of the work of the Laboratory and of his appreciation of the support given to the Institution by our neighbors. This part of the meeting was followed by an exhibition of colored movies of insect life made by Dr. A. L. Melander, Chairman of the Department of Biology, The College of the City of New York. These beautiful moving pictures, taken with telephoto lenses, are altogether unique, and were greatly appreciated by those present.

The demonstrations held in the dining room of Blackford Hall were about as extensive as those shown last year. Dr. Fricke showed methods for detecting the chemical effects of irradiation on proteins and other substances, and Dr. Abramson and Mr. Ochs gave two demonstrations, the first of the movement of red cells in an electric field, and the second of the movement of histamine ions through the skin under an applied electric field, the result being the production of wheals. Dr. Climenko demonstrated the action of drugs on various types of muscle, and Mr. MacLeod gave a demonstration of the methods used for measuring respiration in cells and tissues. Dr. Spieth set up an exhibit, in a series of salt water aquaria, of a number of interesting invertebrates and fishes found in the neighborhood of Cold Spring Harbor and used as material for the course in Marine and Fresh Water Zoology. Dr. Davenport exhibited a number of charts illustrating the growth of the human nose and the instruments whereby the nose is measured. The exhibit also included a demonstration of microscopical objects, principally bacteria, an assembly for the extraction of lysins from blood, and a collection of beautifully mounted local seaweeds, prepared by Dr. Cain. Altogether between 150 and 200 people attended the exhibit and it is hoped that these demonstrations of the work of the Laboratory will be a permanent feature in future years. Our best thanks are due to all those who so willingly undertook to make it a success.

LABORATORY BUILDINGS

As I reported last year, it has been becoming more and more apparent that our laboratory space and equipment would have to be improved if we are to take full advantage of the increasing number of scientific visitors and to give proper instruction to an increasing number of students.

Last spring we took steps in this direction by making extensive changes in the interior of the John D. Jones Laboratory. This laboratory, originally devised as an auditorium, had been divided up by low temporary partitions into a number of small rooms; these have been ripped out and replaced by internal partitions reaching almost to the vaulted roof and making two classrooms, each sufficiently large to hold 10 or 12 students, and four research rooms for independent investigators. This partitioning leaves in the center of the building a large hall, which is used for lectures in the classes of Marine and Fresh Water Zoology and Plant Sociology, and another hallway running from east to west and containing the fresh water and sea water tables on which the aquaria are placed. The rooms for independent research workers have been fitted with sinks and cold water, and we hope to add a hot water supply next year. Large though the building is, it only comfortably accommodates the Marine and Fresh Water Zoology and the Plant Sociology classes; indeed, if these increase in size, we may be somewhat cramped for space once more.

Much needed alterations have been made in the Davenport Laboratory, which consists of a ground floor where the course in Experimental Endocrinology is taught, and an upstairs floor for the course in Surgical Methods in Experimental Biology. All the rooms have been painted throughout, and adequate sinks with hot and cold water have been built in. A small but efficient animal room has been added in the basement; this contains sufficient racks and cages to hold a moderately sized rat colony and the other animals used in the classes.

As a result of a special contribution made by Mrs. Acosta Nichols, we have been able to make considerable improvements and renovations in the George Lane Nichols Memorial. The building has been largely repainted and the seven laboratories have been put in excellent working order. Five of these are available for visiting investigators throughout the year. In addition, in order to avoid reduplication and waste, the small wing at the south end of the building has been turned into a permanent stockroom, where all apparatus not in immediate use is centralized. This stockroom acts as a supply room during the summer.

Since the Wawepex Laboratory was renovated last year for Dr. David R. Climenko, associated with the Calco Chemical Company of Bound Brook, N. J., all the laboratory buildings have undergone renovation within the past two years and are in good working order. Mr. Edward S. Harkness has generously made a special contribution which covers about two-thirds of the sum spent on laboratory improvements.

While our present laboratory buildings are now being utilized to their fullest extent, I must call attention to the fact that we still do not have the space which we require. In saying this I am not thinking of any program of expansion, but merely of the fact that when each summer's Symposium

brings to us from 30 to 60 scientists, a considerable number of whom want to do research work in collaboration with each other, we ought to be able to offer them sufficient laboratory space. This we are not now able to do, and in the future the condition promises to become serious. When the Rockefeller Foundation increased the Symposium grant from $7,000 per annum to $10,000 per annum for 1938 and 1939, this was done with the understanding that at least part of the increased appropriation should be used to encourage selected investigators to carry out cooperative research at the Laboratory. In accepting the grant, it seems to me that we are bound to take steps to supply the necessary space. In order to illustrate the point, I may remark that five of the most productive of the endocrinologists who took part in last summer's Symposium wish to come back again this year to work together throughout a large part of the summer. Our present free laboratory space would scarcely accommodate them, particularly when we remember that members of the 1938 Symposium and also various visiting investigators require laboratory space too.

What is needed is a laboratory building similar to the George Lane Nichols Memorial, although of simpler internal construction, and intended for use in the summer months only. There is room for such a building either beside the George Lane Nichols building or behind the Biophysics building, and it would be possible to construct it for something in the neighborhood of $6,000.

A second desirable addition to our present plant would be a small animal house which could be kept at a constant temperature throughout the year. The heated animal rooms in the basement of the Wawepex Laboratory are already overcrowded, and the small partially heated animal room in the basement of the Davenport Laboratory is sufficient only for the needs of the Surgery and Endocrinology classes. Every summer visiting investigators run into difficulties because there is no building in which their animals can be properly kept. The proper care of animals is an exceedingly important thing to the experimentalist, and one of the best ways to remove the objections from the minds of those who dislike the necessity of animals being used in experimental work is to show them that the creatures are kept under comfortable conditions. The most convenient location for a new animal room would be beside the proposed new laboratory, the two being connected by some sort of passageway. The construction would probably not exceed $1,000.

While speaking of our needs and requirements, I may also add that the living accommodation provided by our various houses and cottages is scarcely adequate. Even though the number of people present at the Symposia does not increase, the number of students is annually becoming greater and the members of the Symposia are tending to stay for longer and longer periods at Cold Spring Harbor. Another cottage similar to the Urey Cottage, and perhaps a small extension on the cottage known as No. 2, would ease the situation greatly; these needs, however, are not as urgent as our need for more laboratory space.

During the last year Mrs. Harris has been in charge of work on the house and grounds, and she gives the following report of what has been done during the year: "Through the generosity of Mrs. Merle-Smith, and

following the advice of Mr. Lawrence Noyes as to type of construction, we have built a fence to mark the entrance to the Laboratory grounds. As much labor as could be spared has been used for maintenance of roads, pruning of shrubs, and cutting of grass. A new incinerator is a great help in keeping the place behind the garages tidy. The splendid response to the appeals sent out by Mrs. Percy Jennings, Chairman of the House Committee, made it possible to replace much of the worn out furnishings in the apartments and dormitories. Because of a fire last spring, the Stewart Cottage had to be repaired and redecorated, and refurnishing was necessary in some of the rooms. The house is still inadequately furnished. The old paint in Williams House must be removed, and some of the walls have already been scraped and repainted. Many minor repairs and redecorating were necessary in other buildings. Our demand for household furnishings remains, as most of our furnishings are old and require constant replacements. Linoleum was laid the length of the dining room and hall in Blackford Hall. This was a gift of Mrs. Acosta Nichols."

ACKNOWLEDGMENTS

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

The National Research Council through its Committee on Radiation has again provided further for the carrying out of an investigation on the effects of X-irradiation on proteins. The American Association for the Advancement of Science has furnished a grant for the study of in vivo hemolysis. The Women's Committee has contributed money and furniture, and the Laboratory is under a special debt to Mrs. Merle-Smith and the officers of the Committee for the interest taken in the arrangements for the Exhibit, and for the many ways in which they have aided us in meeting problems relative to our houses and grounds. Our special thanks are due to Dr. M. L. Crossley of the research staff of the Calco Chemical Company, from whom we have received much help in many directions.

ERIC PONDER.

REPORTS OF INSTRUCTORS

SURGICAL METHODS IN EXPERIMENTAL BIOLOGY

The course on Surgical Methods in Experimental Biology had an enrollment of thirteen, of whom two were teachers holding doctors' degrees, five graduate students in biology and psychology, and six pre-medical students. In general standing and order of ability, last year's class compared very favorably with the high standard of previous years. The instruction was greatly benefited by the presence during the summer of several experienced workers who generously gave demonstrations and talks to the class. These included Drs. Haterius and Gaunt of the staff of the course in Endocrinology, Dr. R. L. Zwemer, Dr. Warren O. Nelson, and Dr. S. R. M. Reynolds. Members of the class all availed themselves of the opportunity to attend the lectures in endocrinology, and indeed the two courses ran in very close and successful cooperation.

GEORGE W. CORNER.

EXPERIMENTAL ENDOCRINOLOGY

This course combined, for the first time, formal lectures and daily laboratory work. The arrangement afforded students the opportunity of putting to test experimental methods and of acquiring practical experience with technical procedures. Lectures included a survey of the physiology and chemistry of the reproductive and gonadotropic principles, together with the experimental physiology of the adrenal cortex. With a brief historical approach and discussion of the classical contributions, stress was placed upon the current status of progress in these fields, with emphasis upon the original literature. Ten students were enrolled, and included recent college graduates, medical students and graduate students. Lectures were attended as well by the students in Surgical Methods.

Laboratory work proved gratifyingly successful. Working in teams, the students acquired a degree of proficiency in routine test procedures, including, for example, methods of vaginal smear determinations, physiological and histological effects of extirpation of the glands considered, physiological effects and bio-assays of gonadotropic and oestrogenic principles, pregnancy tests, experimental control of lactation, studies of the adrenalectomized animal, viz., deficiency syndrome, hormonal administration, salt therapy, water balance, electrolyte and fluid shift. Responsibility was shared by the respective teams in the preparation of histological material recovered and, through a system of exchange, each student upon completion of the course possessed a representative set of slides:—tissues of the hypophysectomized animal, before and following restorative therapy; mammary tissue, inactive, proliferated, and lactating; accessory organs under various experimental conditions, and so on.

The summer of 1937, by virtue of the Symposium subject, offered an additional unique advantage in that guest lecturers were available and obligingly consented to lecture upon their respective specialties at such times as the subject matter proved relevant to the course schedule—a circumstance

which afforded the classes the unparalleled privilege of hearing at first hand an authority in each field under consideration—a specialist, moreover, whose own contributions have been outstanding. Guest lecturers included Drs. Willard M. Allen, R. W. Bates, George W. Corner, F. A. Hartman, W. R. Ingram, W. O. Nelson, Eric Ponder, J. P. Schooley, and W. W. Swingle.

HANS O. HATERIUS,
ROBERT GAUNT.

MARINE AND FRESH WATER ZOOLOGY

The objectives of the Marine and Fresh Water Zoology course for 1937 were as follows: (1) to have the students get a first hand knowledge of living marine and fresh water animals by collecting as many species as possible in different ecological habitats; (2) to bring living specimens into the laboratory and maintain them in a healthy, normal condition; (3) to perform a number of simple but fundamental experiments upon this living material. In addition the students did sufficient reading to become acquainted with a considerable body of zoological literature.

Naturally the field trips are the fundamental basis for all of the work, and during the past summer seven fresh water and eleven marine collecting trips were conducted to various habitats. These trips averaged three per week. In addition, plancton catches were taken at various times, so that the students could become acquainted with the various marine larvae.

Lectures were given to the entire group from time to time, and were supplemented during the laboratory periods by numerous individual conferences between students and teachers. Each student conducted experiments on regeneration, axial gradients, metamorphosis, tropisms and chromatophoral responses.

HERMAN T. SPIETH
WILLIAM A. CASTLE

PLANT SOCIOLOGY

The work of the class in Plant Sociology was continued essentially along the lines emphasized in the last few years. The work falls logically under two heads which are described briefly below.

Field Work: Numerous half-day and whole-day trips enabled the class to become acquainted with the principal types of vegetation of Central Long Island. Occasional long trips extended as far as Fire Island, the Shinnecock Hills, and Montauk, where additional types of plant communities were observed. In all cases notes on the plant communities and field lists of species were made. Plants of all species requiring more careful consideration were collected for laboratory study and addition to the Herbarium. Following this procedure the composition and structure of each community became familiar to the student.

However, the course at the Biological Laboratory is not distinguished by the above procedure, which is usual in field courses, but by the introduction of detailed phyto-sociological studies on selected communities. In the

latter phase of the work statistical sampling methods are critically considered as tools for the better description of plant communities. In addition to the obtaining of data to illustrate the concepts of density, frequency, coverage, constancy, fidelity, sociability, etc., of species, and the structure of phytocoenoses in terms of life-form groups (synusiae), careful consideration was given to the sampling methods themselves. Problems in the determination of minimal area for associations, minimum quadrat size, minimum quadrat area, etc., by the method of the species-area curve were studied. Such procedure offers the student training in the methods of community study as well as in the concepts concerning the community itself.

For the first time in recent years, studies on habitat factors were added to the field work. The students collected soil samples for a study of acidity and the mechanical analysis of their structure. It is planned to extend this phase of the work in future years, concentrating more and more on a few selected communities which will be given detailed study, not only as to their structure and composition, but as to the causes of their occurrance.

Laboratory Work: The value of field work is largely lost if there is not a close follow-up in the laboratory. This work falls into two phases. Unknown or critical species (in all the taxonomic groups) are identified by the use of the standard manuals and reference to the Herbarium. Many of the students here obtain their first experience with the more difficult groups which are frequently avoided in local flora courses (as for example: algae, mosses, liverworts, grasses, sedges, etc.). It is our impression that the course is successful as a local flora or taxonomic course, although this phase of the work is only incidental inasmuch as a knowledge of the flora of a community is fundamental and preliminary to the phyto-sociological considerations.

The second phase of the laboratory work is concerned with the development of the sociological data obtained from community sampling, the results of which are expressed in tabular and graphical forms for the description of individual communities and the comparison with other types and conditions.

During 1937 especial attention was given to marine algae, the grasslands of the Hempstead Plains, the salt marshes, swamp forests, and the vegetation of the sandplains near Seldon.

STANLEY A. CAIN.

REPORTS OF INVESTIGATORS

Mr. Jules C. Abels' Report

New York University College of Medicine

1. A simple and rapid micro-method for the determination of organic (aliphatic) chloride in biological fluids was developed with the assistance of Mr. Irving Ochs. The technique is principally that which I have used for microdeterminations of alcohols and ketones (Proc. Soc. Exp. Bio. & Med. 34, 504, 1936; J. B. C. 119, 663, 1937). A 0.5 ml. sample of blood, urine, etc. is pipetted onto a cm. length of cotton pencil which is suspended from the under side of a cork by means of a pin with a beaded head. This cork fits very tightly in a 50 ml. Erlenmeyer flask. On the bottom of this flask there has been previously pipetted alcoholic KOH; the cotton roll with its absorbed blood (etc.) is thus suspended about 1 cm. above the alkali. The flask is heated to 100° C., the volatile organic chloride is absorbed by the alcoholic alkali and hydrolysed to inorganic KCl under the developed pressure of about 2 atm. After the alkali is made acid to methyl orange with HNO_3, $AgNO_3$ is added and the haze of AgCl developed is estimated turbidimetrically by comparing with standards made from known amounts of KCl in KNO_3. By this means we have thus been able to estimate as little as 5 gamma of aliphatic chloride with an accuracy of about 5 per cent. We expect to present this method for publication in the near future.

2. With the above technique, Mrs. Elsie Moyer and I have attempted to quantitatively estimate $CHCl_3$ specifically in blood and urine. The chloroform is here absorbed by a mixture of conc. NaOH and pyridine, with the consequent production of red colors. This color is matched against standards made from basic fuchsin. It is possible by this means to detect 2 gamma of $CHCl_3$, but as yet we have been unable to make satisfactory recoveries. This work will be continued during the winter at New York University Medical College.

3. The investigation begun last summer with Dr. Laurence Moyer, of the acid (base) binding and electric mobility of egg albumin solutions at constant ionic strength was completed. We have been able to calculate the charge on this protein molecule both by an electrokinetic and thermodynamic method. The evident agreement obtained between these two means has evidently substantiated the electrokinetic theories. We have also been able to study the effect of ionic strength on the electric mobility at given pH, and to develop a theoretical expression which allows the calculation of electric mobilities of proteins from their acid (base) binding curves. This work will soon be published in the Journal of Biological Chemistry.

Dr. Harold A. Abramson's Report.

College of Physicians and Surgeons, Columbia University.

The mechanisms of skin reactions (wheals) ordinarily observed in the clinical study of the allergic individual have been believed for many

years to be dependent upon the liberation of histamine. As part of a comprehensive program of research for the examination of the role played by histamine in producing allergic wheals the following problems were begun or continued in the summer of 1937:

1. With Mr. Irving Ochs has been developed a micro-method for the assay of histamine. This assay depends upon the introduction of histamine into the skin by electrophoresis. Histamine has been recovered from the blood of animals dying of histamine shock.

2. With Mr. Arnold Sookne and Dr. Lawrence Moyer the physical chemistry of ragweed protein has been investigated.

3. With Dr. Moyer, a study of the surfaces of simple ampholytes was continued, especially in connection with the chemistry of the surface of the pores of the skin.

4. The theory pertaining to the iontophoretic and electroosmotic introduction of drugs into the skin was briefly developed.

5. With Dr. Moyer further attention was given to the correlation of the electric mobility of proteins like the serum proteins and the capacity of the proteins to combine with acids and bases. A simple method of calculating the radii of proteins from their electric mobilities was developed.

6. Using the method described above, the histamine content of ragweed wheals, (Dr. V. Lubkin) as well as wheals produced by light and by stroking was investigated. Reversed iontophoresis and diffusion were employed. The commonly accepted belief that histamine is liberated in the allergic wheal could not be confirmed.

Miss Lora Bond's Report

(Dorothy Frances Rice Scholar)

The University of Tennessee

Cain, Nelson and McLean (Amer. Midl. Nat. 18: 334-350, 1937) published a phytosociological study of the "Andropogonetum Hempsteadi: a Long Island Grassland Type" in which they described the structure and composition of this eastern prairie. The present investigation, looking toward an explanation of this grassland, concerns the soil conditions and general ecology of this vegetation-type as represented by an original stand located near Locust Grove (Station VI of the above report).

The vegetation was recorded on a strip transect 10 m. wide extended from the upland prairie across a shallow post-glacial valley. The coverage of the dominant species was recorded by 100 sq. m., blocks along this transect. The density of larger forms, such as Baptisia, and the presence of all other species was also indicated for each block. From soil wells, located at 10 m. intervals and dug to the underlying gravel-pan, samples were taken from each natural horizon. At these levels the following studies were made: (1) soil acidity; (2) mchanical analysis by the hydrometer method; (3) root concentration. Preliminary study of root types relative to competition with the dominant grass was started. Publication on this investigation awaits further work.

Dr. Stanley A. Cain's Report

The University of Tennessee

Most of the month of July, preceding the opening of the class in Plant Sociology, was spent at the Laboratory on completion of the manuscript of the work done with Dr. Penfound on "Aceretum rubri the Red Maple Swamp Forest of Central Long Island." This study presents a detailed phytosociological analysis of the structure and composition of all of the synusiae of this forest phytocoenosis which is common along the sluggish streams of the South Shore of Long Island and about some of the inland lakes and ponds.

Activities for the remaining six weeks are presented under the report concerning class work in Plant Sociology.

Dr. David R. Climenko's Report

Pharmacological Laboratory
Calco Chemical Company

During the course of the last year the scope and the personnel of the laboratory has widened considerably. Dr. Ellis J. Robinson has joined our staff to take over the work on the toxicity of simple cyclic compounds. A bacteriological laboratory has been established, and Mrs. R. L. Schmidt and Miss N. S. Gibby have taken over the technical bacteriological work.

1. Sulfanilamide derivatives. A series of sulfanilamide derivatives synthesized by Drs. M. L. Crossley and E. H. Northey of Bound Brook have been examined in a search for a non-toxic, effective chemotherapeutic agent. It is felt because of the high degree of specificity associated with sulfanilamide itself in the treatment of infectious diseases of streptococcal and gonnococcal origin that similar types of substances will be equally effective in the treatment of other infectious diseases of bacterial origin.

In the course of the last few weeks it has been pointed out that one of these derivatives is a specific therapeutic agent in the treatment of dog distemper. Dog distemper is a virus disease and this represents one of the first attempts at handling virus disease from a chemotherapeutic point of view. The success which has been met with in treating dog distemper encourages further application of this group of compounds to those virus diseases such as influenza, anterior poliomyelitis, measles, etc., encountered in human pathology. The first publication of results of this investigation will be given at the meetings of the American Chemical Society late in March at Dallas, Texas. Subsequent publication will be made at the American Medical Association meeting.

2. Pharmacological and toxicological studies of benzol and the simple cyclic compounds, such as aniline, toluol, nitrobenzol, etc., which have been made over a period of almost two years at this laboratory, are being continued. The work up to the present time has been confined almost entirely to the effects of benzol in order to afford a base line for comparative results. Changes in the blood, the blood-forming organs and in

the resistance of the blood cells have been studied extensively by Dr. Robinson, and these results are to be described at the meeting of the American Physiological Society in Baltimore on March 30.

An extended study has been carried out on the effects of these compounds on cardiovascular reflex mechanisms and a specific effect of benzol has been observed: benzol is capable of paralyzing the peripheral vasoconstrictor mechanism of the body even when present in minute concentrations. This reaction is a very temporary transient one, but it is of extreme importance from a practical point of view.

3. Cardiac Glucosides. A study has been carried out on the cardioactive glucosides of squill (Urginea maritima) and this action has been compared with that of the more commonly known glucosides of digitalis and strophanthus. It has generally been supposed that the action of the cardioactive glucosides is identical regardless of their source. We have been able to show a number of qualitative as well as quantitative differences in the activity of these substances. For example, the rate of cumulation and the ease of reversibility varies tremendously. The results of this investigation have been submitted for publication in the Journal of the American Pharmaceutical Association.

4. Quinoline derivatives. A series of chlor-derivatives of quinoline have been synthesized at Yale University under the direction of Professor A. J. Hill and have been examined here for their bactericidal and bacteriostatic properties. The objective in this search was the production of a germicidal substance containing the active oxychloramine group associated with an equally active nucleus. Up to the present time no practical substance has been found.

5. Depressant drugs. An investigation has been carried out on the effects of a series of urethane, biuret and barbituric acid derivatives in the search for a more effective sedative and narcotic agent than is available at the present time. These substances have been synthesized at New Haven. At the present time no definite conclusions may be made regarding their effectiveness.

Report of Dr. Bert Cunningham, Miss Margaret Fishburne, and Miss Elizabeth Huene

Duke University

During the past summer studies were made here and at Duke University upon quantitative replacement therapy in thyroidectomised rats. The data have been assembled and a paper prepared which will appear in Endocrinology. The standard used to determine the complete replacement was the heart rate. Similar experiments using the basal metabolic rate as a standard are being planned for the coming summer.

We were very fortunate in securing a sufficient number of the eggs of the painted turtle, the snapping turtle, and the box turtle to complete our studies on water absorption by these eggs during incubation. The data have been assembled and a paper prepared which is now ready to

be submitted for publication. So far as the species available at the Laboratory are concerned this study may be considered as completed.

The second problem under investigation by us, namely, the endocrines of the turtle pituitary, has progressed satisfactorily. We collected some 300 painted turtles and shipped them to Duke University where they have been assayed from time to time. The results are as yet too incomplete to make any definite report but there are indications that another (other than prolactin which we reported last year, and concerning which a paper has already appeared) of the common endocrines is present in assayable quantity. Final reports can not be made until the histological preparations have been studied. These studies should be continued upon other hormones which may occur in turtle pituitaries.

Dr. Hugh Davson's Report

University College, University of London

The problem of the nature of the residue from hemolysis, commonly called the "ghost" was investigated. Work done hitherto indicated that the ghost had many points of similarity to the erythrocyte, and consequently there was a tendency to consider the process of hemolysis as a reversible one. If this view holds, the most characteristic property of the erythrocyte, namely its impermeability to cations, might be expected to be retained by the ghost. Experiments carried out, however, indicate that this is not so, the ghost being rapidly permeable to both Na and K. The problem was further investigated by studying the fading times of erythrocytes subjected to varying hemolytic conditions, and the conclusion was reached that the process of hemolysis in hypotonic solutions is by no means a simple bursting of the cell membrane, but is to be considered as the result of an irreversible deformation of the membrane, whereby the size of the pores becomes great enough to allow the passage of the large hemoglobin molecule. This work was done in collaboration with Dr. Eric Ponder.

Dr. Hugo Fricke's Report

Biological Laboratory

The effect of X-rays on solutions of a great number of different organic compounds has been studied by potentiometric acid analysis. An extensive study has been made of the effect of irradiating crystalline egg albumin by gas analysis, acid analysis, different types of amino acid analysis, and finally by heat precipitation analysis. The effect of the rays on the protein in solutions containing other organic materials was also investigated. Dielectric constant and electric conductance measurements on amino acids and on different proteins, native and denatured, were made at different values of frequency, concentration, pH, and ionic strength. The work with Dr. Demerec on the effect of X-rays on Drosophila was finished and published in the Proceedings of the National Academy of Sciences, Vol. 23, p. 320, 1937. The work on the dielectric properties of water-dielectric interphases was also finished and published in the

Journal of Physical Chemistry, Vol. 41, p. 729, 1937. A report of the work on the chemical effects of X-rays was presented at the Symposium on Biological Effects of X-rays at the Atlantic City Meeting of the American Physical Society. An abstract of this report will be found in Physical Reviews, Vol. 51, p. 376, 1937. An extensive report of this work was submitted for publication in the Journal of Chemical Physics. Dr. William Preston, Harvard University, used our equipment for X-ray dosage measurements during the summer months in work on chemical dosimetry. Dr. N. S. Royston Maloeuf used our soft X-ray radiographic apparatus in work on the excretion of metal salts in insects. Work on the effects of X-rays on leucocytes determined by gas metabolism studies was carried outin cooperation with Dr. Ponder. During the spring of 1937 our Standard X-ray dosage apparatus was placed at the disposal of Dr. G. Bucky, New York City, for use in his work on developing a practical clinical dosimeter for soft X-rays.

Dr. A. J. Grout's Report

Biological Laboratory

Volume 1, part 1, of the Moss Flora of North America, was issued late in October 1936. Volume 1, part 2, was issued in August 1937.

In late 1936 and early 1937 I prepared manuscript to finish the Dicranaceae. Dr. Flowers furnished the new and original illustrations for this group, covering all those not previously illustrated in other works and available for reproduction. Mr. H. N. Dixon loaned four full page cuts from his Handbook of British Mosses to illustrate several species common to both countries.

This part of Volume 1, part 2, was in print by April, but Dr. Frye did not send in his manuscript on the Polytrichaceae until May; however, he furnished a full set of original illustrations, excellent in every way.

I have prepared manuscript for several genera of the Pottiaceae. Dr. Wm. C. Steere of the University of Michigan is at work on other genera of the same family. Dr. Flowers has already completed manuscript and illustrations for the Encalyptacee. Dr. Andrews thinks he will be ready with the rest of the Bryaceae by next summer, completing Volume 2. Thus it seems that the manuscripts for the remaining three parts will be ready early in 1939, and at least one part in 1938.

Of Volume 1, part 2, I printed only 1,000 copies instead of 1,500 as before, and this is the number I propose to print of the remaining parts, bringing the cost under $1,000 for each part.

The usual large number of plants for colleges and students has been determined, including very interesting collections by Pere Dutilly in Northern Quebec and northward and by Dr. E. T. Bodenberg on Mt. Hood. Many rare and unusual arctic-alpine plants were collected by each.

Prof. R. T. Wareham of the Ohio State University and Dr. John E. Potzger of Butler University spent about seven weeks at Newfane studying Ohio and Indiana mosses respectively. Many interesting extensions of range were made. Dr. Guilord J. Ikenberry of the Portales, New

Mexico Junior Teacher's College and Miss Mildred Wickes of the Huntington High School, N. Y., also passed a portion of the summer studying with me. One of the professors at the University of Montreal wished to come for the summer, but I did not feel equal to a larger number. All four students were very competent, and expressed pleasure as to the surroundings and studies.

Incidentally the Moss Flora and the knowledge obtained in its preparation has enabled the identification of many plants heretofore unidentifiable, and has extended the ranges of many interesting species and contributed to our knowledge of plant distribution. A check up of my herbarium shows about 25,000 specimens, including some of the important American and European exsiccati. These and more than 3,000 microscope mounts, together with a relatively large bryological library, offer students an excellent opportunity for study. I strongly feel that it is to the interest of the Laboratory as well as myself that the Newfane Summer School of Bryology be continued under the Laboratory's auspices.

Mr. Charles E. Lloyd's Report

(Temple Prime Scholar)

University of Rochester School of Medicine and Dentristy

This work was undertaken to test an hypothesis tentatively suggested by Robson and Henderson of Edinburgh. They attempt to compare the pro-estrous bleeding of bitches to menstrual bleeding of Primates. They find that the histological changes occurring at pro-estrus are dependent on the action of estrin, and that the amount of hormone secreted under normal conditions must be considerable, since the large amounts they used failed to elicit comparable changes in the genitals of bitches. Meyer and Saiki (1931) concluded that the mechanism producing uterine bleeding in the bitch and in Primates is not similar because of differences in the temporal relation between estrin secretion and bleeding. Robson and Henderson found an analogous temporal relation in the two genera and conclude from the evidence available that uterine bleeding occurs only when there is a diminution in the rate of secretion of estrin which has caused the initial alterations in the uterus, external genitals, etc.

When the corpus luteum is formed following ovulation, a different condition pertains in the bitch than in the Primate, according to Robson and Henderson. It is probable that in the bitch the luteal proliferation of the uterus is not accompanied by any great estrin secretion and hence the cessation of luteal activity is not followed by any great diminution in estrin secretion and therefore no very profound degenerative changes occur in the uterus. In Primates, on the other hand, the luteal phase of the menstrual cycle is accompanied by a high rate of estrin production and a large part of this estrin is from the corpus luteum itself. Therefore, degeneration of the corpus luteum is accompanied by a large fall in estrin secretion which accounts for the degenerative changes constituting menstruation.

Robson and Henderson state that a condition of estrus when the

luteal activity is decreasing can be artificially produced in the bitch and that withdrawal of estrus produces uterine bleeding. The purpose of our experiment was to attempt to produce the same condition in rabbits and to study the degenerative changes in the uterus produced by withdrawal of the hormones.

The rabbits were castrated at the beginning of the experiment, since it would have been impossible to control or accurately ascertain the amount of hormones produced by their own ovaries. The animals were divided into three groups, two groups, of controls, and one experimental group. The procedure for the three groups was identical. The animals received daily injections of the hormones for ten days and then the hormones were withdrawn. Animals were killed on different days after withdrawal of the hormones and the uteri were preserved for histological study. Daily vaginal washings were made of all the rabbits and examined for red blood cells as indication of uterine bleeding. One group of 5 controls was injected daily with 125 International Units of estrin (Amniotin) alone. The second group of 5 control rabbits received daily 1 mgm. of progesterone alone. The experimental animals received daily 1 mgm. of progesterone and 125 International Units of estrin. These amounts of hormones were chosen as being within the probable physiological limits of secretion by normal animals.

The control rabbits were killed on the 3rd, 4th, 5th, 6th and 9th days after withdrawal of the hormones. The experimental animals were killed on the 3rd, 4th, 5th, 6th, 7th, 8th and 9th days after deprivation of the hormones. During the experiment it was noted that nearly all the experimental rabbits showed red blood cells in the vaginal washings, generally on or about the 4th or 5th days of deprivation. None of the control animals had any red blood cells in the vaginal washings at any time. Histological section has shown that the estrin controlled uteri underwent a typical castrate retrogression, and that the progesterone rabbits also underwent a retrogression, as was to be expected after the progesterone proliferation. Some of the experimental rabbits showed a considerable amount of degeneration.

No conclusions can be drawn at this moment, since the great degeneration shown by the uteri deprived of progesterone and estrin must be confirmed and explained by further histological study. The final results will be reported in detail in a paper to be published in the near future.

Thanks are due to Dr. Corner of the University of Rochester, and to Drs. Willard M. Allen and Samuel R. M. Reynolds.

John MacLeod's Report

The Biological Laboratory

During the twelve months from October 1936 until September 1937, the blood smears of 20 individuals in residence at the Carnegie Institution and the Biological Laboratory were taken at regular monthly intervals, and the differential blood picture in each case was studied carefully. We were interested, particularly in the polymorphneutrophils and the polynuclear count. For many years, Dr. Ponder and his co-workers have analysed

the polynuclear count in various conditions in humans and, experimentally, in animals. The general conclusion is that the polynuclear count in health is remarkably steady, but that it is exceedingly sensitive to any deviation in the healthy state. However, recent work done in various parts of the world by workers using the Cooke and Ponder criterion has shown that the individual count varies with the environment. In other words, the healthy polynuclear count in Great Britain differs from that in China, and the count in Egypt is not the same as that in Denmark. The general opinion is that climatic differences are responsible. Therefore, it seemed possible that the count in any one locality might be subject to seasonal variation, and we decided to investigate this possibility in a fixed population in Cold Spring Harbor where climatic changes during the year are of the extreme variety.

The results showed that there is a marked shift to the left in the polynuclear count during the winter months and that this shift is not due entirely, if at all, to infected states. During the spring and summer months the count slowly returns to "normal", reaching the healthiest mean in September.

The ratio between the various types of white cells (the differential white blood count) was also studied, but no seasonal variation was found. In fact, it remained remarkably steady during the course of the investigation.

This paper is in press and will appear in an early number of the American Journal of Physiology.

While Dr. Reynolds was participating in the Symposium of 1937 he took advantage of the special facilities of the Laboratory to study a problem relating to the action of estrin on the uterus and, in particular, the effect of estrin on the 0_2 consumption of uterine muscle. Two main groups of experiments were performed. In one, the 0_2 consumption rate was measured in a group of untreated rabbits ($3\frac{1}{2}$ to 4 months of age), five of which were intact and the remainder of which (21 animals) were ovariectomised for periods varying from 2 to 15 days. In the second group, the 0_2 consumption rates were measured at intervals of 5, 10, 18, and 24 hours after a single intramuscular injection of 500 I. U. of estrin to rabbits ovariectomised from 9 to 11 days. It was found that a progressive increase in 0_2 consumption (up to 60 per cent) takes place after the administration of estrin to an ovariectomised animal, the 0_2 consumption being maximal at 24 hours. The initial hyperemia produced by estrin precedes any appreciable rise in metabolic activity of the tissue and at the tenth hour, when the muscle commences rhythmic activity, the metabolic effect is two-thirds complete.

It was found, also, that the rate of 0_2 consumption in the uterus of normal, intact animals is higher (28 per cent) than that of uteri taken from rabbits ovariectomised for about ten days. The removal of the ovaries from immature rabbits deprives the uterus, immature and undeveloped as it is, of a potent substance which activates the 0_2 consumption of the uterine tissue. This agent is presumably estrin.

This paper appears in the February 1938 issue of the Proc. Soc. Exp. Biol. and Med.

Dr. N. S. R. Maloeuf's Report

Yale University

The work completed during this summer is contained in two published papers.

1. The Basis of the Rhythmic Flashing of the Fire-fly. The tracheal end-cell theory of the control of flashing in fireflies is shown to be quite untenable. Cogent evidence is presented to show that flashing is the result of a rise and fall in the osmotic pressure of the photogenic cells. This phenomenon is under spontaneous cerebral control in the normal animal but can be imitated by the injection of hypertonic solutions or by partial asphyxiation, thus producing a continuous glow. (Ann. entom. Soc. Amer. 1938, in press.)

2. Water and Electrolyte Exchange in the hermit-crab, Pagurus longicarpus. When hermit-crabs, of the small species P. longicarpus, of 0.5 gm., or below, in wet weight are placed in 50 per cent sea water they rapidly increase in weight for approximately the first two hours (ca. 28°C) after which they decrease in weight even to values below the initial. These individuals can live in 50 per cent sea water indefinitely. The blood becomes isotonic with the sea water by the time the maximum weight is attained, and the subsequent decline in weight is due to the discharge of an isotonic body fluid, presumably urine. The crabs do not behave like perfect osmometers because they lose salts to the exterior. This loss is not rapid at the outset, i.e. when the animals are absorbing water. They do not drink, water loss and salt seepage occurring by way of the body wall.

Animals above 0.5 gm. generally cannot discharge a copious isotonic body fluid when in 50 per cent sea water and consequently perish after maintaining an approximately constant weight for some time. All sizes can generally regain their initial weight in 75 per cent sea water.

The rate of oxygen consumption is decreased by hypotonic sea water even after equilibrium is attained. In animals which regain their initial weight in hypotonic sea water the heart rate is not noticeably reduced. Heart rate does not, therefore, necessarily parallel metabolic rate.

Like other completely stenohaline Crustacea, their kidneys have no urinary canal. Probably for the first time in the Brachyura, a pair of renal nerves, issuing from the tritocerebral nerve of the brain, is described. (Arch. intern. de Physiol. 1938, in press).

Dr. Laurence S. Moyer's Report

(John D. Jones Scholar)

University of Minnesota

During my tenure of the John D. Jones scholarship last summer, I engaged in the following work:

1. The electrophoresis of adsorbed egg albumin. (L. S. Moyer). The properties of protein surfaces have become of increasing importance, especially in biological systems. In view of their complexity, it seemed

desirable to examine in some detail the characteristics of absorbed surface films of proteins, of protein particles, and the influence of the adsorption process on the electric properties of the surface. Abramson had noted a small discrepancy between the electric mobility of dissolved egg albumin investigated by the U-tube method of Tiselius and his own experiments on adsorbed egg albumin. The present experiments deal with solutions of highly purified egg albumin adsorbed on a number of particles. Under our conditions, the isoelectric point of egg albumin adsorbed on "inert" surfaces, such as quartz, glass, mineral oil, carbon and collodion, lies at pH 4.82, in complete agreement with the results of Smith. The electrophoretic mobility-pH curve is identical for all of these surfaces when coated with egg albumin and suspended in buffers of constant ionic strength. The curve is parallel to the curve given by Tiselius for dissolved egg albumin, but shifted upward on the pH scale. Egg albumin adsorbed on silica gel or aluminum oxide particles yielded the same isoelectric point, but the curve slopes were shifted. The electric mobility curve of surface-denatured egg albumin freely dispersed or adsorbed on quartz is correspondingly displaced, with pH 5.02 as its isoelectric point, but parallel to that of egg albumin on "inert" surfaces. The shift of the curve slope by adsorption on silica gel or aluminum oxide is looked upon as an effect on the radius, whereas the shift in isoelectric point on adsorption is probably due to a removal of electrons incidental to the protein adsorption. If denatured, the egg albumin surfaces which are isoelectric at pH 4.82 are not identical with egg albumin denatured at an air-water interface. These results emphasize the importance of investigations of the properties of adsorbed materials at liquid-liquid or solid-liquid interfaces in the interpretation of absorbed films on biological surfaces. This paper has been presented before the Fourteenth Colloid Symposium at its Minneapolis meeting (J. Phys. Chem. 42, January, 1938).

2. Electrokinetic theory in the calculation of the charge of proteins. (L. S. Moyer and J. C. Abels). It is of importance in the determination of the charge of protein molecules to arrive at the desired result by two independent methods. The method usually employed is the thermodynamic method; knowing the effective molecular weight of the protein and the number of grams present in a given solution whose pH and activity coefficient are known, the charge can be determined. Another method, not thermodynamic, but which depends upon conductance, rests upon direct measurement of the mobility of the protein ion itself.

The titration curve of solutions of crystalline egg albumin was determined at constant ionic strength. From these data the charge was calculated by the thermodynamic method. Electric mobility data of Tiselius for dissolved egg albumin at the same ionic strength were employed to calculate the charge per molecule by the use of electrokinetic theory. Comparison of these calculated charge-pH curves over the range, pH 4.0-pH 5.5, shows excellent agreement between the two methods thus substantiating the electrokinetic theories in their simple form. Electrokinetic theory also permits the determination of the effect of ionic strength on the electric mobility at given pH. Application of these considerations to data for egg albumin and phycoerythrin has completely confirmed the

theoretical assumptions. (J. Biol. Chem., 121, 331, 1937).

3. The ratio of electroosmosis to electrophoresis. (L. S. Moyer). The demonstration that the electrophoretic mobility of inert particles coated with protein is the same as the electroosmotic mobility of the solution past the wall of the electrophoresis cell when it too is coated with the same protein is of considerable importance in the verification of the theories of electrokinetic phenomena. A previous paper by Moyer and Abramson had shown that the ratio of these mobilities was equal to unity at concentrations of gelatin down to 0.02 per cent, even in the absence of added salt. Recently, however, it has been claimed that lower concentrations of gelatin (at about 0.01 per cent), although sufficient to give a complete coating to the surfaces involved, yield ratios which are greater than 1. Following our usual technique, quartz particles were placed in strong solutions of gelatin until coating was complete. The solution was then diluted to 0.001 per cent gelatin and introduced into an electrophoresis cell whose walls had been coated by contact with strong gelatin solutions. Under these conditions, the ratio was not significantly higher than unity, although the protein concentration in the solution was very dilute indeed. These results substantiate the validity of the equations of electrokinetics in their simpler form. It is probable that divergent values of other workers have been secured through the presence of incompletely coated surfaces. This paper has been submitted to the Journal of Physical Chemistry.

4. Comparison of the isoelectric points of dissolved and crystalline amino-acids. (H. A. Abramson and L. S. Moyer). An investigation of the electric mobilities of crystalline amino-acids has been carried out. The results show that although the isoelectric points of dissolved cystine, tyrosine, and aspartic acid molecules lie at widely differing pH values, the isoelectric points of the surfaces of these substances in the crystalline state are all near pH 2.3. This was found to be true in solutions of hydrochloric acid and in acetate buffers. When suspended in gelatin, tyrosine and cystine crystals adsorb the protein and attain a surface identical in behavior with gelatin-coated quartz or collodion particles. Albumin ions at low concentrations reduce the electric mobilities of tyrosine crystals to zero in a manner analogous to their effort on other surfaces. It seems probable that these phenomena may be accounted for by the return of dissolved amino-acids to the surfaces of the crystal lattice. At pH values below the isoelectric point of the dissolved amino-acid, the entering molecules would bring with them a positive charge which, together with the adsorption of ions by the crystal, would account for the change in its sign as the pH is reduced. These results are discussed in connection with the concept of the general definition of the isoelectric point and the behavior of certain insoluble proteins such as wool and silk fibroin.

5. Comparison of the electric mobilities of adsorbed and dissolved proteins. (L. S. Moyer). Abramson had shown that the electrophoretic mobilities of quartz particles coated with adsorbed serum albumin molecules were the same as the electric mobilities of the dissolved protein itself. Adsorbed egg albumin was found to be very close, although not identical in behavior with dissolved egg albumin molecules. This

comparison afforded the best evidence for the accuracy of the microscopic method of electrophoresis, comparing as it did data obtained by the microscopic and the U-tube methods. Since then the writer has demonstrated that the agreement between the data for adsorbed and dissolved egg albumin is less than had been supposed. This left the agreement between the two sets of data for serum albumin as one of the few checks of the validity of the microscopic method. It was therefore desirable to reinvestigate the problem in some detail. The electric mobilities of collodion particles when coated with solutions of crystalline serum albumin were found to be in complete agreement with the data of Abramson on the mobilities of quartz particles coated with this protein and also with the data of Tiselius for the dissolved serum albumin measured in a U-tube. In addition, complete agreement was secured over the range, pH 4 to pH 8 between the mobilities of adsorbed pseudoglobulin and the mobilities reported by Tiselius for this protein in a dissolved state (J. Biol. Chem., in press).

6. Mixed protein solutions. (L. S. Moyer and E. Z. Moyer). It was found that in solutions containing both gelatin and egg albumin, the gelatin confers its electrokinetic properties to the surfaces of quartz or collodion particles when these are introduced to the solution. If quartz particles initially coated with egg albumin are placed in solutions of gelatin or if quartz particles coated with gelatin are placed in solutions of egg albumin, the result is in both cases the same: the quartz particles take on the electrokinetic properties of the gelatin. In mixtures of serum albumin and gelatin, the result appears to be more complicated. At low concentrations of gelatin, the surface is virtually serum albumin in its properties, but as the gelatin concentration is increased, the particles assume the electrokinetic properties of gelatin. The electric mobilities of serum albumin, pseudoglobulin, and the total globulin from horse serum have been determined over the range pH 4 to pH 8 at an ionic strength of 1/10. This permits the comparison of serum proteins in mixtures. Preliminary results show that the total globulin and the pseudoglobulin are selectively adsorbed when either of these proteins is mixed with solutions of serum albumin. The nature of the underlying surface appears to have very little effect upon the final result. Further experiments are in progress, and it is expected that a more detailed report will soon be published.

7. The behavior of quartz particles in aqueous extracts of ragweed pollen. (H. A. Abramson, A. Sookne and L. S. Moyer). Preliminary investigations have been carried out on this problem and indicate that quartz particles placed in extracts of giant ragweed pollen adsorb a component which has all the electrokinetic characteristics of protein with regard to its isoelectric point and the shape of its electric mobility-pH curve. This adsorption takes place from solutions of low nitrogen concentration, further increases in concentration having little effect. Titration curves of the extract have a shape quite similar to the electric mobility curves. The component taken up from the solution by the particles appears to be quite definite; for both mineral oil droplets and quartz

particles, although markedly different in their own electric mobilities, in the presence of the optimal concentration of the extract lose their own characteristics and become identical in electric mobility. Scratch tests on patients sensitive to ragweed indicate that the component adsorbed by the quartz is an active agent in wheal formation. These results are of a preliminary nature, but we hope to pursue further investigations in this field.

8. Relationship between electric mobility and titration curves of serum proteins. (L. S. Moyer and H. A. Abramson). A comparison of the curves of base (acid) binding and electrophoresis of horse serum albumin and of pseudoglobulin showed close agreement in shape over the range pH 4 to pH 8, thus confirming the conclusion of Abramson that in solutions of the same ionic strength the two curves should be proportional. It was found that electrokinetic theory offers a means of calculating the effective radius of protein molecules from electric mobility data at two ionic strengths. Use of the fact that the charge is nearly identical when calculated from titration curves and electrophoretic measurements on proteins permits the estimation of the molecular weights of proteins from data of this sort. Radii and molecular weights were calculated for several proteins for which data was available and it was found that the results agree closely with values obtained with the ultracentrifuge by Svedberg and his colleagues.

Dr. Eric Ponder's Report

The Biological Laboratory

The investigations carried out by myself and my collaborators are best reported on under the heads of the papers in which they are being published.

1. Peritoneal exudates in rabbits. For some time past we have been producing peritoneal exudates in rabbits, by injecting large volumes of saline, for the purpose of obtaining large numbers of leucocytes. The amount of fluid injected is very large, and greater than the blood volume of the animal, and the number of white cells which leave the blood stream of the animal to appear in the exudate is greater than the number which the blood stream normally contains. The bone marrow, however, promptly produces new cells of a juvenile type, and the blood stream is flooded with these. This response of the marrow is probably the result of the absorption of products of breakdown of the cells in the exudate. If the exudates are repeatedly produced at sufficiently short intervals, the cells produced by the marrow become younger and younger, so that the blood stream becomes successively filled with very young polymorphs, metamyelocytes, and myelocytes. Finally the animal dies with a severe leucopenia.

Studies of 0_2 consumption of the cells of the exudate, made in Fenn respirometers, show that the 0_2 consumption per million cells per hour steadily increases as the cells become more immature. Under conditions where the predominating type of cell is the metamyeloctye and the myelocyte, the 0_2 consumption may be increased to over 4 times the normal.

This work is part of an investigation which is being carried on under a grant from the Ella Sachs Plotz Foundation, and will shortly be published as two papers in the Journal of Experimental Medicine and the Journal of General Physiology.

2. Acceleration of hemolysis by benzene derivatives. A study of the hemolytic effects of the triphenylmethane dyes by Blum, both in the dark and in the light, has shown that the order of activity of these substances is fluorescein, eosin, erythrosin, rose bengal, and the same result has been obtained in an investigation of the concentration of the dyes necessary to produce prolytic spheres. Since fluorescein contains no halogens, while eosin has four Br, erythrosine four I, and rose bengal either four I and two Cl or four I and four Cl, increasing the number and reactivity of the halogens attached to the rings increases the lytic activity of the whole molecule, and Dr. Astbury suggested to me that the addition of more and more polar groups confers on the molecule the ability to react with the side chains of the proteins of the red cell surface, thus exerting a solvating effect. This suggestion is so interesting that one would like to find other lysins in the case of which the relation of lytic activity to the addition of reactive groups could be studied, but a difficulty immediately presents itself in that most active lysins (e.g. saponin, the bile salts, and even the soaps) are structurally too complex for the desired substitutions to be made.

Many accelerators, on the other hand, are simple substances, and so, instead of trying to relate lytic activity to molecular structure, one can try to relate accelerating power to the structure of the accelerator. The two problems are somewhat similar, for while a lysin breaks down the red cell membrane, the accelerator modifies the membrane so as to make it more easily disintegrated by the lysin, added subsequently.

As a starting point benzene was selected; this substance has quite a considerable accelerating power for saponin and bile salt lysis. Addition of one Cl, to form mono-chlor-benzene, increases the accelerating power 7 times, adding one Br, 8 times, and adding one I, 25 times. If two Cl are added, the resulting acceleration depends on their relative position, the order of decreasing effectiveness being ortho, meta, para. Two Br are better than two Cl, and two I better than two Br; three halogen atoms on the ring are also more effective than two. Addition of a second ring to give naphthalene, increases the accelerating power of benzene 48-fold, and the halogenated derivatives of naphthalene are still more powerful accelerators. Anthracene and phenanthrene are not more, and probably less, effective than naphthalene, and the 4 and 5 ring compounds containing only carbon and hydrogen are so insoluble that no accelerating effect can be detected.

Experiment shows that the action of an accelerator such as benzene is a two-fold one. The substance is taken up at the cell surfaces, although acceleration can be observed when there is not sufficient accelerator to form even a monolayer. By washing the cells immediately with saline, the accelerator can be removed, leaving no permanent effect upon the resistance of the cells. If the accelerator is allowed to remain in contact with the cells for some time, however, irreversible effects upon resistance

are produced and these effects may even in some cases result in hemolysis.

3. Structure of the red cell membrane. During the past year a number of investigations have been carried out on the structure of the red cell surface. Dr. Davson and I investigated the permeability properties of ghosts produced by saponin hemolysis, hemolysis by hypotonic solutions, and hemolysis by freezing and thawing. After these forms of lysis, the cell membrane is apparently permeable to cations, although by conductivity methods the cells appear to be virtual non-conductors. This paper will shortly be published in the Biochemical Journal.

Dr. Neurath and I investigated the change from disc to sphere which occurs between closely opposed slide and coverglass, and the way in which this phenomenon is affected by covering the surfaces with hydrophobic monolayers. Sphere formation is completely prevented by covering the surface with monolayers of calcium stearate. At the same time, however, the stearate produces lysis, even under conditions where there are obviously far too few stearate molecules to cover the cell surface. By studying the "fading times" of the cells which hemolyse on these monolayers, we were able to show that the escape of hemoglobin is about that which one would expect if the cell surface broke down in patches of about 250 A in diameter. These experiments have a bearing on the hypothesis put forward by Abramson that lysins act on "key spots" on the cell surface. The paper will be published shortly in the Journal of Experimental Biology.

For some time past it has been apparent that there is a discrepancy between the results of various methods of arriving at the thickness and structure of the red cell membrane. Gorter and Grendel's results, based on chemical analysis and surface spreading, show that the lipoids present are only sufficient to form a bimolecular layer at the cell surface. Fricke's conductivity measurements initially indicated the same thing, but within the last few years new interpretations and measurements at higher frequencies have made it seem possible that the membrane may be considerably thicker. Lastly, polarization optics suggest the presence of a complex ultrastructure composed of oriented lipoid and protein. In order to reconcile some of these differences, Dr. F. O. Schmitt, Dr. Bear and I have reconsidered the implications of the optical measurements in a paper published in the April issue of the Journal of Cellular and Comparative Biology.

The whole question of disc-sphere transformations and their relation to the structure of the red cell and of its surface has been reviewed with the addition of recent observation in a paper read at a Symposium on cell membranes held by the Faraday Society (Transactions of the Faraday Society, April, 1937).

4. Red cells in pernicious anemia. At the Symposium this summer Dr. C. P. Rhoads called attention to the fact that an intravascular hemolytic process is involved in pernicious anemia, and produced evidence that indol or some similar substance may be involved. The hemolytic properties of indol and related compounds have accordingly been investigated quantitatively by the methods employed in this laboratory. In saturated

solution, indol is a feeble lysin and in this, and also very much more dilute solutions, it is a powerful accelerator for lysis by saponin and the bile salts. If one admits the existence of a normal intravascular hemolytic process, administration of indol would presumably accelerate the process and so bring about an anemia, such as Rhoads finds. It is interesting that skatol is not a lysin, although it is an accelerator, and that indican, the normal conjugated form of indol, is neither lytic nor acceleratory. An account of these investigations is published in the Proceedings of the Society for Experimental Biology and Medicine.

During the course of this work it seemed advisable, if only as a routine measure, to examine the red cells from cases of pernicious anemia by the methods developed in this laboratory. Examination of the time-dilution curves for lysis by saponin of the washed red cells from cases of pernicious anemia revealed the fact that such curves differ profoundly from the normal. The principal difference lies in the fact that the curves in pernicious anemia rise to a higher asymptote than do the curves for normal blood. Usually, in addition, the curves cross each other, so that the cells from the subject with pernicious anemia hemolyse more slowly than do normal cells in high concentrations of saponin, whereas they hemolyse more quickly in low concentrations. This difference in the time-dilution curve has now been found in each one of the 28 cases of pernicious anemia, some in remission and some in relapse. In relapse the phenomenon is much more striking, but it apparently remains even when the patient is in clinical remission. So far we have been content merely with making observations, but it will be clear that the change in the form of the time-dilution curve corresponds to a change in the surface chemistry of the cells, and that red cells whose resistance to lysins is about normally low would be vulnerable to the intravascular lytic process which Rhoads believes to be accelerated in cases of pernicious anemia. This investigation is now ready for joint publication from The Biological Laboratory and the Hospital of the Rockefeller Institute.

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The Relation of the Reticulo-Endothelial System to the Antihormones
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