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THE BIOLOGICAL LABORATORY

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LONG ISLAND BIOLOGICAL ASSOCIATION

INCORPORATED 1924

ANNUAL REPORT

OF

THE BIOLOGICAL LABORATORY

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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 the following report for the year 1933.

The Biological Laboratory has continued to hold and to strengthen its position and work as an advance post of biological research, through

1. Inauguration of a new method in biological research, namely extended conference-symposia on quantitative biology.
2. Its all year research upon the chemical activity of X-rays.
3. Studies of electrical properties of red blood corpuscles.
4. Publication and distribution of Volume I of Cold Spring Harbor Symposia on Quantitative Biology.
5. Continuing to aid in the compilation of knowledge in bryology.
6. Providing opportunities for study and research here during the summer to some eighty professors and advanced students from thirty-six institutions.

Conference-Symposia on Quantitative Biology

During 1933 the Laboratory has taken over for biology a method which quite likely may be very useful to biological research. This method has its physical expression in conference-symposia which take place during the summer.

There is, of course, nothing new in conferences or in symposia, but the union of the two during a month and the inclusion of mathematicians, physicists, and chemists in the conferring group will, we hope, partially realize for biology an opportunity which it has had for some time.

People in general are just beginning to get a well rounded, first hand acquaintance with something which has been an everyday experience of workers in scientific research, namely almost continual change in those matters which form a basis of daily activity. Thus bankers and other men interested in foreign trade and foreign exchange have had an opportunity to become somewhat accustomed to carrying on their daily work with a dollar which frequently changed in value. Men interested in sugar and in certain public utilities in Cuba have been carrying on their work with frequent changes in governments, policy and public attitude toward their holdings and interests. Merchants and laborers have adjusted their work to changes in conditions such as codes affecting hours and wages. Indeed there is scarcely any human activity or region in the world which has not lately been surrounded by conditions in which change seems to be generally characteristic.

Now this is like scientific research. There is little or nothing that is orthodox. Scientific workers are continually trying to find something new, to prove or disprove a hypothesis or conjecture. In the United States alone there are about 22,000 persons listed in a bibliographical directory called American Men of Science (Fifth Edition 1933). All of these 22,000 are supposed to be somewhat actively engaged in research. At least they have all published research work. Judging from previous editions of this same directory over 40% are engaged in work in the biological and medical sciences, not including chemistry and physics. This means that in this country alone there are approximately 10,000 people engaged in building up and changing the knowledge upon which research in biology rests. If at the same time we consider the chemists and physicists we must add another 5,000. The speed at which these numbers are increasing is indicated by the fact that the directory which had 22,000 names in 1933 had in its edition of 1927 not more than 13,500, in 1921 9,500, in 1910 5,500, and about 4,000 in 1906.

From this it can easily be seen that established facts and projected hypotheses in research are continually increasing and changing. This is further shown by the following facts. The authors' index of abstracts of chemical scientific papers for one year (1932) indicates that there are about 50,000 titles. A single monthly issue of Biological Abstracts contains over 2,500 abstracts of papers on biology. I pick up at random a scientific journal, Protoplasma, published in Germany. The issue I am looking at was received October 30, 1933. The first paper I turn to was received for publication March 18th. I find in the November 1933 issue of Biological Abstracts an abstract of a paper published in 1927. This is in no way meant to reflect upon the important and excellent work of scientific journals, but to give an indication of how difficult it is for anyone engaged in scientific research to keep abreast of the changes, an appreciable number of which may be of significance to him. I glance at papers on mitogenetic rays (rays concerning the very existence of which there is considerable discussion and disagreement) in one volume of one journal, and look at the source of the papers. One is from Moscow, one from Rome, one from Berlin, one from Princeton, one from Kiev. Thus the changes in even a very limited aspect of research originate in widely separated areas.

Scientific societies and individuals do much to help overcome the difficulties already indicated. But neither can, of course, effectively provide itself with a news service which approaches that in use in business, diplomacy, finance and other human activities. The result is that scientific research is continually working under appreciable handicaps.

Marine biological laboratories were first established in part to help overcome these difficulties at a time when there were possibly 2,000 scientists of all kinds in the United States, and probably not over 800 men who had actually published results of research in biology and medicine. Under those conditions it was comparatively easy for leaders of biological research to gather at Woods Hole or at Cold Spring Harbor during the summer and to exchange news concerning their work and their ideas.

But, as we have seen, the number of research workers has increased enormously; and other complications have entered. Outstanding among these is a great broadening of the field actually covered by biological research until it includes biometry, biophysics and biochemistry. Indeed as research in biology becomes more mature it becomes increasingly quantitative and moves more and more into territory previously almost wholly occupied by mathematics, physics and chemistry.

Trying to take all of these things into consideration the Biological Laboratory this year inaugurated a method which probably will be of considerable value in fostering a closer relationship between biology and the so-called exact sciences, mathematics, physics and chemistry, and will provide a limited though important news bureau of changes taking place in biological knowledge.

The procedure is to invite each summer to the Laboratory a small group of outstanding workers in one given aspect of biological research. These men are chosen so that they will represent not only biological aspects of a given problem, but those of mathematics, chemistry and physics, the idea being to try to cover each year all of the aspects of a limited subject from mathematics to application to medicine. These men are in residence at the Laboratory for one month during the summer. Their small number is supplemented by others who come from time to time to present papers and to take part in symposia and discussions. The papers presented at these symposia, together with the carefully edited discussion which takes place after the presentation of a paper, are published as soon as possible. This year over half of the papers were given preliminary publication in the news bulletin of the Woods Hole and Cold Spring Harbor scientific communities (The Collecting Net) within two weeks after they were presented, and a volume containing all of the papers and edited discussion is already being distributed. It is planned that the papers and discussion shall be participated in not only by Americans but also by scientists from other parts of the world, this being done by letter when personal attendance is not possible.

I wish to stress particularly the fact that we do not consider that this is a panacea for the increasing difficulties of the exchange of methods and ideas, but we do think that the method has important values and possibilities, and that its cooperative adoption by other laboratories that may be in a position to carry on something of this sort will be of considerable advantage to biological research.

The list of men who took part in the conference-symposia of 1933, and a list of the papers presented, occur elsewhere in this report, while the Report of the Scientific Advisory Committee contains certain details.*

X-rays

(The Laboratory Director's report of the research is written with the hope of being of interest to lay-members of the Association. Scientist-members who wish a more detailed or technical account of the work should refer to the reports of individual workers appended to this report).

Within every living cell there are ultramicroscopic units which are the determiners of heredity. These genes are apparently so constant in their chemical composition and in their action upon the cell in which

* We have just learned that the Rockefeller Foundation has made a grant of $5,000 specifically for the symposia of 1934.

they are located and possibly upon other cells, that not only does a cat produce a cat, but an alley cat produces an alley cat. At times, however, the constancy of action of a given gene is upset. An animal is produced which is unlike others which had identical inheritance for a period long enough to insure complete similarity of inherited characteristics. A mutation has occurred which, together with cross breeding, forms the basis of inherited differences and possibly of evolution. Since the mutation which we see has its origin in one or more genes we can only assume that the gene or genes have undergone unusual chemical change.

It happens that small fruit flies have been used enough for experiments in heredity for us to know that if the flies are exposed to certain dosages of X-rays at certain critical periods in the reproductive cycle, the frequency with which mutations appear will be appreciably increased; let us say mutations will occur one hundred times as often as they would under normal conditions.

What effects have the X-rays had upon the genes? What effects do X-rays have upon living cells and tissues in general, and upon the chemicals which have a vital role in these cells?

The answers to some parts of these questions are common knowledge. Everyone knows that X-rays are cumulative as far as their action upon living cells is concerned. Thus dosage, the total amount of X-rays received, is very important, whether the rays are received at one treatment or many treatments. In man a small dose of X-rays will cause possibly only reddening of the skin, while a large dose may cause death.

Another factor: intensity, the amount of X-rays, received per minute, is also a determining factor in producing biological effects.

Indeed the purely physical variables connected with X-rays are fairly well understood. But the general problem of what is happening within a cell is scarcely more than approached.

When an investigator turns X-rays upon a fly with the hope that one or more of the rays will penetrate a gene and alter its chemical composition enough to produce a mutation he is trying to hit something he has never seen, whose chemical structure is unknown to him, and whose very existence is dependent solely upon mathematical and deductive reasoning.

Fortunately the chemistry of a biological cell is somewhat better known, but even there the best chemist can make only the wildest guess as to what happens within cells when they come under the influence of X-rays.

The problem is far too complex, and present ignorance far too great to justify a direct attack. Hence Doctors Fricke and Brownscombe's systematic studies of the chemical activities of X-rays.

Water is a simple chemical substance and it forms a very high percentage of the material in living cells. This year Doctors Fricke and Brownscombe have made a surprising discovery concerning effects of X-rays upon water. Until this year it had been thought, as a result of the researches of many men, that X-rays had the power of breaking up water into its chemical constituents: hydrogen and oxygen. This, however, is not the case. Doctors Fricke and Brownscombe have found this year that if the water is absolutely pure, X-rays have no observable effects upon it.* The effects previously seen are found to be due to the breaking down of organic impurities in the water. This does not mean that workers here and elsewhere have been careless in their previous work. As Dr. Fricke points out in his report, "It is interesting to note that water, purified by several distillations (from permanganate and chromate) and distilled directly into sealed off pyrex glass vessels, cleaned chemically, may still give by irradiation as much as 10 micro-equivalents of carbon dioxide, showing the presence in the water of a corresponding amount of organic matter." Only when additional precautions are employed, involving a pre-heating to the softening point of all glassware used, and a heating of the water to 1000 degrees Centigrade do we obtain water so pure as to be chemically no longer altered by X-rays.

This discovery is a convincing indication of the value of extreme care and exactness in certain scientific work, and of the fact that such care is used in our biophysical laboratory.

This new finding will probably have immediate practical application in addition to its theoretical significance. A possible application, for example, lies in analyzing deep sea water. Indeed Professor August Krogh of the University of Copenhagen, who is collaborating with the Oceanographic Institute at Woods Hole is interested in using the method arising from Doctors Fricke and Brownscombe's work.

(The question of the amount of organic chemicals in deep seawater has interested oceanographers for some time because it is intimately connected with life in the ocean depths. It is already known that life in the ocean decreases rapidly as the distance from the surface increases. Thus according to the results of some surveys (Hentschel) there are 40 times as many minute organisms (nannoplankton) at the surface of the sea as at a depth of 400 meters, while there are nearly 1000 times as many at the surface as at 5000 meters. The significance of these figures is indicated by the fact that the average depth of the Atlantic Ocean is 4000 meters, and that over one-quarter of its area is more than 5000 meters deep. . . . . Since the whole cycle of all kinds of animal life at ocean depths probably depends upon the amount of food available for minute organisms, and since an appreciable percentage of the food of these small organisms may be organic chemicals in solution in the sea-water at great depths, the reason for oceanographers' interest in this matter is apparent).

Doctors Fricke and Brownscombe's findings provide a very much quicker and simpler method of making a quantitative determination of organic substances in sea-water than the previously employed chemical analysis. With the aid of X-rays, the amount of organic matter in a small sample of water can be determined in a short time.

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* We have also found, contrary to previous belief, that beta rays from radium do not break down water.

Moreover, the same method can probably be applied to testing for organic substances in any chemical solution—this is certainly true of all chemicals thus far tested for that purpose in this laboratory.

Indeed, as far as our present experience goes, it seems likely that X-rays can break down an organic substance until only carbon dioxide and hydrogen remain. We have found this to be immediately true in the case of certain relatively simple organic substances such as formic acid and oxalic acid, in which Doctors Fricke and Brownscombe have completely ascertained the nature of the reaction. Among more complex substances, however, such as sugar, and urea (and other of the higher hydrocarbons) the final complete breaking down is achieved through a series of intermediate chemical compounds, a fact which must be considered in biological studies.

Oxygen, a substance whose importance to living animal cells is comparable with that of water, has also been studied during the year. We have found that its presence apparently always greatly increases the effects of X-rays.

The relative acidity of the solution also affects X-ray activity. This has been true of all of the substances which we have studied thus far, provided a sufficiently wide range of acidity is used.

The effects of X-rays upon hydrogen peroxide are also particularly interesting because of certain characteristics which this substance has in common with biological material. Thus, the activity of X-rays upon hydrogen peroxide is dependent upon intensity: the number of X-ray units to which the peroxide is exposed per minute. Indeed here for the first time we find a purely physical reason for a part of the intensity-dependence found in biological material. Coexistent with this physical reason there are unquestionably purely biological factors, such as the fact that cells may be dividing and otherwise changing during any given period of time.

While usually X-ray effects are found to be independent of temperature, we have found that the activity of X-rays in hydrogen peroxide is greatly influenced by changes of temperature, and it is quite possible that the same may be true in the case of biological material.

Thus we see being built up in our laboratory for biophysics a structure of knowledge which may at some future time serve as a point of departure for a thorough understanding of the activity of X-rays in living cells.

Meanwhile, I cannot point out too clearly that, all of these studies attempting to find physical reasons for X-ray activity, which are susceptible to mathematical treatment and hence favorable for building up generalizations or "laws", involve the greatest labor and care. This year has seen many improvements in our technique. The matter of pure water is one example. The studies of dependence upon relative acidity, upon gaseous oxygen, upon intensity, and upon temperature are other examples. All of this has necessitated our building up intricate apparatus and improving and extending our methods of analysis. In particular, gas analysis (carbon dioxide, oxygen, and hydrogen peroxide) has been included besides the original electrometric titration. That such care and labor, however, are absolutely essential in certain types of scientific research is demonstrated in the results of the year in our studies upon the chemical activity of X-rays.

Electrical Properties of Cells. The studies concerning electrical properties of red blood corpuscles have continued to be very productive of results during the year.

From previous studies, conducted in this Laboratory and elsewhere, we know that when death approaches, or occurs, the electrical resistance of a cell drops to zero. This, of course, indicates that the membrane surrounding the cell has become completely permeable: substances in solution may pass through it freely in either direction. During the life of a cell, on the contrary, the cell membrane is only semi-, or selectively, permeable. Certain substances are allowed passage in, others out, and others cannot pass the membrane in either direction. This semi-permeability is of the greatest importance to all forms of life. Without it such an every day occurrence as a fish-egg developing in the ocean would be wholly impossible, indeed, life, as we know it, could not exist.

Red blood corpuscles fall into this general category of biological cells. Within these corpuscles there is, among other substances, haemoglobin, a rather complex chemical which easily takes on and gives off oxygen, permitting the red corpuscles to ventilate, and promote combustion in, the innermost cells of the body. Incidentally haemoglobin makes red corpuscles red. Under certain unfavorable conditions, however haemoglobin escapes from the corpuscles in a process called haemolysis. This process can be seen under the microscope as a "fading" of the corpuscle.

Haemolysis brought on by freezing and thawing, and by certain other physical and chemical agents produces a condition in the corpuscular membrane which is similar to that which accompanies death. Selective permeability is lost and the electrical resistance falls rapidly. But Doctors Fricke and Curtis have found during the year that haemolysis may be produced by water, and by other substances in low concentrations, and still the electrical properties of the membrane are the same after haemolysis as they were before. Since a change must have occurred during haemolysis, the membrane becoming permeable in order for the haemoglobin to pass out, the electrical evidence indicates that the permeability is temporary and is reversed within a short time. The time required for haemolysis is probably approximately two seconds, according to Professor Eric Ponder of New York University who is collaborating in certain physiological aspects of this work.

During the year Doctors Fricke and Curtis have obtained further convincing evidence of the existence of a membrane surrounding the red blood corpuscle. With the improved bridge which has been constructed during the year we can now measure both the capacity and resistance with considerable accuracy over a range of cycles per second from 250 to 16,000,000. With this new apparatus it has been found that electric capacity shows a slight dependence on the frequency used: the capacity of the corpuscular membrane increasing with lower frequencies, a fact typical of non-conducting membranes.

We have also obtained a better value for the electrical conductance of the contents of red blood cells. This is found to be about twice as large as that of the blood serum surrounding the cells, a fact which is accounted for by the non-conducting haemoglobin within the cell. When haemolysis occurs and the haemoglobin escapes from the corpuscles into the serum, the conductance of the serum and of the interior of the corpuscle become the same.

Ultra-violet dosimetry

Studies in ultra-violet dosimetry were continued during the summer by Mr. R. F. Baker, working under Doctor Fricke's supervision.

Bryology

Volume II, Part 1 of the "Moss Flora of North America North of Mexico" was published in November. This part, written by George N. Jones, of the University of Washington, and edited by Dr. Grout, deals with the Grimmiaceae. The part consists of 65 pages and 25 full page plates. Further progress in the preparation of the Moss Flora is indicated in Doctor Grout's report.

Other Work

Research conducted at the Laboratory during the summer by visiting scientists embraced such subjects as the connection between presbyopia and length of life; regeneration in **Fundulus** (killifish) embryos; the direct determination, by microinjection of indicators, of the level of oxidation-reduction intensity in the interior of **Ameba dubia** (a protozoa) under strict anaerobiosis; electric resistance and reactance of the common kelp, **Laminaria**; the vegetation of this region from the sociological standpoint; the development of the nervous system in the lower vertebrates; studies on adrenalectomized rats; further study of the Malpighian tubules of the earwig **Annisolabis maritima**; study of certain parasitic infections in oligochaete worms; movements of human leucocytes (white blood corpuscles); the effects of electrolytes on the rhythm of the oyster heart.

Instruction

The courses of instruction were maintained upon an advanced basis. Thirty-six students, from twenty-one colleges and universities, were admitted. A special grant of five thousand dollars for equipment from the Carnegie Corporation permitted us to greatly improve facilities for student work, particularly in the course in General Physiology. Indeed, with the help of the grant we are able to place the course in General Physiology upon a basis where it will compare favorably this coming year with any similar course in the country. See Dr. Taylor's Report.

List of Evening Lectures

Dr. Harold Abramson, Department of Biochemistry, College of Physicians and Surgeons—"Electrokinetic Potentials in Biology and Medicine."

Legends for Figures

Figure 1.

Something of the difficulty of trying to obtain pure water (referred to in the report of the work upon X-rays) is indicated in the distilling apparatus pictured herewith. This combination of stills is designed to produce water free from organic impurities. Ordinary distilled water contains as much as 10-20 microequivalents of organic matter per quart. Even with the complicated apparatus shown, one microequivalent may remain after several distillations and unusual precautions.

Ordinary distilled water from a tin still is lead by way of an all glass reservoir and ungreased stopcock into the still to the right. Here it is digested with potassium permanganate and sodium hydroxide. This strong chemical oxidation breaks down many organic substances into carbon dioxide and water. The water is then forced by oxygen (which is also used for another purpose) from the reservoir of this still into the still on the left. Any permanganate or hydroxide carried over as a spray is retained in this still.

The steam is now mixed with air or oxygen and passed through the quartz oven which may be heated to nearly 1000° Centigrade (approximately 1800° Fahrenheit). This usually burns out the last trace of organic substances.

Provision is also made for removing inorganic impurities, and the apparatus is so arranged that the whole glass surface with which the steam which is used comes in contact may be heated to the softening point to burn off any adsorbed impurity.

The water is drained from the reservoir, after the final distillation, by breaking a drawn-out glass tip, as it would be impracticable to burn off impurities from a stopcock.

Discoveries resulting from such careful distillation of water are described in the text.

Figure 2.

One of the groups of apparatus for research in quantitative biology in use at the Laboratory last summer was the Chambers micromanipulator shown on the opposite page. Doctor Chambers, who developed the manipulator, is Professor of Biology at New York University. He took part in the symposia last summer and is a member of our Board of Directors. The apparatus shown here was used by Professor Barnett Cohen and Doctor T.-T. Chen, of the department of Physiological Chemistry of the Johns Hopkins School of Medicine.

With the aid of this apparatus a skilled investigator can learn something of the chemistry of a living cell, more particularly he can establish facts concerning oxidation and reduction within a living cell. Doctors Cohen and Chen were studying such processes in a one-celled animal, an ameba.

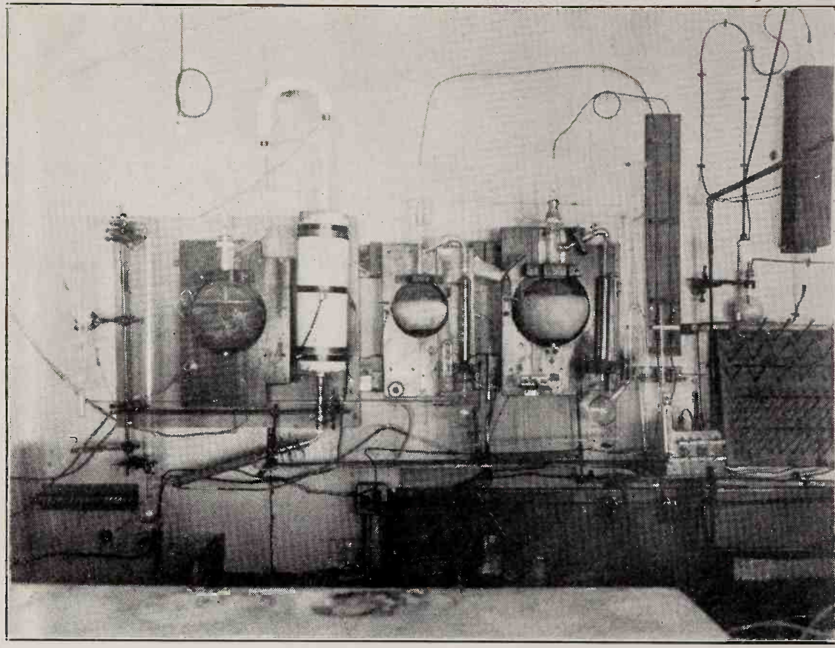

Figure 1

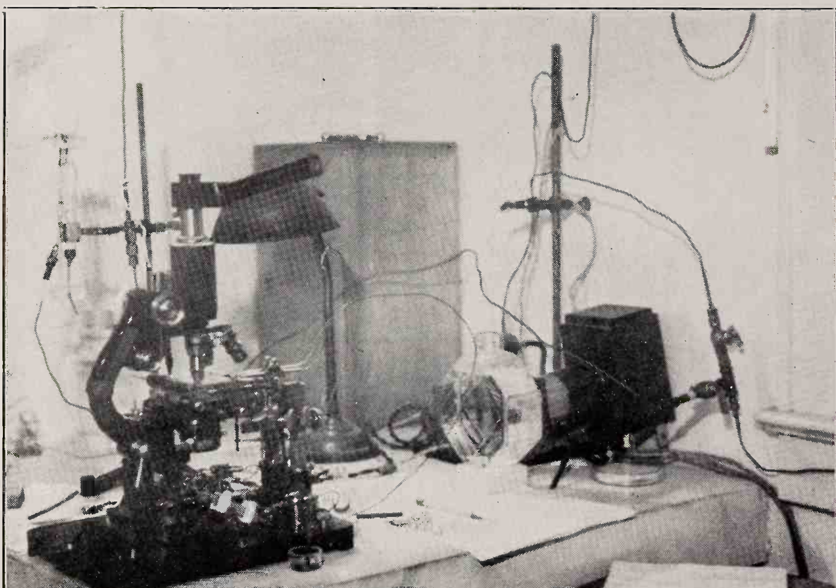

Figure 2

The technique consists of placing the ameba, in suitable surroundings, under the microscope. By means of minute needles mounted on the framework of the microscope, injections of pure dye substances which change in color when oxidized or when reduced are injected into the interior of the ameba. Ingenious arrangements make it possible to move the needles slowly in any direction by means of controlling thumb screws. The black arm projecting from the top of the microscope allows not only the experimenter, but an assistant or colleague, to watch the whole process.

In the work conducted this summer the apparatus was built to make possible control of the gas available to the ameba from its environment. (Unfortunately, this part of the apparatus could not be included in the photograph.) In this case the amebae were kept within a chamber in which oxygen was not available.

Dr. A. M. Banta, Brown University and Carnegie Institution of Washington—"Some Newer Data on Control of Sex in Cladocera."

Dr. Felix Bernstein, Investigator, The Biological Laboratory—"The Connection Between Presbyopia and Length of Life."

Dr. H. S. Conard, Professor of Botany, Grinnell College—"A Criticism of Succession."

Dr. Charles B. Davenport, Director, Department of Genetics, Carnegie Institution of Washington—"Which Came First in Evolution, Farm or Function?"

Dr. M. Demerec, Investigator, Department of Genetics, Carnegie Institution of Washington—"Genes—An Element Essential for the Life of the Cell."

Dr. S. I. Kornhauser, Professor of Anatomy and Embryology, School of Medicine, University of Louisville—"Studies on Anisolabis."

Dr. Stuart Mudd, Professor of Bacteriology, School of Medicine, University of Pennsylvania—"Infection and Resistance."

Dr. Oscar Riddle—Investigator, Department of Genetics, Carnegie Institution of Washington—"Prolactin and Other Anterior Lobe Hormones."

Dr. A. A. Schaeffer, Professor of Biology, Temple University—"Protoplasmic Organization of Movement."

Dr. W. W. Swingle, Professor of Biology, Princeton University—"Functional Studies of the Adrenal Cortex."

Women's Auxiliary

The Women's Auxiliary under the fine leadership of Mrs. Van Santvoord Merle-Smith continued to take a very useful and important part in the work of the Association, through the raising of two thousand dollars to help provide for a chemist in the biophysics laboratory, and through the donation of furniture and money for the improvement of living quarters. If we were to make full acknowledgment to all who helped make these splendid results possible it would be necessary to name nearly every member of the Auxiliary, and many who are not members. However, special acknowledgment and appreciation are due to the sustained work of the President, Mrs. Merle-Smith, the Treasurer, Mrs. Walter Jennings, and, since her election last summer, the Chairman of the House Committee, Mrs. Percy Jennings. Mrs. J. H. J. Stewart, as chairman of this committee previous to the Annual Meeting in August, gave her help generously, and Mrs. Fairman Dick, Secretary, has been very helpful to the Auxiliary and its President.

The improvement in the arrangement, decoration and furnishing of small apartments was an important part of the preparation for holding the first of the Cold Spring Harbor Symposia on Quantitative Biology. In the success obtained by the Auxiliary in this part of the preparation too much credit cannot be given to the taste and hard work of Mrs. Reginald G. Harris. Furnishing must be carried farther during the coming year, notably in respect to the two cottages being completed this winter. The Auxiliary is already at work to make sure that this additional need will be met with characteristic success.

Wawepex Society

The Wawepex Society, Charles M. Bleecker, Governor, Jesse Knight, Scribe, and T. Bache Bleecker, Custodian, continued its very helpful support of the Laboratory through the granting of fifteen hundred dollars to the general fund. In addition the Society voted to place the awarding of the John D. Jones Scholarship in the hands of the Scholarship Committee of the Biological Laboratory. Previously this Scholarship has been awarded by the Department of Zoology of Columbia University. In making the change the officers of the Society believed that the Laboratory would have a wider range of selection in awarding the Scholarship than one department of any given university. The John D. Jones Scholarship was established by the Society in honor of its Founder, and the Founder of the Laboratory, Mr. John D. Jones. The annual stipend is two hundred fifty dollars. Last year, due to unusual conditions, the Scholarship was divided among three men: R. F. Baker, graduate of Pennsylvania State College, now at Harvard University, Harry C. Elliott, graduate student at the University of Toronto, and Edward McC. Walzl, graduate student at the Johns Hopkins University. All of these men carried on research at the Laboratory during the summer.

During the year the Society lost by death its former Custodian, Walter J. Whipple, who has long been an officer of the Society.

Contributions and Grants

The work of the Association could not have been conducted upon the high level it has attained had it not been for the continuance of an emergency grant of $20,000 from the Rockefeller Foundation. The Foundation also made a special grant of $2,300 in support of the work of Dr. Felix Bernstein.

The Carnegie Corporation made a special grant of $5,000 for scientific equipment.

The unemployment committees of Oyster Bay and of Huntington, particularly the latter, were very helpful in detailing skilled and unskilled labor to the value of some $3,000. Mrs. Ellen Mary Chouteau generously contributed her services as Librarian.

Contributions from members reached their lowest level since 1926. This was due in large measure to the forced further reduction in the amount contributed by many of the Laboratory's largest contributors, to the loss by death of certain generous friends of long standing, and to the difficulty of making new friends in times such as we have been experiencing. It is, however, essential that the low of 1933 should be the bottom. We do not wish and we cannot expect the Rockefeller Foundation to continue a general grant for emergency purposes. We must immediately begin the climb back to normal receipts from individual contributions. Fortunately, we already have received indications that our friends will support us in this climb, while the Rockefeller Foundation, appreciating the circumstances surrounding the Laboratory, has adopted a policy of gradual reduction of its emergency support of the work rather than removing all of the prop at once.* This encouraging and understanding action will be of great help to us in regaining our normal support from members of the Association.

Contributions for 1933 were received as follows: $1,000 to $22,300; Carnegie Corporation, Mrs. Leonard K. Elmhirst, Marshall Field, Wilton Lloyd-Smith, Mrs. Van Santvoord Merle-Smith, Rockefeller Foundation, Wawepex Society; $500 to $1,000: Mrs. Ethel Clyde, Mrs. W. Emlen Roosevelt, John Schiff; $100 to $500: Frank L. Babbott, W. R. Coe, Mrs. H. P. Davison, Henry W. de Forest, Mrs. Walter Jennings, Mrs. Russell C. Leffingwell, Ogden L. Mills, Acosta Nichols, Mrs. Acosta Nichols, George D. Pratt, Harold I. Pratt, John K. Roosevelt, Thomas Roulston, Carl Schmidlapp, Henry L. Stimson, Mrs. Landon K. Thorne, William K. Vanderbilt, Willis D. Wood.

Grounds and Buildings

With the help of unemployed, assigned to the Laboratory by local unemployment committees, we have been able to accomplish needed work in building maintenance. Three buildings have been given one coat of paint, one roof has been reshingled, and many minor repairs have been made. Important improvements were made in apartment accommodations, and two small cottages are being built, suitable for summer use.

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* We have just received word that the Rockefeller Foundation has reduced its emergency grant, in respect to 1934, from $20,000 to $15,000. The Foundation has also informed us of a grant of $5,000 in support of the Symposia on Quantitative Biology.

Recommendations and Acknowledgments

Your Laboratory Director heartily approves of the recommendations contained in the reports of the Scientific Advisory Committee and of the Committee on Biophysics. A physiologist in residence at the Laboratory throughout the year would be of considerable value to both the all-year and summer work. The recommendation for the appointment of a biologist to carry on research in connection with the work of the biophysics laboratory should be followed with the least possible delay.

Acknowledgment is made of the continued help of President Page. The thought, time, work and advice which he gives so wholeheartedly are reflected in the accomplishments of the Laboratory every year. Much the same should be said of the President of the Women's Auxiliary, Mrs. Merle-Smith. Mr. Acosta Nichols, Mr. John K. Roosevelt and Mrs. Walter Jennings, Treasurer of the Women's Auxiliary, have worked splendidly for the Association. I have called frequently for advice in scientific matters upon Vice-President Osterhout. His help has been invaluable.

I wish to take this opportunity to thank the many not specifically mentioned above whose contributions of money, work and advice have made possible the fine record of this year.

REGINALD G. HARRIS.

Report of Scientific Advisory Committee

The Scientific Advisory Committee has observed with satisfaction the way in which the first conference-symposia on quantitative biology developed at the Laboratory last summer. The gathering seemed to achieve the basic ideas which caused us to approve giving the method a trial at the Committee's meeting of December 1932. Outstanding among the basic ideas, it will be recalled, was that of fostering a closer relationship between biology and the basic sciences, and of covering a specific aspect of quantitative biology over the whole range from theoretical mathematics and physics to medicine. A glance at the list of those taking part in the discussion shows that this was accomplished. (Thus theoretical physics was represented by Prof. Mueller of Massachusetts Institute of Technology; physics applied to biology by Prof. Cole of the College of Physicians and Surgeons, and Dr. Fricke of the Laboratory; physical chemistry by Drs. MacInnes and Michaelis of Rockefeller Institute; chemistry by Dr. Briggs of the Otho S. A. Sprague Memorial Institute; biochemistry by Dr. Abramson of the College of Physicians and Surgeons; physiological chemistry by Dr. Cohen of Johns Hopkins Medical School, and by Dr. Van Slyke of Rockefeller Institute; physiology and biology by Drs. Chambers and Ponder of New York University, Dr. Gasser of Cornell University Medical College, and Dr. Osterhout of Rockefeller Institute; and bacteriology and medicine by Dr. Mudd of the University of Pennsylvania Medical School.) The Committee approves of the method in which discussions were edited and published together with the papers, the almost immediate publication of some of the papers in THE COLLECTING NET, and the publication of all of the papers in a volume. The addition of papers by Professors Hill and Svedberg provides what we believe is a fortunate precedent of including a limited number of pertinent papers in the volume which cannot be presented before the group.

The Committee recommends that the symposia, and publication of papers and discussions be continued.

The Committee is pleased to note the progress of the Laboratory's work in Biophysics and to endorse the recommendation of the Chairman of the Committee on Physiology and Biophysics that a biologist be appointed to carry on research in connection with the work of the biophysical laboratory.

The Committee also urges that the Laboratory take steps as soon as possible to carry on work in physiology on an all-year basis. It is believed that by doing this the Laboratory will not only strengthen its all-year work but will be in a better position to be of service to the increasing number of physiologists who come to the Laboratory during the summer.

J. H. BODINE,
Chairman

Report of the Committee on Biophysics

Studies of the chemical effects of X-rays have included: Gas-free water (published), and water containing oxygen (ready for publication), certain organic compounds, ferro-sulphate, chromic acid (published). Hydrogen peroxide. The influence of the hydrogen ion, the hydroxyl ion and certain other ions as well as of the addition of organic substances in small concentrations has been determined. The analysis has been broadened to include gas analytic determination of oxygen, carbon dioxide and hydrogen. Through the cooperation of the Memorial Hospital, a series of measurements of the effects of beta-rays from radium has been made. The use of X-rays as an oxidizing agent for organic matter in aqueous solution appears to have practical value in the analysis of water for traces of organic matter, and it is planned to so use this method in analyzing deep sea water, in co-operation with Dr. Krogh of the University of Copenhagen.

The dielectric constant and power factor of the red corpuscle membrane have been determined over a wide range of frequencies. The measurements have been carried to an accuracy which gives them value as a means of characterizing the normal corpuscle membrane and the method has been so used in a study of hemolysis. Preliminary studies have been made of the impedance of artificial membranes and of the dielectric constant of different proteins in aqueous solution.

In co-operation with Dr. Demerec of the Carnegie Institution measurements of the heredity effects of very soft X-rays have been initiated.

The Committee is convinced that the time has arrived to take a step which is in accordance with previous plans and with Dr. Fricke's program, and we strongly recommend the appointment of a biologist to carry on research in connection with the work of the biophysics laboratory.

W. J. V. Osterhout,
Chairman

Report of The Secretary

During the year 1933 there was held one annual meeting of the Association (July 25) and one meeting, the 29th, of the Board of Directors.

The 29th meeting of the Board of Directors was held at Cold Spring Harbor, July 14. Eleven members were present. The President spoke on the death of Mr. Walter Jennings, and it was voted to incorporate in the minutes the telegram of condolence sent to Mrs. Jennings on learning of his death:

> "The Executive Committee of the Biological Association desires to express its sense of irreplaceable loss in the death of your husband, for forty years valued advisor aud supporter of the Laboratory, upbuilder of the community, wise counsellor, genial companion, steadfast friend."

The action of the Finance Committee in selling certain securities of the Association was voted. The sum of $200 was appropriated to enable Dr. Salant to prepare for publication the data collected at the Laboratory during the last few years, and Dr. Salant was appointed to the honorary staff of the Laboratory. The treasurer's report showed receipts during the first seven months of the fiscal year of $32,432.57, including cash on hand November 30, 1932, $864.78. The receipts included the renewal by the Rockefeller Foundation of its appropriation of $20,000. The Laboratory Director made a report stressing the importance of quantitative biology, and the role that the Laboratory is to play in its development. He looked forward to strengthening the present work of the Laboratory by developing the biochemical, physiological and mathematical sides of biology, particularly by means of the conference-symposia being held at the Laboratory, and stated that for the year 1933 certain phases of surface phenomena would be discussed. The Laboratory Director was authorized to undertake the establishment of a Department of Physiology as soon as funds for the physiologist could be secured. It was voted to print the Symposia of the Summer of 1933.

The Executive Committee was elected as appears on other pages of this report and President Page appointed a special committee on the development of the Laboratory for the next three years, as follows: Henry C. Taylor, Chairman, Marshall Field, Wilton Lloyd-Smith and Willis D. Wood.

The thanks of the Board were extended to the Rockefeller Foundation for its grant.

The tenth annual meeting of the Long Island Biological Association was held at Blackford Hall, July 25, 1933, about 24 members being present. Dr. Robert Chambers was elected chairman of the meeting. On motion of the nominating committee the following were elected to serve on the Board of Directors until 1937: C. B. Davenport, R. G. Harris, Henry Hicks, Stuart Mudd, Acosta Nichols and John K. Roosevelt. Director Harris called attention to the fact that it is now ten years since the Long Island Biological Association was organized. He gave tables showing the growth of the Laboratory in investigation and financial support.

The stated meeting of the Board of Directors was called for December 22, 1933, at the Dr. Walter B. James Memorial Laboratory, to be adjourned to meet on call of the President.

C. B. Davenport,
Secretary.

February 6, 1934.

THOSE TAKING PART IN SYMPOSIA AND IN DISCUSSIONS (1933)

Abramson, Harold A.—Department of Biochemistry, College of Physicians and Surgeons, Columbia University.

Barron, E. S. Guzman—Assistant Professor of Medicine, University of Chicago.

Bates, Robert—Investigator, Station for Experimental Evolution, Carnegie Institution of Washington.

Blinks, L. R.—Associate, Division of General Physiology, Rockefeller Institute.

Briggs, D. R.—Investigator in Chemistry, The Otho S. A. Sprague Memorial Institute, University of Chicago.

Brownscombe, E. R. — Chemist, Biological Laboratory, Cold Spring Harbor.

Chambers, Robert—Research Professor of Biology, Washington Square College, New York University.

Chen, T.-T.—Graduate Student in Physiological Chemistry, (Received Doctorate 1933), Johns Hopkins Medical School.

Climenko, Robert—Assistant Professor of Physiology, University of Alberta.

Cohen, Barnett—Associate Professor of Physiological Chemistry, Johns Hopkins Medical School.

Cole, K. S.—Assistant Professor of Physiology, College of Physicians and Surgeons, Columbia University.

Curtis, H. J.—Physicist, Biological Laboratory, Cold Spring Harbor.

Fricke, Hugo—In Charge of Dr. Walter B. James Laboratory for Biophysics, Biological Laboratory, Cold Spring Harbor.

Gasser, Herbert S.—Professor of Physiology, Cornell University Medical School.

Harris, R. G.—Director, Biological Laboratory, Cold Spring Harbor.

***Hill, A. V.**—Foulerton Professor of the Royal Society; Department of Physiology, University of London.

Irwin, Marian—Associate, Division of General Physiology, Rockefeller Institute.

Kornhauser, S. I.—Professor of Anatomy and Embryology, University of Louisville Medical School.

MacInnes, Duncan—Associate Member, Division of Physical Chemistry, Rockefeller Institute.

Michaelis, L.—Member, Division of Physical Chemistry, Rockefeller Institute.

Mudd, Emily B. H.—School of Medicine, University of Pennsylvania.

Mudd, Stuart—Professor of Bacteriology, School of Medicine, University of Pennsylvania.

Mueller, Hans—Assistant Professor of Physics, Massachusetts Institute of Technology.

Osterhout, W. J. V.—Member, Division of General Physiology, Rockefeller Institute.

Ponder, Eric—Professor of General Physiology, Washington Square College, New York University.

Reiner, L.—Instructor of Bacteriology, New York University.

Riddle, Oscar—Investigator, Station for Experimental Evolution, Carnegie Institution of Washington.

Shedlovsky, T.—Associate, Division of Physical Chemistry, Rockefeller Institute.

Stiehler, Robert D.—Graduate Student in Chemistry, (Received Doctorate 1933), Johns Hopkins University.

***Svedberg, Theodor**—Professor of Physical Chemistry, University of Upsala, Sweden.

Van Slyke, D. D.—Member, Division of Physiological Chemistry, Rockefeller Institute.

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* Did not take part in the symposium but presented a paper in the volume.

TABLE OF CONTENTS OF COLD SPRING HARBOR SYMPOSIA ON QUANTITATIVE BIOLOGY, VOLUME I, (1933).

Partial List of Publications by Members of the Staff and Others Who Worked at the Laboratory. (In addition to Volume I of Cold Spring Harbor Symposia on Quantitative Biology.)

†**Bernstein, Felix**—"Foundation of Probability in the Natural Sciences." American Mathematical Society.

†**Birnie, J. H.**—"Regeneration of the Tail-fins of **Fundulus** Embryos." Am. Soc. Zool.

***Fricke, Hugo and Eugene R. Brownscombe, 1933**—"The Reduction of Chromate Solutions by X-Rays." Journal of the American Chemical Society. Vol. 56, p. 2358-2363.

***Fricke, Hugo and Eugene R. Brownscombe, 1933**—"Inability of X-Rays to Decompose Water." The Physical Review. Vol. 44, p. 240.

Gaunt, Robert, 1933—"Adrenalectomy in the Rat." American Journal of Physiology. Vol. 103, p. 494.

***Gaunt, Robert and Wm. M. Parkins, 1933**—"The Alleged Interrelationship of the Adrenal Cortical Hormone and the Gonads." American Journal of Physiology. Vol. 103, p. 511.

***Grout, A. J., 1933**—See Jones, George Neville.

***Harris, R. G., 1933**—"The Biological Laboratory at Cold Spring Harbor." The Collecting Net. Vol. 8, p. 29-32. And numerous news notes concerning the Laboratory, published in various scientific journals.

†**Hodge IV, Charles** — "Nutrition of **Melanoplus Differentialis** Thomas (Orthopetra: Acrididae) on a Diet of Boiled Wheat Leaves, Alone and Supplemented with Accessory Food Stuffs."

***Hollander, Franklin, 1932**—"Studies in Gastric Secretion. IV. Variations in the Chlorine Content of Gastric Juice and Their Significance." The Journal of Biological Chemistry. Vol. 97, p. 585-604.

***Hollander, Franklin, 1934**—"Studies in Gastric Secretion. V. The Composition of Gastric Juice as a Function of its Acidity." The Journal of Biological Chemistry. Vol. 104, p. 33-42.

***Jones, George Neville; edited by A. J. Grout**—"Moss Flora of North America North of Mexico." Vol. II, Part 1. Published by A. J. Grout, Newfane, Vermont. 65 pages and 25 full page plates.

†**Mueller, Hans**—"Experimental Demonstration in Molecular Physics."

†**Pomerat, C. M.**—"Studies on the Femoral Pad of **Triturus Viridescens.**" Am. Soc. Zool.

Taylor, Ivon R. and James H. Birnie, 1933—"A Micro Vessel for Glass Electrode Determinations of Hydrogen Ion Activity of Biological Fluids". Science. Vol. 78, No. 2017.

***Wharton, D. R. A., and Martha Washburn Wharton, 1934**—"Canine Distemper: The Disease and the Nature of the Virus." The American Journal of Hygiene. Vol. 19, pages 189-216.

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* Reference made to the Laboratory in the title.

† Paper presented at the Annual Meeting of the American Association for the Advancement of Science held at Boston.

REPORTS OF RESEARCH WORKERS

The Connection Between Presbyopia and Length of Life

Professor Felix Bernstein, Columbia University

(Prof. Bernstein was assisted this summer by Mrs. Anne H. Gallagher, George Smith, Marion Smith and Cornelia Williamson.)

That the duration of life is hereditary, to a certain extent, is suggested by observation of twins of very great age, and also by data on families. Evidence in this respect was given by K. Pearson and Beston and by A. G. Bell. Dr. W. auf der Nollenburg, of my Institute, made an investigation of the length of life using data of a population of 13 protestant communities. These people have inhabited the low border of the Rhine in the midst of catholics for about 200 years, without any kind of intermarrying with the latter. The mean age, at death, of the children, taken for those over 20, thereby excluding childrens' diseases, varies with the mean age, at death, of the parents, increasing from 52.8 to 65.6. It is of popular interest that natural death prevails as a cause of death among those children whose parents were old when they died. Infectious diseases caused death in only 9% of children, both of whose parents survived 75 years, whereas the percentage increased to 30% when both parents died under 65 years of age. The study of heredity of length of life on an experimental basis was taken up by Raymond Pearl in Drosophila. Pearl showed that mutations, weakening the lives of the larvae, also shortened the life of the insect, the way of heredity here being, of course, according to the simple monogenic Mendelian scheme. Auf der Nollenburg did not find any indication of dominance in his material.

Duration of life is a very complex phenomenon including non comparable items, and is, in the case of man, unsuitable for quantitative studies in families. For this reason I tried to single out the main feature for making a study of aging, rather than length of life.

There are difficulties in measuring signs of aging of the skin or hair, for instance, but measurement is easy in the case of the lens of the eye. Aging of the lens has the effect of hardening the lens, and this can be measured by determining the faculty of accommodation. (Accommodation is made possible through the bending of the lens by a certain muscle.) Age, hardening of the lens, has the effect of increasing the distance between the eye and the nearest point at which clear reading is possible.

A study of the nearest point of reading can be made during the whole life, but clinical records are available only in case of presbyopia, which means that the nearest reading-point has increased to beyond 33 cm., so that eyeglasses are needed.

To find out the connection between presbyopia and length of life, we looked up the records of some University clinics from 1880 to 1910, and followed cases until the death of the patient.

This study revealed the fact that early presbyopia means early death, late presbyopia means late death. As we have extensive data on cases of presbyopia, we can easily group people into three classes, for early age at least, (1) hypernormal, (2) normal and (3) subnormal. If this be done, we get, for all ages, differences of longevity in favor of less presbyopia. If we confine our material to those cases in which the persons died of "heartstroke" or "brainstroke", the differences are nearly doubled. For people of 50 years of age there is a difference in mean expectation of life of more than 12 years, between the hypernormal and the subnormal, with a mean error of 2 years.

For cancer there was a slight difference; for infectious diseases no difference at all. Those results, based on about 2,000 cases, show that early presbyopia is a sign of early arteriosclerosis. Preventative or actual treatment of arteriosclerosis may be tested by the influence on development of presbyopia, or increase of the distance of the nearest point of reading.

The efficiency of the treatment will be shown by diminishing the slope of the curve of this distance depending on age. These results are all based upon eyes which are normal in other respects. In cases of farsightedness, or shortsightedness, or astigmatism, a correction has to be made beforehand.

Hardening of the lens of the eye is caused by deposition mostly of cholesterin and other derivatives of globulin. In order to obtain confirmation, in other parts of the body, of our measurements of aging, we took X-rays of the larynx. In these X-rays, calcification is shown by deepening of the shadows.

To a common factor of aging may be added local factors, acting in different parts of the body; and for mammals the effects of internal secretions play some role.

Aging, as expressed in the color of the hair, has been studied recently by F. Boas.

The study, now going on, concerns the heredity of aging in families, studied by testing the nearest point of reading. As we know the development of presbyopia with age, we can reduce the data to a normal, and thus find out the way of heredity.

There is no sex-difference in aging, measured by presbyopia, a fact that has to be reconciled with the fact that women live somewhat longer than men. No difference was shown in presbyopia by two succeeding generations, so that the well known increase in the average length of life of the past generation, seems to be wholly due to betterment of external conditions of life.

Regeneration of the Tail-fins of Fundulus Embryos

James H. Birnie, Brown University

Embryos of **Fundulus heteroelitus** were removed from the chorion between the twelfth and fifteenth day of development and were operated upon so as to remove half of the basal plate of the tail together with the differentiated and undifferentiated ray material distal to it. New rays had definitely appeared seventeen days later at the cut edge of the basal plate regardless of whether the part of the plate is derived from the spines or from the original central plate. (In these embryos the basal plate of the tail-fin is derived partly from the original central plate and partly from the neural and hemal spines of the four vertebrae immediately anterior to it.) The plate regenerates slowly as compared with the rays returning to its original shape only after fifty days. When the entire basal plate and all parts distal to it are removed no regeneration of the plate or rays could be observed at the end of sixty-five days. It seems that a similarity is demonstrated between regeneration of the tail fins of fishes and the same process in the limbs of **Amblystoma.** The basal plate is apparently like the shoulder girdle, since it may replace itself and give rise to all parts distal to it. It is concluded, therefore, that the rays like the limbs in **Amblystoma,** and the basal plate like the girdle, belong to a self-differentiating mesenchymal system.

Professor Barnett Cohen's Report
The Johns Hopkins School of Medicine

(Includes the work of Dr. Tung-Tou Chen)

I submit below a report of my research work conducted at The Biological Laboratory during the summer of 1933.

The **problem** under examination was the direct determination, by microinjection of indicators, of the level of oxidation-reduction intensity in the interior of **Ameba dubia** under strict anaerobiosis.

Apparatus. The Chambers micromanipulator and special microscopic equipment and reagents were transported from Baltimore. The necessary gas-purifying apparatus was furnished by the Biological Laboratory. The excellent mechanical facilities of the Department of Biophysics, made freely available by Dr. Hugo Fricke, aided in the prompt assembly of the apparatus and early commencement of the experimental work.

Summary of experiments. Lack of suitable indicators has hitherto prevented an examination of intracellular oxidation-reduction intensities by microinjection under anoxybiosis. Earlier attempts by Cohen, Chambers and Reznikoff using phenosafranine were inconclusive because the dye underwent irreversible change during the time required for manipulation and observation. Stiehler, Chen, and Clark and Stiehler have recently made available some new indicators for the more negative regions of reduction potential which we have utilized. Some of these compounds undergo irreversible change but by quick operation (injections made within a few minutes after reduction) we have succeeded in obtaining definite indications.

Observations were made in an improved hermetic chamber through which flowed moistened purified nitrogen or hydrogen. Injection of oxidizing agent or exposure to air were used to check the reversibility of the indicator in the cell interior. Results on immersion of the cells in oxidant or reductant confirmed those obtained by microinjection.

The experiments were restricted to one type of unicellular organism, e. g., **Ameba dubia.** The results are given in the accompanying table.

Indicator	pH 7 E'_0 at (Volt)	Oxidant in cell	Reductant in cell
Dimethylphenosafranine	—0.260	Partly reduced	Reoxidized
Safranine T	—0.289	Partly reduced	Reoxidized
Sulfonated rosindone No. 6 (cf. Stiehler)	—0.380	Not reduced	Reoxidized

These observations are now being augmented by examination of other available indicators (in Baltimore).

This work was conducted with the assistance of Dr. Tung-Tou Chen; and a preliminary report of it was published in the Proc. Soc. Exptl. Biol. Med., 1933, **31,** 115.

In addition, I participated with great profit in the scientific symposia held at The Biological Laboratory and presented two papers entitled: "Reversible oxidation-reduction potentials in dye systems" and "Reactions of oxidation-reduction indicators in biological material, and their interpretation".

I must not leave unmentioned a rather important element of whatever success was attained in our scientific labors at the Biological Laboratory. In the considerable preparations before and during the experimental work, there occurred those unforeseen emergencies that are so prone to be the source of irritating and fatal delays. That such delays happily did not occur was due, in my opinion, to the quietly efficient administration whereby the elementary needs of the experimentalist were met quickly and directly without undue formalities. This situation lent effectiveness to the genuine helpfulness and cooperation of the entire Laboratory staff.

Report of Doctors Kenneth Cole and Bruce M. Hogg

College of Physicians and Surgeons, Columbia University

With the cooperation of Dr. Fricke and Dr. Curtis electric resistance and reactance measurements were made at frequencies from one thousand and two million cycles per second on the common kelp **Laminaria.** The experiments on normal kelp show that (1) these and probably previous resistance measurements at one thousand cycles are not dependent upon the reactance of the cell membranes. (2) the conductivity of the cell interior is approximately that of sea water. (3) the resistance and reactance of the cell membranes are power functions of the frequency with different exponents.

The experiments on kelp progressively injured by exposure to sodium and calcium chloride solutions show that the exponents of the membrane resistance and reactance remain unchanged. The effects of injury on concentration of living cells and the relative magnitudes of the membrane resistance and reactance have not been estimated satisfactorily, so that no physiological conclusions can be drawn at present.

Professor Henry S. Conard's Report
Grinnell College

(Includes the work of Miss French and Professor Sargent)

The staff and class in Botany devoted all attention to the preparation of the report on the Vegetation of the region, from the sociological standpoint. Miss French worked on correlation of the two available soil surveys of Long Island in relation to our study, assisted variously in the preparation of the preliminary manuscript, and worked on identification of plants. Miss Horner made an excellent series of photographs of the associations studied, as a part of her class work. Miss Sargent took responsibility for identification of all the more difficult and doubtful material. She had entire care of the class during the afternoons, thus leaving half of the time of Mr. Conard free for preparation of the manuscript report for special investigations. A number of examples of the marginal societies along the Sound were critically examined by Mr. Conard and Miss French. As a result of this united effort much progress was made upon the Vegetation project, thirty associations were diagnosed, and the study was brought within sight of completion. For so productive a season, major credit must be given to the Direction of the Laboratory in adding a competent assistant to the staff.

H. C. Elliott's Report
University of Toronto

The subject undertaken for study was the development of the nervous system in the lower vertebrates, especially **Fundulus.** The eggs of the latter were prepared at various developmental levels and young recently-hatched fish were also studied.

Unfortunately owing to difficulties in technique and an unexpected curtailment of my stay at the laboratories I was only able to lay the foundations for the intended work. These, however, were most valuable to me; I became expert in the handling of eggs, hatching and dissecting them under the binocular microscope, also in the peculiarities of technique required in making serial sections of the material; I obtained some excellent serial sections of various stages stained to show the general embryonic development for comparison with later neurological stains.

On this basis I am continuing my studies during the Winter of 1933-34, using the Lake Trout.

Doctor Fricke's Report

Studies of the chemical activity of X-rays have been made for pure water and water containing (1) oxygen, (2) hydrogen peroxide, (3) ferrous sulphate, (4) chromic acid, and (5) certain simple organic compounds, particularly formic, acetic and oxalic acid. The influence of oxygen, of the hydrogen ion concentration and of certain other ions has been studied for each of these reactions. The analytic procedure has been widened to include analysis for carbon dioxide, oxygen and hydrogen. The dependence of the X-ray action on the presence of minute traces of organic materials has necessitated the working out of special methods for keeping the irradiated solutions free of such impurities. It is interesting to note that water, purified by several distillations (from permanganate and chromate) and distilled directly into sealed-off pyrex glass vessels, cleaned chemically, may still give by irradiation as much as 10 microequivalents of carbon dioxide, showing the presence in the water of a corresponding amount of organic matter. The present method of purification involves a pre-heating to the softening point of all glassware used and heating of the vapor of the water to 900° C. The power of X-rays to cause the complete decomposition of organic matter, dissolved in water, may have a practical usage in the analysis of water for traces of organic matter. We may, during the coming year, be able to attempt to use this method in analyzing deep ocean water in conjunction with the work carried out by Professor A. Krogh of the University of Copenhagen. Irradiation with X-rays has also provided a rapid method of detecting organic impurities in the chemicals, which we are using. The amount of such impurities sometimes present in the highest grade of chemicals obtainable is rather saddening and has necessitated considerable work in developing suitable methods of purifying these chemicals. It was with some surprise that we found that pure, gas free water is not decomposed by X-rays, since it appears to be a well established fact that decomposition of water has been accomplished by irradiation with radioactive rays, and it is usually assumed that no qualitative difference would exist between these types of rays. Through the courtesy of Dr. G. Failla of the Memorial Hospital, New York City, 500 Millicurie of radon was placed at our disposal for irradiation with the beta-rays from radium, and it was found that no decomposition of the water took place, in opposition to the results of earlier workers. We expect later to be able to test the effect of the α-rays from radium.

A particularly interesting case of the influence of the hydrogen ion has been found for the production of hydrogen peroxide by irradiating water containing gaseous oxygen. The graphical representation of this relationship shows the simple form of a dissociation curve.

While over wide ranges of irradiation intensity most biological effects of X-rays are found independent of the intensity, in some cases a dependence is found to exist. It has been a much debated question whether this dependence has its origin in the primary effect of the rays or is due to the change which takes place in the living material during the irradiation. Usually we find the chemical effects of X-rays to be independent of the X-ray intensity, but for hydrogen peroxide we have for the first time met a reaction which did show such a dependence.

The determination of the electric impedance associated with the red corpuscle surface has been brought to a perfection which gives it value as a means of characterizing the normal red corpuscle. The results have given a very convincing evidence that the impedance is due to the presence of a membrane, acting as a static condenser. The method has been used in a study of hemolysis which has demonstrated that a red corpuscle may be hemolyzed without change of the electrical properties of the surface membrane. Since undoubtedly at the moment of exit of the hemoglobin the membrane must be permeable, it would appear that this permeability is temporary only, reversed within a very short time. This type of hemolysis takes place with water and saponin and complement-amboceptor used in low concentrations. When saponin is used in higher concentrations, a complete destruction of the corpuscle membrane takes place. Hemolysis by freezing-thawing is of a different type, resulting in a marked change in the impedance of the corpuscle membrane. The problem as to what changes take place in the membrane during hemolysis has also been considered in a theoretical study, in which the time of "fading out" of a corpuscle was calculated on the basis of the diffusion constant of hemoglobin. Comparison with data by Dr. E. Ponder indicates that there is a considerable permeability of the membrane during hemolysis, although data available for the diffusion constant of hemoglobin are not accurate enough to allow an accurate calculation to be made. In connection with the work on the red corpuscle membrane, studies have been made of the impedance of artificial membranes, particularly the collodion membrane. It is found that collodion membranes may be prepared which simulate the whole range of electric phenomena occurring at biological membranes, from the red corpuscle membrane to that of tissues. Changing the concentration and kind of salts used in the fluid in which the collodion membrane is placed, provides one means of effecting changes of such a wide range. Work has also been done on the dielectric constant of solutions of various proteins in water and on the influence of ions of high valence on the dielectric constant of water. During the summer Mr. Richard Baker continued our earlier work on ultraviolet dosimetry. This work is carried out in collaboration with Dr. Harry Goldblatt of Western Reserve University and is particularly concerned with the development of a dosimeter useful in studies of the antirachitic effect of ultraviolet light. Studies of the hereditary effects of very soft X-rays have been instituted, in conjunction with Dr. M. Demerec of Carnegie Institution.

Studies on Adrenalectomized Rats
The report of Professor Gaunt and Jo Howland Gaunt
College of Charleston

I Lactation

We began a study of the effects of adrenalectomy on lactation in rats under varying conditions. This work is still incomplete and only tentative conclusions can be drawn. It appears, however, that rats suffering from adrenal insufficiency, even though that insufficiency may be of only slight severity, will rarely lactate at all and never in normal amounts.

Adrenalectomized rats kept in good condition by injections of the **cortical hormone** will lactate sufficiently to raise small litters in the majority of cases, but this lactation is not in normal amounts and results in undersized young. The addition of rather large amounts of sodium chloride to the diet together with cortical extract administration permits a more adequate lactation. A sodium chloride-rich diet by itself is fairly but not totally effective in maintaining lactation in adrenalectomized rats.

II Survival after Cortical Hormone Treatment.

We had previously established that in our colony only 5% of our rats survived indefinitely after adrenalectomy. Such survival when it occurs is brought about by the hypertrophy of accessory cortical tissue.

When these animals are treated from one to three weeks after operation with cortical extracts, approximately 50% of them survive indefinitely after the extract is withdrawn. At autopsy in all such cases of indefinite survival microscopic cortical accessories can be found.

Professor A. J. Grout's Report

In November 1933 Vo. 2, pt. 1 of the Moss Flora was published. It is a monograph of the N. Am. Grimmiaceae by Geo. N. Jones of the University of Washington.

Vol. 3, pt. 4 was completed in Ms. and mostly in type. The Leskeas and Pseudoleskeas were monographed by Prof. A. J. Sharp of the University of Tennessee. Dr. Winona H. Welch of DePauw University is monographing the very difficult Fontinalaceae. As soon as she is able to complete the task, pt. 4 will be issued.

Material (i. e. specimens) has been loaned and other assistance has been given by the Laboratorie Cryptogamie of the Museum d'histoire Naturelle in Paris. This corresponds to our U. S. National Museum. Also from The German Berlin-Dalheim Botanishes Museum, a corresponding German institution.

Types were loaned from Paris and from the Canadian National Museum at Ottawa.

Our own National Museum, Harvard University, Yale, The New York Botanical Garden and numerous other institutions have co-operated.

Dr. T. C. Frye of the University of Washington is at work on the Polytrichaceae for Vol. 1. The eminent American bryologist, Mr. E. B. Bartram, is monographing the Pottiaceae for Vol. 1. Mr. Bartram is a relative of the early American botanist of the same name. He has made the most important bryologic discoveries of the present century, so far as North America is concerned, and has written a Manual of the Mosses of Hawaii.

Dr. A. LeRoy Andrews of Cornell University is at work on a monograph of the difficult Bryaceae for Vol. 2. Dr. Andrews is in Switzerland for a year and I understand is to consult in person some of the older herbaria of Europe to consult types of American species to be found there.

Mr. H. N. Dixon, the well known British bryologist, has been very helpful in determining doubtful specimens and in looking up references at Kew and elsewhere.

As before, hundreds of specimens have been determined for colleges and private students. In this way many unique specimens have been obtained.

The exsiccati which I have been issuing have had a larger sale this year than in any preceding, except during the first year of issue. A steadily increasing number of colleges are using my books as texts.

Due to the financial condition and the relatively high price, necessitated by the copious illustrations, sales of the Moss Flora have not been as large as hoped. However reviews have all been appreciative and foreign orders have been relatively large.

At the beginning I estimated that the work would take at least ten years; by the end of 1934 I hope the work will be half completed.

With the assistance now in sight, the work should proceed as fast as funds are available for publication. For the past two years all funds advanced by the Laboratory have gone into publication.

Professor S. I. Kornhauser's Report
School of Medicine, University of Louisville

My work consisted of a further study of the Malpighian tubules of the earwig Anisolabis maritima, especially with reference to the development of the tubes and the method of elimination of vital stains into the intestine.

It was discovered that the removal of the intact digestive tube with the attached tubules was advantageous in studying the living tubules and in actually seeing the dyes pass into the intestine. Details of structure could also be preserved by prefixation of the tissues in osmic acid fumes, fixation in Carnoy's fluid, and preservation in Euparal vert.

Besides the three segments of the tubes previously described, namely, the free end (distal segment) with its clear non-staining cells, the long middle portion with cells showing clear vacuoles and osmophilic bodies, and third, the shorter proximal portion (concentrating segment), there was discovered a muscular valve-like connecting piece which emptied into a muscular saccule of the intestinal wall. From the latter each tube is developed as a small sprout which grows by mitosis.

Colloidal dyes such as trypan blue, isamine blue, and lithium carmine, when injected into the coelom, are not eliminated by the tubules but the particles are phagocytized and accumulate in large quantities in the cells of the dorsal body cavity just ventral to the heart and show the outline of the entire heart nicely under the microscope.

Indigo carmine is rapidly eliminated and is the best dye to show the act of emptying of the tubules. Ten minutes after injection long needle-like particles of the dye are seen in the lumina of the tubes.

The movement of the dye toward the intestine can be followed because the cells do not stain. The muscular segment and saccule undergo active circular and longitudinal muscular contractions injecting the dye into the intestine where it passes caudad along four longitudinal grooves.

Brilliant cresyl blue, methylene blue, and neutral fuchsin are also eliminated but not so rapidly as indigo carmine. The first two also stain the osmophilic bodies of the middle segment and by their metachromatism bring out the interstitial cells nicely. Neutral fuchsin seems to pass through the cytoplasm of the middle segment cells. It shows up the brush borders clearly and is concentrated in the proximal segment where it may be seen as solid particles which are eliminated into the intestine.

In the adult insect there are on the average forty-eight tubules arranged in four groups ninety degrees apart around the caudal end of the mesenteron. In the first instar there are only eight tubules in four groups of two each. In the second instar there are sixteen tubules; two new ones have grown in each clump. This process of increasing the number of sprouts from the saccules continues throughout development. Thus the tubules are of different ages, the older ones being larger and darker due to the cytoplasmic structures in the cells of the middle segment. Young tubules are clear with small lumina and few vacuoles or osmophilic bodies. Not until these latter structures appear and the five segments are distinguishable are dyes like methylene blue taken up and eliminated. We have here material to study the ontogeny of cell structures associated with the function of excretion.

A Study of Acephaline Gregarines (Protozoa, Sporozoa) Found in Some Oligochaete Annelids

Report of O. G. Rybachok, Temple University

The study of acephaline gregarine infection among the oligochaete annelids as **Lumbricus terrestris,** Linn., **Lumbricus castaneous,** Sav., and **Helodrilus foetidus,** Sav. begun in the spring of 1933 at Temple University was continued. Comparatively little work has been done on the gregarine forms existant in the olegochaetes of America.

Specimens were gathered from various sections of the Laboratory grounds and thoroughly examined for infections. The data obtained enabled me to make a comparative study of geographical as well as seasonal distribution of the gregarine fauna. During the same period a marine annelid of the genus Pontodrilus, from Key West was examined.

Observations in all cases were made on living organisms. The living gregarines taken from the seminal vesicles or coelom of the oligochaete worms were studied in fresh smears made with .65% saline solution. Small pieces of glass were used to support the cover slip so that it did not exert a pressure on the organisms, while the edges of the cover slip were sealed with paraffin oil to prevent evaporation of the smear. This procedure enabled me to study methods of locomotion, the process of encystment, gamete formation, and union of gametes. The observations on living organisms were checked by a study of preserved materials which consisted mainly of cover slip smears fixed in Schaudinn's fluid, and stained in Mayer's haemalum.

This study is being continued especially from the point of view of seasonal and geographical distribution.

Report of Professor A. A. Schaeffer, Temple University

The summer of 1933 was spent in the study of the movements of human leucocytes. The work on leucocytes is another step in a program of research directed toward the establishment of correlation between the stereo characteristics of movements and of the fundamental stereo structure of organisms. Such correlations have been established for several groups of organisms: ciliates, flagellates, rotifers, et cetera. Previous work indicates that there exists a mechanism in the higher animals which produces a predominance of right spiral turns in leucocytes of these animals when the leucocytes are experimentally placed in such conditions that they can move spirally in three dimensions. The leucocytes(polymorphonuclear neutrophiles) of the hen have been found to move predominantly to the right. Preliminary tests on the leucocytes of the rat and the mouse also indicate a similar predominance. The technique of studying the leucocytes of the latter two mammals is much more difficult than that of the hen, and the data are correspondingly harder to secure. The same is true of human blood. Since this summer was the first time that human leucocytes were studied with reference to their spiral movements, the greater part of the time was necessarily spent in developing technique. Although the technique was not perfected by any means, it nevertheless was developed far enough to state confidently that human leucocytes move predominantly right, as predicted, but the data are too few to state a quantitative ratio between right and left turns, except to say that it appears to be lower than that of the hen which came out about 1:1.7.

Professor Ivon R. Taylor's Report
Brown University

At present a concentrated effort is being made to place the work of the course in General Physiology on such a basis that it will be possible to offer instruction in those phases of the subject in which most college students do not ordinarily have the opportunity to gain experience. The content of the course is being designed so that those who plan to enter biology or medicine may profit by the work. Development is being planned along lines that are fundamental and, in keeping with the modern trend in general physiology, the work is being placed on as quantitative a plane as possible. The acquisition of additional good equipment prior to and during the season has brought these objectives considerably closer towards achievement.

An unusual opportunity was afforded to the members of the class due to the fact that the symposia on "The Potential Differences at Interfaces and Their Bearing upon Biological Phenomena" were held at The Biological Laboratory while the course was in session. A great advantage was derived from the courtesy and interest shown by the prominent group of men taking part in the symposia in giving special lectures and, in some cases, demonstrations to the class in their fields of investigation. Members of this group who lectured were Dr. H. A. Abramson, Dr. David R. Briggs, Dr. Barnett Cohen, Dr. Kenneth S. Cole, Dr. Hugo Fricke, Dr. Stuart Mudd and Dr. Hans Mueller. Mr. Ernest Victoreen and Mr. Edward Walzl, investigators at The Biological Laboratory, also presented lectures. The cooperation which was received was highly gratifying and deeply appreciated.

Of special value and interest to the group in general physiology was the work on X-rays and ultra violet light, including dosimetry, offered at the Biophysics Laboratory through the kindness of Dr. Hugo Fricke who directed the work. The cooperation of Mr. Ernest Victoreen and the efficient assistance of Mr. D. M. Gallagher and Mr. R. F. Baker were also received in this connection.

It is felt that students can derive the most benefit from the course in general physiology by doing the regular work during the first season in which they are associated with the course. Research problems which await investigation are brought to the attention of the group from time to time and the opportunity is given to students to return to The Biological Laboratory another season to continue the work of the course and to carry on research. In keeping with this policy Mr. Edward Walzl returned to The Biological Laboratory to work on the effect of ions on the oyster heart, a problem which arose during his first season in the course.

This summer I considered it impractical to move my rather delicate research equipment from Brown University to The Biological Laboratory on account of the time involved in setting it up and the likelihood of breakage. Accordingly after the course was over I did not remain at The Biological Laboratory to do research but returned to Brown University in order that the work might be placed under way as soon as possible.

A paper was published entitled "A Micro Vessel for Glass Electrode Determinations of Hydrogen Ion Activity of Biological Fluids" by Ivon R. Taylor and James H. Birnie in Science, August 25, 1933, Vol. 78, No. 2017.

The Effect of Electrolytes on the Rhythm of the Oyster Heart

Edward Walzl
The Johns Hopkins University

The past summer was spent in continuing and extending the work started last year at The Biological Laboratory on the effects of certain ions on the rhythm of the isolated, perfused heart of the oyster, **Ostrea virginica.**

Since the water of Oyster Bay, from which the oysters were obtained, is diluted by fresh water springs, and since it has been reported that the body fluids of marine invertebrates are in equilibrium with the salt concentration of their environment, it was desirable to ascertain the osmotic pressure of the blood of the animals used. This was measured by cryoscopic and conductivity methods.

The non-electrolyte fraction of the blood was found to contribute 1.5-2.5% of the osmotic pressure, the total osmotic pressure corresponding to a freezing point lowering of $\Delta = 1.37°$.

Van't Hoff's solution, diluted until its osmotic pressure was equal to that of the oyster blood, was used as the control perfusion fluid. Perfused with this solution, the excised hearts of healthy animals could be kept beating with normal rhythm for several days.

All solutions were made up so as to be isotonic with the oyster blood, to be of the same pH (as compared with a bicolorimeter), and to contain the same ionic ratios as are present in Van't Hoff's solution, with the exception of the ions to be tested.

As it was found that the characteristics of the heart beat were altered by slight changes in the hydrostatic pressure, this was maintained constant by means of a flowmeter and other apparatus. The temperature was maintained at 18° C $\pm$ 0.1°.

Experiments on the excised hearts showed the following:

1. When the heart of the oyster is perfused with an isotonic solution of $CaCl_2$ there is immediate arrest in diastole with extreme loss of tone. In this condition it is hyperexcitable, going into a state of extreme systolic tonus on slight mechanical stimulation. Recovery is slow (1-2 minutes) on return to the control solution, and preceded by marked rise in tone.

2. Perfusion with an isotonic solution of KCl always causes immediate arrest in systole with greatly increased tone. Recovery on return to the control solution, is slow, beats not being resumed until the tonus has returned almost to the normal level.

3. An isotonic solution of NaCl causes decreased frequency of beat, decreased amplitude, and after about 1.5 minutes, systolic arrest with slightly increased tone.

4. Van't Hoff's solution lacking potassium causes the heart to stop immediately in systole.

5. Some variation was observed in the effect of a modified Van't Hoff's solution which did not contain calcium salts. In about one-third of the specimens studied, the heart stopped immediately in systole. In the others, the heart continued beating slowly with greatly prolonged period of contraction. There was loss of tone after each beat until the heart stopped in diastole. In these latter cases, replacing this solution with one lacking potassium, caused immediate rise in tone and arrest in systole, which was maintained until return to the control solution.

6. Solutions containing different concentrations of KCl from O up to its concentration in the modified Van't Hoff's solution, were

tested. The concentration of the other ions were the same as in the control solution.

It was found that the amplitude of contraction increased in proportion to the concentration of KCl. The extent of contraction remained the same, the increase in amplitude being due to more complete relaxation.

7. Increase $CaCl_2$ in the control solution causes decreased frequency of beat, and increased amplitude, i. e. the systolic tonus is increased, the diastolic tonus is unaffected.

8. Different amounts of an isotonic solution of $CaCl_2$ were added to pure, isotonic solutions of Dextrose, and the resulting solutions tested as to their effects on the excised hearts.

The solutions containing the higher concentrations of $CaCl_2$ caused decreased frequency of beat, increase of the period of relaxation, with attendant decrease of diastolic tone, and almost immediate arrest in diastole.

In the lower concentrations of $CaCl_2$ (about the same as that in the control solution, i. e. one part $CaCl_2$ in 44cc of solution) opposite results seemed to obtain. There was increased frequency, increased diastolic tone, and ultimate arrest in systole.

9. A few experiments with a like series of solutions using KCl Dextrose instead of $CaCl_2$ and Dextrose, indicated that KCl gives opposite effects to $CaCl_2$, both in the higher and lower concentrations.

In the higher concentrations there was systolic arrest with increased systolic tone, while in the lower concentrations there was decreased frequency, and increased extent of relaxation.

10. Perfusion with Mg-free Van't Hoff's solution produces at first systolic arrest. However, some of the hearts tested quickly became adapted to this solution and began again to beat quite strongly, though with increased systolic tone, and increased frequency over that shown in Van't Hoff's solution containing Mg.

11. Removal of both calcium and Mg (i. e. the solution containing only Na and K salts) causes decreased frequency, increase of systolic tone, and decreased amplitude with ultimate arrest in systole.

12. Removal of both K and Mg (i. e. the solution containing only Ca and Na salts) causes increased frequency, increase of systolic tone, and decreased amplitude with arrest in systole.

In general it may be concluded that Na and K are stimulatory in their action on the heart of the oyster, while Ca and Mg are inhibitory.

K and Ca are opposite in their effects, and antagonize one another.

Ca and Mg are similar in effect, but also seem to be antagonistic in at least some respects.

The influence of single ions on tonus seems to depend in part on the concentration of that ion.

Persons in Residence at the Laboratory in 1933
Including Members of the Staff.
Investigators, Assistants and Technicians

Abramson, H. A.—Research, dept. of biochemistry, College of P. and S., Columbia.

Bacon, Annette L.—Research, ass't., Temple.

Baker, R. F.—Research, grad. stud., Penn. State.

Bernstein, Felix.—Research, formerly director Institution for Mathematical Statistics, Univ. of Goettingen, Germany. Now at Columbia Univ.

†**Birnie, James H.**—Research, grad. ass't. Brown.

Briggs, David R.—Research, investigator in chemistry, The Otho S. A. Sprague Memorial Institute, Univ. of Chicago.

***Brown, Catherine R.**—Secretary, Biological Laboratory.

***Brownscombe, E. R.**—Research, chemist, Biol. Lab.

***Chouteau, Ellen M.**—Librarian, Biol. Lab.

Chen, T.-T.—Research, grad. stud., physiol. chem., Johns Hopkins Univ. School of Medicine.

Cohen, Barnett—Research, assoc. prof. physiol. chem., Johns Hopkins Univ. School of Med.

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