# Annual Report

OF

# THE BIOLOGICAL LABORATORY

1957-1958

LONG ISLAND BIOLOGICAL ASSOCIATION
COLD SPRING HARBOR
LONG ISLAND, NEW YORK

LONG ISLAND BIOLOGICAL ASSOCIATION

Incorporated 1924

# ANNUAL REPORT

of

# THE BIOLOGICAL LABORATORY

Founded 1890

Sixty-Eighth Year

1957-1958

The Biological Laboratory was organized in 1890 as a department of the Brooklyn Institute of Arts and Sciences. It was financed and directed by a Board of Managers, consisting mainly of local residents. In 1924 this group incorporated as the Long Island Biological Association and took over the administration of the Laboratory.

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# A MESSAGE TO THE MEMBERS

We ask you, as a member of the Association, thoughtfully to consider the financial report shown later in this pamphlet. Your contribution, and the contributions of our 385 other members, amounted to $8,840.00. This, together with the contributions from the Wawepex Society and Walter B. James Fund, and our net return from investments, totalled just under $13,000, and represented the only unrestricted income available to the Laboratory last year. The major item of our expenses, $205,611, was paid from grants and contracts attracted by the abilities of the laboratory staff; a minor amount of our other expenses is covered by self supporting items such as the book sales and summer activities. Your contribution therefore is multiplied more than 15 times in its effect. Unfortunately this is too high a multiple to be practical for the future. It is a matter of grave concern in that it is a serious and understandable deterrent to the replenishment of the Staff.

Three months ago our Laboratory Director prepared and presented to your Board a memorandum on the "Present Status and Plans for Future Development" of the Laboratory. He pointed out that to continue to maintain an adequate staff and plant necessary to attract the grants and contracts it is necessary to have an assured unrestricted income of about four times our present figure. To meet this requirement, your Board has undertaken a program to develop gifts to an endowment fund, and to expand its membership and the size of the annual gifts. The members, at the annual meeting in June, following a recommendation of a Policy Committee of our Board which was chairmanned by your retiring president, Amyas Ames, approved a change in the by-laws of the Association creating the new office of Chairman. This officer will work with the President in accomplishing these purposes.

As your new executive officers, we concur in the findings of the Policy Committee that a rehabilitation of the plant and creation of stability for the work and staff of the Laboratory are essential. These matters have been discussed with the administration of our close collaborator and neighbor, the Carnegie Institution of Washington's Genetic Laboratory. We feel such a program is necessary to continue the basic research in genetics, and the annual symposia and summer courses through which the Laboratory has developed a world-wide reputation and standing. You have helped to make this vital work possible in the past, and we hope we can count on your enlarged and continued support to insure the distinguished future of the Laboratory.

NEVIL FORD
Chairman

WALTER H. PAGE
President

# REPORT OF THE DIRECTOR

This year we began a concentrated effort to work out long-range plans for the functioning of the Biological Laboratory. At the 75th meeting of the Board of Directors of the Long Island Biological Association, held on November 3, 1957, a Special Policy Committee was appointed to review the activities of the Association, make recommendations as to its future policy, and suggest means for putting such plans into operation. The Committee held a series of meetings, and consulted with Dr. Caryl P. Haskins, president of the Carnegie Institution of Washington, regarding the relationship of the two laboratories. In a report to the Board of Directors, the Policy Committee made the following recommendations:

1) That strong support be given to the research program, which is the backbone of the Laboratory's operation, to work in teaching, and to the efforts to promote interchange of scientific thinking, all of which activities are soundly conceived and effectively carried out.

2) That every effort be made to secure an endowment fund to be used for the salaries of key research personnel.

3) That plans for modernization of the Laboratory's facilities be prepared by the director, and that funds for that purpose be searched out.

4) That the Biological Laboratory and the Department of Genetics of the Carnegie Institution of Washington continue to be coordinated in broad policy, under the director of the Department of Genetics, and that the research and administrative work of the Biological Laboratory be in charge of an assistant director, who will report to the director.

5) That a drive be organized to raise the funds needed for endowment and modernization.

Last winter President Ames assumed new and major business responsibilities, which would preclude his undertaking the extra activities ensuing from the recommendations of the Policy Committee. Therefore, in March 1958 it became necessary for him to resign the presidency of the Association. Mr. Walter H. Page was elected to succeed him, and Mr. Nevil Ford was elected chairman of the Members' Council. Mr. Page, although not previously active in the affairs of the Association, has long been familiar with its work through the participation of his father, Mr. Arthur W. Page, and his wife, Jane N. Page, who have played prominent roles as directors and officers.

As a first step in carrying out the recommendations of the Special Policy Committee, a firm of architects, Ferrenz & Taylor, has been engaged to prepare preliminary plans for a new laboratory building; and an effort is under way to secure funds for its construction.

## RESEARCH

The research work of the Laboratory is carried on in four sections: Population Genetics, Microbial Genetics, Tissue Culture, and Psychobiology. A detailed progress report of the work in each section is presented later in this Report by the member in charge of the project. Here I will summarize briefly.

Bruce Wallace has finished the preliminary phase of his study of the viability effects of radiation-induced mutations in herteozygous condition. This study is attempting to differentiate between two hypotheses regarding the genetic structure of Drosophila populations. According to one hypothesis, there is a "normal" or "type" gene at each locus; all other alleles are somewhat deleterious. According to the other interpretation, there are no "normal" genes; the normal condition results from the action of two different alleles in heterozygous condition. Dr. Wallace's data indicate that randomly induced mutations in heterozygous condition increase the viability of otherwise homozygous individuals. These results offer no support for the theory of "normal" genes, and are best explained on the basis of the second hypothesis.

A completely exploratory project, for investigation of the effect of genetic heterozygosity on developmental variation, has been undertaken by James C. King, in the hope of bringing together the fields of population and developmental genetics. A really effective intergration of these fields would be of great value to both, and would very probably contribute substantially to progress in research in such diverse areas as plant and animal breeding, studies of abnormal growth, and gene chemistry. The first year of the project has been devoted to the adaptation of existing techniques, both biological and statistical, and their development and refinement to make them applicable to the specific problems envisaged. No spectacular breakthrough has yet been made, but the work done to date indicates the project has promise. Embryos of different populations do show characteristic differences in development as early as the blastular stage, and there appear to be differences between heterozygous and homozygous populations in developmental rates. The immediate problem is to devise precise methods of measuring these differences and of analyzing the measurements, so that significant differences can be demonstrated with a reasonable expenditure of time and effort. This work is being pressed as rapidly as possible.

Hermann Moser during the past year continued his studies of spontaneous and induced genetic changes in human cells grown in vitro. Applying the methods developed by Puck and his associates for the plating of single cells, he developed an analytical technique for the accurate determination of survival fractions of single cells. This technique takes account of the fact that some fraction of the single cells plated on culture dishes reaggregates during the plating procedures, depending on the density of cells on the plates. Dr. Moser studied the effects of ultraviolet light

on the fate of single cells, and determined and analyzed the ultraviolet survival curves in a number of established cell strains. He also carried out experiments designed to detect, among the survivors of large populations of irradiated human cells, induced mutations affecting biochemical functions.

Ellis Englesberg and his collaborators, working with bacteria, continued a study of what is known as diauxie, a phenomenon whereby living cells, when supplied with two nutrients which they can utilize, use one of them first, and then, when the supply is depleted, adapt so as to grow on the other. The cell thus possesses a mechanism for reacting with its environment, internal and external, and producing enzymes as required for the utilization of various compounds. Bacteria placed in an environment where several compounds are available usually are not induced to synthesize enzymes involved in the utilization of all these compounds at once. It is in general found, for instance, that if glucose is present as one of the several carbon sources, the cells do not utilize other carbon sources that are present, and as a result multiplication proceeds with glucose as the sole carbon and energy source. Dr. Englesberg and his co-workers found that diauxie is controlled by the genetic mechanism of the cell, and their studies included various genetically variant strains of bacteria.

P. D. Skaar continued his analysis of a small region of bacterial (Escherichia coli) chromosome containing the genes concerned with tryptophan synthesis. It appears probable that the arrangement of four tryptophan genes in E. coli is identical with the arrangement of four similar genes in Salmonella, in that it coincides with the sequence of successive steps in the synthesis of tryptophan. A gene concerned with sensitivity of the bacteria to phage T1 is located near the tryptophan loci.

M. Demerec and Joan Sams, working with bacteria, studied the relation between X-ray dose and the frequency with which mutations are induced by the treatment. Since large numbers of bacteria can be treated in such experiments, very small percentages of induced mutations can be detected. Small doses of X-rays were applied, and the objective was to find out whether or not there is a threshold dose for genetic effects of radiation—that is, a dose below which treatment is not effective in inducing mutations. The smallest dose was 8.5 roentgens; and it was able to induce mutations. Thus, within the limits of the experiments, no threshold was detected.

Harold A. Abramson and his collaborators have continued their studies of the psychobiological effects of LSD-25 (lysergic acid diethylamide) as well as many of its derivatives such as ALD (1-acetyl LSD), OML (oxymethyl LSD), and DAM (lysergic acid dimethylamide). Comparative bioassays were conducted with Siamese fighting fish as the test animals. These fish have been found suitable for conducting bioassays in which extremely small concentrations of the various compounds need to be determined. Studies of reaction to these compounds were also carried

out with volunteer nonpsychotic human subjects, as well as with shizophrenic patients at the Central Islip State Hospital. An effort is being made to find a substance that will block LSD reaction.

Alexander Sokoloff has been pursuing two problems, one dealing with inheritance of eye color in the flour beetle and the other with the genetic structure of horseshoe crab populations. His detailed report of the results so far obtained is included in this volume. In observing horseshoe crabs on the Sand Spit beach during the season of 1957, he estimated the size of the adult population then as between 2,500 and 17,000. Dr. Sokoloff also noted that there were about four times as many males as females; that males seemed to locate the females by sight; and that one female deposited at least 11,000 eggs in one nest on a strip of beach exposed at low tide. Eggs are laid in the sand during the mating season, in late May and early June. The eggs hatch into trilobite larvae, which after the first moult acquire the appearance of adult horseshoe crabs. At low tide, thousands of young crabs can be observed in shallow pools on the beach, feeding on small organisms found in the sand. In order to identify the individuals which he had recorded, Dr. Sokoloff marked them with thumbtacks. This method of tagging appears to be painless to the crabs. Dr. Sokoloff would appreciate it if anyone seeing one of the marked horseshoe crabs would report the location to him.

Katherine Brehme-Warren has been working on an extensive revision of a reference book which describes the known mutants of Drosophila melanogaster. The book was published in 1944, was reprinted in 1950, and is at present out of print. Dr. Brehme-Warren and the late Dr. Calvin B. Bridges were the authors of the first edition.

Again during the summer of 1957, visiting scientists were particularly active in research with microorganisms (viruses and bacteria), a type of experimentation for which our laboratories are well equipped. A group from the University of Illinois, consisting of S. E. Luria, E. Calef, Nina Calef, Sylvia Smith, R. C. Ting, and H. Uetake, worked on analyses of the genetic structure of bacterial viruses and the genetic changes found in certain viruses and bacteria. R. D. Hotchkiss and Audrey H. Evans, of the Rockefeller Institute for Medical Research, studied the properties of various pneumococcal strains, in an effort to find suitable material for further research on the transfer of genetic properties from one bacterial strain to another.

N. E. Melechen of Saint Louis University, and L. Mindich of the Rockefeller Institute, studied the synthesis of deoxyribonucleic acid in virus T2 of a colon bacillus. A. Novick of the University of Chicago began an investigation, also using colon bacteria, of the origin of an enzyme involved in utilization of the sugar galactose. A. Bernheimer and Lois Schwartz, of New York University College of Medicine, tested extracts prepared from 40 species of plants growing in Cold Spring Harbor in an effort to find specific inhibitors for a certain enzyme. L. Y. D. Hamilton, of the Sloan-Kettering Institute for Cancer Research, continued experiments on the incorporation of thymidine by certain cells in rats.

S. Granick of the Rockefeller Institute spent the summer preparing review articles for publication.

In addition to these summer guests, a number of scientists came to Cold Spring Harbor for shorter periods of time to give lectures, attend conferences, or visit with friends.

## SYMPOSIUM

The twenty-third Cold Spring Harbor Symposium on Quantitative Biology was held for nine days, June 3rd to 11th, 1958. It was attended by more than 250 scientists. The purpose of this meeting was to bring together outstanding investigators in the field of genetics, so that experimental and analytical methods and conclusions could be shared, and discussed. This year's Symposium dealt mainly with the nature and properties of the mechanisms of heredity, and with the processes that are responsible for different recombinations of parental traits in offspring. The experimental results presented for discussion by scientists working with viruses, bacteria, fungi, insects, and plants, strongly indicated that these recombination processes are similar in different groups of organisms. Thus they gave additional support to a conclusion reached earlier, namely, that the basic mechanism of inheritance is the same in all living organisms, from the smallest and simplest known, viruses, to the most complex, including man. About sixty of the participants were from 17 foreign countries, including Australia, Denmark, Finland, Germany, Great Britain, Greece, India, Ireland, Israel, Italy, Japan, New Zealand, Poland, Sweden, Switzerland, and Yugoslavia, as well as Canada. The Laboratory financed the conference with funds obtained from the National Science Foundation, the United States Public Health Service, the United State Atomic Energy Commission, the Carnegie Corporation of New York and the Rockefeller Foundation.

## TEACHING

The Nature Study Course in the summer of 1957 was supervised by Mr. Marvin Rosenberg, of Northport Central High School, Northport, N.Y. Other teachers were Mr. Otto Heck of Island Trees High School, Levittown, N.Y., and Mrs. Jill A. Lamoureux of Port Washington, N.Y. They were assisted by the Misses Donna Granick, Gail Geraghty and Elizabeth Pierce. Children in these classes are trained to improve their observation of the many animals and plants in the area in which we live, and instructed in methods of finding answers to the many questions which are raised by these stimulated observations. Their knowledge of natural phenomena is expanded and through these methods an interest in nature study is aroused and intensified in the young people of the community. The children ranged from six to thirteen years of age, and were divided into seven classes according to age and experience. At the conclusion of the first class, which was held in July, a new one was organized for the month of August, by special request. A total of 168 children attended the courses this year.

A two-week course for elementary school teachers, Workshop in Nature Study, was offered for the second season in 1957. Lectures, discussions, individual projects and field trips were included in the curriculum; and two in-service credits were awarded by the New York State Education Department to the 14 teachers who successfully completed the requirements.

For the thirteenth consecutive year the course on techniques and problems of research with bacterial viruses was given in 1957, with Dr. G. Streisinger of the Carnegie Institution of Washington, and Professor S. E. Luria of the University of Illinois, in charge. They were assisted by Sylvia Smith and Robert Ting, of the University of Illinois. Eighteen students and one auditor were enrolled.

Genetics of Fungi was taught for the second year in 1957. Instructors were Drs. R. W. Barratt of Dartmouth College and E. Käfer of McGill University, Montreal. There were nine students and one auditor in attendance. This year the program was expanded from three to four weeks. This comparatively new course is designed, as are the other courses—for research workers and advanced graduate students; and it complements the courses on Viruses and Bacterial Genetics by greatly extending the teaching program in genetics of microorganisms.

The three-week course in Bacterial Genetics, in its eighth year, was conducted by Drs. M. Demerec, V. Bryson, Irving Goldman, E. M. Witkin and P. D. Skaar, in collaboration with Dr. H. Moser and Messrs. W. D. Cannon, E. L. Lahr and H. Ozeki. Enrollment in the course was eighteen students and one auditor.

## LECTURES

As part of the 1957 program of courses, invited speakers presented a series of seminars, which were open to all members of the Laboratory, and of neighboring scientific institutions. The seminar schedules are listed in the reports of the courses in this Report. In connection with the Nature Study Course, films were shown on two evenings to students and their parents and friends.

Following the 1958 Symposium, the Laboratory collaborated with the Planned Parenthood Group of North Suffolk in organizing a public panel discussion on "Education." With Dr. R. Gordon Hoxie of the C. W. Post College of Long Island University as moderator, six panelists opened a discussion in which the audience participated. The panel was composed of Professor D. G. Catcheside of Birmingham, England, Professor M. Westergaard of Copenhagen, Denmark, Dr. Edward Borowski of Gdansk, Poland, Mr. Jacob G. Michael of Jerusalem, Israel, Professor Chozo Oshima of Misima, Japan and Professor Russell Kirk, Editor of "Modern Age" and "The Conservative Mind." The meeting was held in the Lecture Hall of the Laboratory and about 150 persons were present.

The Lecture Hall was used by several local groups during the year. On October 16, 1957 the American Association of University Women,

Huntington Township Branch, held a fellowship meeting, addressed by a former fellow of the the A.A.U.W. The annual meeting of The Nature Conservancy, Long Island Chapter, was held on December 6. On January 8, 1958 the Foreign Policy Workshop of the Huntington League of Women Voters sponsored several lectures on atomic energy. Dr. Demerec spoke concerning the work of the laboratories at a meeting of the Southwood Association on May 13, 1958; and on May 16 there was a public meeting on the subject of substandard housing in Huntington Township, sponsored by the Unitarian Fellowship of North Suffolk and the Social Justice Committee. From May 25 to May 29 both the Lecture Hall and the living facilities of the Laboratory were used by Columbia University for a Symposium on Comparative Endocrinology, which was attended by 142 scientists in that field.

## SPECIAL EVENTS

On Sunday, September 29, 1957, more than two hundred and fifty members and friends of the Association attended a demonstration and tea in Blackford Hall, followed by a talk in the Lecture Hall. This open-house demonstration, which has become an annual fall event, is held so that members may learn informally about current research at the Laboratory, and become more closely acquainted with the work they help to support. The scientific exhibits included projects of the regular staff of the Laboratory and staff members of the Department of Genetics of the Carnegie Institution. A brief lecture, entitled "Genetic Experiments with Human Cells," was given by Dr. Hermann Moser, who described the potentialities of new techniques for growing human cells in laboratory cultures for the study of certain problems of human heredity. The serving of the collation was efficiently organized by Mrs. Edward S. Blagden.

## SCHOLARSHIPS

The funds available for scholarships in 1957 were distributed among the following summer investigators and students, to help toward the living expenses of the former and the tuition fees of the latter:

John D. Jones Scholarships—Helen Clugston, University of Rochester, Rochester, N.Y.; Murray Coulter, University of California, Los Angeles, Calif.; Robert J. Doyle, Windsor, Ontario, Canada; William Firshein, Rutgers University, New Brunswick, N.J.; Julian Gross, Institute of Animal Genetics, Edinburgh, Scotland; Ann Heuer, Rutgers University, New Brunswick, N.J.; William Ogata, Dartmouth College, Hanover, N.H.; Minna Rotheim, University of Rochester, Rochester, N.Y.; Hassan Rouhandeh, University of Teheran, Iran; Richard Schneider, Dartmouth College, Hanover, N.H.; Dr. P. R. Srinivasan, College of Physicians and Surgeons, New York, N.Y.; Dr. Tsutomu Watanabe, Keio University School of Medicine, Tokyo, Japan; and Dow O. Woodward, Yale University, New Haven, Conn.

Temple Prime Scholarship—Students: Hassan Rouhandeh, University of Teheran, Iran; Richard Schneider, Dartmouth College, Hanover, N.H.

Dorothy Frances Rice Fund—Student: Helen Clugston, University of Rochester, Rochester, N.Y.

Biological Laboratory Scholarships—Two research workers: Dr. Leonard Hamilton, Sloan-Kettering Institute for Cancer Research, New York, N.Y.; and Dr. Karl Mamorosch, Rockefeller Institute for Medical Research, New York, N.Y. Four students: Dr. Tsutomu Watanabe, Keio University School of Medicine, Tokyo, Japan; Dr. Nathan Entner, New York University Medical School, New York, N.Y.; William Ogata, Dartmouth College, Hanover, N.H.; and Hassan Rouhandeh, University of Teheran, Iran.

## BUILDINGS AND GROUNDS

A number of needed improvements have been made to the buildings during the past year. In Wawepex, where our Nature Study courses are conducted, we have added two new washrooms which were made necessary by the increase in registration in the children's nature study courses, which last year exceeded the hundred and fifty mark. We have also added an outside stairway from the third floor of Wawepex, which was used for the first time for the Nature Study classes, so that children may reach the outside directly by means of these stairs.

An additional room has been added to Cole Cottage. In the Carpenters' and General Workshop we have installed a new heating system, which makes it possible to work there in greater comfort during the wintertime, and in inclement weather. A much needed new heating system has also been placed in Urey Cottage.

New leaders and gutters have replaced the old ones in both the Nichols and DeForest buildings, and two of the apartments in Hooper House have been redecorated.

Among other improvements may be included the purchase of three new refrigerators and a number of new beds for the various apartments on the grounds.

## FINANCES

The expenses of full-time research and of the Symposium are being met by grants received from the organizations mentioned in the following section.

The Laboratory continued to receive the support of the Wawepex Society and annual contributions of members of the Long Island Biological Association. These funds were used to meet expenses connected with the administration of the Laboratory, summer research, scholarships, and the upkeep of buildings and grounds.

## ACKNOWLEDGMENTS

The Laboratory gratefully acknowledges the grants received from the following organizations in support of its scientific activities:

For research of the Population Genetics section: the Atomic Energy Commission and the National Science Foundation.

For research of the Microbial Genetics section: the Atomic Energy Commission, U. S. Public Health Service and the National Science Foundation.

For research of the Psychobiology section: the Josiah Macy, Jr. Foundation.

For revision of "Mutants of D Melanogaster": National Science Foundation.

For the Symposium: the Carnegie Corporation of New York, U. S. Atomic Energy Commission, U. S. Public Health Service, National Science Foundation, and the Rockefeller Foundation.

For the summer courses: the U. S. Public Health Service.

For re-equipment of summer laboratories: the National Science Foundation.

For purchase of a new electron microscope: the U. S. Public Health Service.

Our sincere thanks are expressed to the following members of the Long Island Biological Association who entertained our guests at dinner parties during the Symposium: Mr. and Mrs. Robert L. Cummings, Jr., Mr. and Mrs. Paul Cushman, Mr. and Mrs. Ferdinand Eberstadt, Mr. and Mrs. Maitland A. Edey, Mr. and Mrs. Joseph R. Eggert, Jr., Mr. and Mrs. Richard S. Emmet, Mr. and Mrs. Nevil Ford, Mrs. George S. Franklin, Mr. and Mrs. George S. Franklin, Jr., Mr. and Mrs. Augustin S. Hart, Jr., Mrs. Burton J. Lee, Sr., Mr. and Mrs. Grinnell Morris, Mr. and Mrs. William B. Nichols, Miss Juliet L. Nourse, Mr. and Mrs. Arthur W. Page, Sr., Mr. and Mrs. John H. Page, Mr. and Mrs. Walter H. Page, Mr. and Mrs. H. Irving Pratt, Mr. and Mrs. Cooper Schieffelin, Mr. and Mrs. William S. Smoot, Mr. and Mrs. Franz Schneider, Mr. and Mrs. Eugene S. Taliaferro, Mr. and Mrs. Taggart Whipple, and Mr. and Mrs. Cornelius Wickersham, Jr.

It gives me pleasure also to acknowledge the help of members of the Women's Committee who provided refreshments for the Open House Tea and Demonstration in September and helped to organize the dinner parties for the Symposium participants.

Once again we wish to thank the Wawepex Society especially for its annual gift, and express our appreciation to the many friends who during the year contributed as members of the Association.

M. DEMEREC
Director of the Laboratory

# REPORTS OF LABORATORY STAFF

## INDUCTION OF GENETIC CHANGES BY X-RAYS

M. Demerec and Joan Sams

It is well known that ionizing radiations, such as X-rays and gamma rays, are potent inducers of changes in living cells which are exposed to them. Among the changes that can be induced are those affecting the hereditary (genetic) mechanisms, that is, alterations in the units of heredity (the genes) and in the chromosomes that carry the genes. Gene changes are called mutations, and there is a considerable amount of evidence showing that the frequency with which mutations are induced by radiation is proportional to the radiation dosage; in other words, the relation between dose and effect is linear. Such evidence has been obtained by a number of scientists, working in different laboratories and experimenting with different animals and plants. In all the earlier experiments, however, data could be obtained only if fairly high doses were employed in treatment, for the frequency with which mutations are induced is in general so low that with the materials previously used it was technically impossible to obtain data for low doses.

A question therefore arose as to whether the conclusion reached by geneticists regarding proportionality between dose and effect applied to low doses as well. Those who raised this question pointed out that with regard to a number of biological reactions a threshold for irradiation dosage had been observed—a dose below which radiation treatment was not effective. At present it is of considerable interest to know whether or not there is a threshold dose for the genetic effects of radiation. Under ordinary circumstances, the amount of radiation to which the human race is likely to be exposed in connection with atomic energy activities is small; and if a threshold does exist it may well be above that amount. If this is so—it is pointed out by those who question the complete linearity of the dose-effect relationship—genetic damage is not likely to be produced by the atomic radiation to which the general population will be exposed.

In connection with our studies of mutation and mutability we have developed, employing bacteria as the experimental material, a very sensitive method for detecting mutants that are present in small numbers in large populations of organisms. Sometimes we can detect mutants occurring with a frequency of one per hundred billion ($1 \times 10^{-11}$). This technique therefore makes it possible to study the mutability of individual genes, even though the rate is extremely low. We have been studying dose-effect relations in several mutants of Escherichia coli and Salmonella typhimurium treated with low doses of X-rays. We are measuring reverse mutability in individual genes that control the synthesis of essential compounds, that is, reversions from auxotrophy to prototrophy. Tests have been completed with three mutants of E. coli: argenine-3 (ar-3), methionine-2 (me-2), and threonine-1 (thre-1). The lowest doses applied in

these experiments were 8.5 roentgens (r) with ar-3 and 17 r with the other two mutants; and the highest dose was 4320 r. The data show a linear dose-effect relationship for these three genes. Thus, within the limits of the experiments, no threshold was detected.

These results support the view of geneticists, who believe that even a low dose of radiation is effective in inducing mutations. Experiments with several genes of Salmonella are in progress, and the results so far are similar to those obtained in experiments with E. coli.

It has been determined that the spontaneous-mutation rates per $10^{10}$ bacteria are 10.8 in ar-3, 15.8 in me-2, and 2.3 in thre-1. From the curves of graphs plotting the dose-effect data it is estimated that the X-ray dose which doubles the spontaneous rate ("doubling dose") is 68 r for ar-3, 186 r for me-2, and 85 r for thre-1. The number of additional mutants induced by treatment with an X-ray dose equivalent to the doubling dose is the same as the number of mutants occurring spontaneously—without any treatment and for causes unknown. The doubling doses for the three genes studied are very much higher than the natural radiation. Thus it is evident that natural radiation could account for only a small fraction of the spontaneously occurring mutations.

# VIABILITY MUTATIONS

Bruce Wallace, Carol Madden, Gloria Gillies, and Ellen McMullen

During the past year we have concentrated entirely upon the viability effects of randomly induced mutations in heterozygous condition. Last year, it may be recalled, we reported on the first experiments of this sort; we have now greatly increased the body of data available. Our conclusion of last year remains unchanged: It appears as if the effect of a random mutation in heterozygous condition on an otherwise homozygous genetic background is to improve viability. We will describe the logic of these experiments and summarize the results in this report.

Why do we emphasize the fact that the mutations studied are random mutations? Because we are attempting ("groping" may be a better term) to learn something of the nature of the genes found at different loci on a chromosome obtained from a large population. Imagine that we are separated from a house by a high wall and we want to describe the house without being able to see it. One approach would be to throw stones over the wall and listen to the noise they make when they strike the house. If our control were perfect and we threw each stone in precisely the same manner we would learn very little. On the other hand, with perfect control we could vary each throw and "scan" the house; we could then estimate its size and, for example, plot the positions of windows in the walls. If our aim were not perfect, however, and we threw the stones so that they hit the house at random we could still obtain an estimate of the size of the house as well as the relative areas of windows to walls. If nearly every stone thrown resulted in a broken window, we would conclude that the unseen house was the proverbial glass house or, more realistically, a greenhouse.

Essentially the same line of reasoning underlies the study of viability effects of new mutations. X-rays cannot be aimed at individual gene loci so the possibility of "scanning" the chromosome is out of the question; these radiations can, however, induce gene mutations at loci anywhere on the chromosome. If we study the effects on viability of a large number of independently irradiated chromosomes, we obtain some notion of the effects of these mutations and, therefore, of the nature of the genes on the non-irradiated chromosomes.

Briefly, the experimental test consists of comparing in each of two series of cultures the viabilities of three classes of flies relative to that of a fourth class which is common to both series. The fourth class serves as a constant standard. The classes of flies are designated as CyL/Pm, CyL/+, Pm/+, and +/+ where CYL/Pm is the standard for comparison. In the control series, the + chromosomes are non-irradiated. In the X-ray series, one of the + chromosomes in the wildtype flies is an irradiated chromosome (these flies are designated +/(+)). Depending upon the experiment, either the CyL/+ or the Pm/+ class carries an irradiated chromosome while the other class carries one which is non-irradiated. There are, consequently, two classes of flies in the cultures

of the X-ray series which should be identical to the corresponding classes of the control series as well as two classes which differ by carrying irradiated chromosomes.

The results of seven experiments which have been completed are summarized below. We will not attempt to describe the different experimental techniques in detail; suffice it is to say that the dose of X-rays administered was either 500r or, in one instance, 1000r; that in some instances males were exposed while in others females were treated, and that in some experiments irradiated "background" chromosomes were introduced into the control series to make this series resemble somewhat more closely the irradiated cultures. In all experiments the viability of CyL/Pm flies is 1.000 by definition.

Experiments in which the irradiated series of cultures contain CyL/(+) and +/(+) flies:

| Designation | Series | CyL/+* | Pm/+ | +/+* | Mean number of flies per culture | Number of cultures |
|---|---|---|---|---|---|---|
| 2- 9 (M) | Control | 1.094 | 1.146 | 1.008 | 375.81 | 766 |
| | X-ray | 1.115 | 1.137 | 1.033 | 375.90 | 764 |
| 6-14 (F) | Control | 1.093 | 1.139 | 1.000 | 393.91 | 676 |
| | X-ray | 1.108 | 1.140 | 1.007 | 390.85 | 672 |
| 15-22 (F) | Control | 1.105 | 1.137 | 0.989 | 380.11 | 636 |
| | X-ray | 1.110 | 1.145 | 1.015 | 381.82 | 637 |
| 19-26 (M) | Control | 1.100 | 1.143 | 0.979 | 325.73 | 596 |
| | X-ray | 1.108 | 1.136 | 0.989 | 327.13 | 598 |

Experiments in which the irradiated series of cultures contained Pm/(+) flies and +/(+) flies:

| | | | | | | |
|---|---|---|---|---|---|---|
| 11-18 (M) | Control | 1.098 | 1.127 | 0.983 | 368.44 | 639 |
| | X-ray | 1.095 | 1.125 | 0.990 | 366.12 | 637 |
| 37-43 (M) | Control | 1.154 | 1.189 | 0.992 | 279.59 | 499 |
| | X-ray | 1.147 | 1.201 | 1.002 | 279.05 | 496 |

Experiments in which the irradiated series of cultures contained CyL/(+) and +/(+) flies but in which the wildtype flies of both control and X-ray series are heterozygous for two different second chromosomes:

| | | | | | | |
|---|---|---|---|---|---|---|
| 23-34 (Het) | Control | 1.185 | 1.215 | 1.143 | 307.08 | 839 |
| | X-ray | 1.182 | 1.222 | 1.155 | 305.60 | 837 |

In each of the experiments, the average proportion of wildtype flies in the X-ray series exceeds that of the corresponding flies in the control series. With one exception (23-34 Het) the same is true for the CyL/+ flies; these also occur in higher frequency in the X-ray than in the control series. If the results are recalculated by setting the class of flies (CyL/+ or Pm/+) which in the X-ray series is free of irradiated second chromosomes as the common standard, the results are unchanged. Again, without exception, the average proportions of wildtype flies in the X-ray series exceeds that of the control. In this case, however, there are two exceptional cases (23-34 Het and 15-22F) in which the CyL/(+) flies of the X-ray series occur in lower relative proportions than the CyL/+ flies of the controls.

The results described here indicate that there exist in populations high frequencies of heterotic mutations, mutations whose presence in a population is determined not by their effects when homozygous but by their interactions with other alleles occurring at the same locus. The results indicate that over half of all loci on a chromosome taken from a large population are occupied by genes which are deleterious when homozygous but which are heterotic with any one of many other alleles to which they can be induced to mutate.

A variety of possible alternative explanations of our results has been examined and discarded as incapable of fitting known facts: (1) **Most mutations are beneficial.** Discarded because the statement implies that natural selection acts to accumulate deleterious genes in a population. (2) **Homozygous individuals are abnormal and, therefore, random mutations have an average beneficial effect on such individuals.** Discarded (a) because the experimental flies were homozygous for chromosomes taken from a large population and therefore not "abnormal" in gene content and (b) because these homozygotes were "better than average" homozygotes. The statement implies that the average result of any set of mutations is that of a regression toward the mean and that the mean viability of a population is stabilized by the random effects of mutation. (3) **The observed increase in viability results from the dominance of "beneficial" mutations and the recessivity of those with "deleterious" effects.** Discarded (a) because the statement is contrary to known facts and (b) because it would require an absurdly large number of mutations per chromosome to produce the observed increase in viability. (4) **Heterotic loci exist but the number of these is limited to a small percentage of all loci.** According to this statement we would expect that flies homozygous for their second chromosomes would have less than the optimal proportion of heterozygous loci; the newly induced mutations partially restore this optimum and, therefore, increase viability. This idea

has been discarded because—unless the optimum proportion of heterozygous loci is approximately 100%—it would predict that $F_1$ strain- or race-hybrids should frequently be less viable than the parental strains themselves; this simply is not so. There are certain other, more complex, ramifications of this statement which can also be refuted.

The picture of a population with which we are left, then, consists of numerous alleles at each locus. Random combinations of these alleles are selectively advantageous; any one of these numerous alleles when homozygous has an average deleterious effect on viability. Presumably the amount by which individuals heterozygous for different alleles exceed the corresponding homozygotes in average fitness is a function of the proportion of heterozygosity at the remaining loci. This statement is based on the rather large (1½%!) increase in viability observed in our experiments; it is obviously impossible that an increment this size can accompany each of thousands of mutations. At the same time, our conclusion that the optimal proportion of heterozygous loci approaches 100% implies that the average effect of heterozygosis does not become negative after a given number of loci are made heterozygous.

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# DEVELOPMENTAL ASPECTS OF POPULATION GENETICS

James C. King

On June 1, 1957 work was begun on a new research project which, it is hoped, may help to bridge a gap existing between developmental and population genetics. Population genetics has made rapid progress in the last decade and a considerable portion of this advance in knowledge has been contributed by this Laboratory. It has been pretty conclusively demonstrated that the genes or genetic factors possessed by a population of cross-breeding organisms are not a fortuitous collection of unrelated elements, but constitute a delicately balanced system undergoing constant readjustment in response to selection. In any population those factors are maintained which, when paired at random, as happens whenever a male and a female nucleus unite to form a zygote, have a high probability of producing in the environment where the population lives viable and fertile individuals. Genes or combinations which tend to produce individuals of lower viability or fertility—which are not good mixers—are reduced in frequency.

Since a new or mutated gene will almost inevitably be tried out in heterozygous condition, its survival in a population will depend on its performance when paired with a dissimilar allele. As might therefore be expected, evidence continues to pile up that most populations are heterozygous to a degree that earlier students of genetics never dreamed of. And this genetic diversity is not a load—a cross—born because some malevolent force is constantly producing degenerative mutations. It is a tool of survival used by the population to keep its members within reasonable limits of variation in form and function—in size, shape, color, length of jaw, digestive ability or what not. Any artificial reduction in the heterozygosity of a population usually produces deviant individuals—flies, chickens, corn plants or mice—which depart from the set of characters most common in the population—from what we call the modal phenotype.

We can define population genetics as the study of those processes which result in a given population having a given set of characters and which account for the persistence or change of that set. Since he deals with populations or groups of individuals, the population geneticist is interested not in the character—weight, for example—possessed by one individual selected as representative or normal, but in the average weight of a sample of a number of individuals and the departure of the different individuals in the sample from the average. To use the more precise statistical terms, he is interested in the mean and the variance. Two populations may have identical means, but different variances; they may have different means, and indistinguishable variances. There are many ways in which two populations may be shown to differ statistically even though one cannot, by looking at any one individual, say definitely from which population it comes. It is often possible to show that these statistical differences between populations are genetically determined.

Almost all the work in population genetics has been done by observing characters of adult organisms. These characters must, of course, result from processes which go on during individual development; differences in adult characters must be produced by different developmental events. Developmental genetics deals with these processes by which, in the embryo or during growth, genes produce their effects. For very good reasons developmental geneticists have not approached their problem from the point of view of the population. Rather, they have worked with organisms carrying single genes of major effect which when present produce clearly observable abnormality. For example, one can compare albino embryos with non-albinos and find at what point in developmen one can demonstrate a difference between the two; perhaps one can determine just what substance, necessary for the production of pigment, fails to appear. Genes which are variable in their expression, which sometimes produce an effect and sometimes not, or which produce a graded series from little to much, are obviously unsuited to this type of work. And so developmental geneticists have largely confined their experiments to "switch" genes, and give us careful descriptions of when and how the first rudiments of abnormality appear, at what point in development a given lethal gene causes the embryo to die or at what stage a given genotype can be shown to be chemically different from another.

The time may have come, however, to attempt a synthesis of these two very different approaches. In order to do this one must devise ways of dealing with embryos or early stages on a statistical basis. One cannot employ qualitative descriptions, however precise, of a few embryos nor accurate chemical assays of homogenates of many developing organisms. One must have measurements of individuals so that one can obtain means and variances and compare these for different strains and for crosses between them.

A beginning in this direction is being tried. Since much of the work in population genetics has been done with Drosophila and since these flies are cheap and efficient material for experimental work they are being used in the present study. Drosophila offer a further advantage in having a long period of development extending from the egg through the larval and pupal stages. So far, however, work has been confined to happenings within the egg.

A great deal of technical exploration is necessary because the procedures of neither population nor developmental genetics, as commonly used, are adequate. The usual embryological procedures—fixation, embedding, sectioning and staining—are elaborate and time-consuming and make observations on hundreds of individual embryos difficult and expensive. We have found it possible to make worth-while observations on large numbers of embryos by killing and fixing them with a few drops of boiling water, removing the shells with dilute Clorox and examining them within twenty-four hours by transmitted light under the microscope at relatively low magnification.

About two hours after D. melanogaster eggs are laid they reach what is known as the blastular stage. At the surface of the egg just inside the vitelline membrane there is an envelope of cells of uniform size and thickness completely enclosing the yolk. This envelope appears as a clear band or ring around the outer edge of the egg because the cytoplasm of the cells is transparent and the yolk is opaque. On looking at embryos in this stage, it was noticed that those of one inbred line had cytoplasmic bands noticeably wider than those in the embryos of another larger, randomly-breeding population. By making camera lucida drawings of embryos from the two populations, it was possible to make measurements and compute the ratio of yolk volume to total volume for each egg. Means and variances for these ratios could then be calculated. With samples of twenty eggs it was possible to show that the two populations could be separated on the basis of this character even though the ratios for individual embryos in some cases overlapped. A cross between the two strains gave $F_1$ embryos with a mean ratio intermediate between those of the two populations but closer to that of the large population. When the hybrids were inbred to produce an $F_2$ and subsequent generations, the mean ratio and its variance remained substantially like those of the $F_1$.

These data suggest that the character of small yolk volume in the inbred line must result from the interaction of a large number of genetic factors. Further experiments will be necessary to obtain a clear understanding of the situation. But we have here a genetically controlled character which expresses itself very early in development. Without doubt other such characters exist. Whether and how embryonic characters may be correlated with adult characters can be revealed only by careful investigation. It is worth pointing out that small yolk volume is not characteristic of all inbred lines. Blastulae from two other such lines were measured and found to have a significantly higher percentage of yolk volume than that of the large, randomly breeding population.

Another approach to the analysis of developmental differences between populations is the investigation of comparative developmental rates. At a given temperature Drosophila of a given species develop at a fairly uniform rate. Ordinarily eggs hatch about twenty-four hours after they are laid; larvae pupate ninety-six hours after hatching, and flies emerge from the pupae about ninety-six hours later. The various embryonic stages may be similarly scheduled. However, if we take a sample of eggs, we find that the developmental race never results in a dead heat. If we take up a position anywhere on the course and clock the individuals as they pass it, one is always ahead, one is at the rear, and the others fall in between. If we record for each individual within a sample the time at which he arrives at a given developmental stage, we have a set of measurements which can be manipulated statistically. We can compute a mean time—that is the time at which half the individuals have reached the stage in question—and we can compute a variance which is a measure of the dispersion of the times of all the individuals around the mean.

This type of approach is being used. Of course it is not practical

to sit and clock large numbers of embryos for hours on end. Instead, one starts a series of different races at carefully fixed intervals and then freezes (with boiling water) all the entries at some carefully selected later time. We then have what would correspond to a series of fast photographs of a race taken at different times during its course and we can figure out just when half the runners had passed a given point, and where all the other entries were at that moment.

A number of technical problems arise in such experiments. One of the most troublesome is that of persuading the flies to lay reasonable numbers of eggs within a series of short periods of time. Nor is the statistical analysis of the data as simple as might first appear. It has turned out that the most efficient statistical procedure is that used by toxicologists in studying the effects of poisons. We say that a fertilized embryo reacts to time as an organism reacts to a drug. Give it a certain dose of time and certain effects will be produced. The toxicologist calculates the dose (of poison) which will produce a given effect, for example, death, in half of his treated sample. We figure out the dose (of time) necessary to bring half of a sample of embryos to a given stage of development; for example, formation of the blastula. One departure from the usual toxicological technique should be mentioned. The toxicologist usually measures dose on a logarithmic scale. In developmental studies more meaningful results are obtained if the time scale is simply arithmetic.

To date, most of the time and effort expended on the project have gone into working out feasible technical and statistical methods for dealing with the problem. Preliminary results indicate, however, that different populations do differ in developmental rates and that these differences show up both in mean time for arriving at a given stage, and in the pattern of individual dispersion around the mean. There is a further suggestion that inbred populations where homozygosity is high have a greater variance of developmental time—that is, wider dispersion around the mean —than large, randomly-breeding populations which have a higher degree of heterozygosity. Present results are, however, very fragmentary and much more experimentation will have to be carried out before we will be justified in making generalizations.

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# INHIBITION AS A NATURAL REGULATORY MECHANISM IN BACTERIA

Ellis Englesberg, Laura Ingraham, and Joanna Hadden

All living things expend a great deal of energy in maintaining a relative constancy of internal environment against ever changing external factors. The mechanisms for temperature control, water balance, acid-base balance, and sugar balance in man are good examples of this. Besides these relatively complex control mechanisms involving in most cases several different tissue-organ systems and mediated by complex organic compounds, we know from investigations with bacteria that these unicellular microorganisms have relatively simpler but nevertheless essential and intricate control mechanisms as well. These cells react individually in many ways to their own intermediary metabolites and to simple organic compounds in their external environment, and these reactions result in regulating the various intracellular processes and provide thereby the necessary integration of the multitude of reactions involved in maintaining life. There is little doubt that some such similar intracellular, self-regulating control mechanisms are functioning in all living cells. Since bacteria have no elaborate organ systems, their regulatory mechanisms are naturally simpler, therefore easier to get at, and we are just beginning to unravel and thereby understand how they function.

In last year's report we discussed three such mechanisms: a methionine feedback control mechanism in Pasteurella pestis, a glucose inhibition or control mechanism (glucose diauxie phenomenon) in Salmonella typhimuriam, and a rhamnose inhibition or control mechanism in Salmonella typhi. This past year we have devoted our efforts to further elucidation of the two latter phenomena.

Bacteria in general have the potentiality to produce many enzymes involved in the breakdown of external carbon and energy sources and in the synthesis of essential components of the cell. However, we know that some of these potentialities are never fully expressd. The specific substrates or closely related compounds required to induce the synthesis of most, or all, enzymes are not always present. The cell thus possesses a mechanism for reacting with its environment, internal and external, and producing enzymes as required for the utilization of various substrate stimuli. This interaction can be complicated by the presence in the environment of several different substrates, all of which may play the same role in the cell metabolism, for instance, provide carbon and energy for growth. Bacteria placed in such an environment usually are not induced to produce enzymes involved in the utilization of all these compounds at once. It is in general found, for instance, that if glucose is present as one of the utilizable carbon sources, the cells are not induced by the other substrates present, and as a result of this, growth proceeds with glucose as sole carbon and energy source. After the complete utilization of glucose, the cells are then able to be induced to produce the factors

required for the utilization of other compounds, and in many cases we find a definite order of induction of enzymes and sometimes simultaneous induction, depending on the compounds present and their relative amounts. In the presence of glucose and sodium citrate, we have found that Salmonella typhimurium will use glucose first for growth, and subsequent to the complete utilization of glucose will go through a growth lag, during which time a factor is synthesized which is required for growth with citrate as sole carbon and energy source. This two-step growth curve is characteristic of the diauxie phenomenon (Monod). This type of control mechanism prevents the cell from running off in all directions at the same time in enzyme synthesis, and thus prevents the wasteful synthesis of enzymes which may not be required. How does this control mechanism function?

The Glucose Inhibition (Diauxie Inhibition) or Control mechanism in Salmonella Typhimuriam. We had the opportunity of studying this phenomenon in detail with the isolation of three mutants of Salmonella typhimurium which are unable to utilize glucose (or other carbohydrates), glycerol, or pyruvate as sole sources of carbon and energy for growth ($C^-$), although they still behave like the parent strain in being able to use various Krebs-cycle compounds for this purpose. Although excellent growth of these mutants occurs in a mineral citrate medium, the addition of glucose severely inhibits growth ($dg^s$) and glucose resistant ($dg^r$) mutants are isolated, which are still unable to use glucose, glycerol, or pyruvate as sole carbon and energy sources for growth ($C^-$ $dg^{r-1}$). By transduction with phage PLT 22, previously grown on the wild type ($C^+$ $dg^s$) it was possible to convert $C^-$ $dg^{r-1}$ to $C^+$ $dg^{r-1}$. The glucose sensitivity or inhibitory effect was found to be a ramification of the glucose diauxie response, and glucose resistance resulted in immunity to the glucose diauxie. We hoped that by a comparative study of glucose sensitive (diauxie sensitive) and resistant (diauxie resistant or immune) strains, a beginning could be made in the elucidation of the nature of this glucose "preference." Glucose sensitivity was found to be very specific. Although other compounds will force a diauxie-type of growth on Salmonella typhimurium, none are as potent in this ability as glucose. $C^+$ $dg^{r-1}$ was found to be resistant to glucose + citrate diauxie and to diauxie with other pairs of compounds containing glucose to which the wild-type exhibited diauxie (glucose + serine, glucose + rhamnose, glucose + maltose, etc.) but not resistant to pairs of compounds lacking glucose to which the wild-type exhibits diauxie (fructose + citrate, fructose + maltose, galactose + citrate, galactose + maltose, gluconate + citrate, glucuronate + citrate, and glucose-6-phosphate + citrate). Furthermore the $dg^{r-1}$ marker was found to lower the growth rate of the otherwise prototrophic strain, specifically with glucose as substrate, and cause a 2-3 fold increase in activity of an acid phosphatase. (We are indebted to Dr. N. Entner who worked in our laboratory during the summer of 1957 and performed the initial experiment with regard to the phosphatase.) With glucose-6-

phosphate as substrate, the phosphatase has an optimum pH of 5.2. It is active against a large variety of phosphomonoesters, such as glucose-6-phosphate, fructose-6-phosphate, mannose-6-phosphate, fructose-1-6-diphosphate, glucose-1-phosphate, ribose-5-phosphate, and phenyldisodium phosphate, but has little if any activity toward adenosine triphosphate or sodium pyrophosphate. The relationship between the increased phosphatase activity and the glucose diauxie phenomenon has yet to be determined.

Besides the potential significance of these findings, the interaction of the $dg^{r-1}$ marker on the prototrophic phenotype presents an ideal model on an enzyme level as to the functioning of modifier genes. The increased phosphatase activity caused by the $dg^{r-1}$ marker can be considered to modify the functioning of the genes involved in glucose metabolism by partly short-circuiting glucose utilization at the glucose-6-phosphate stage, resulting in the lowering of the growth rate with glucose. In this respect, $dg^{r-1}$ is similar to the so-called dilution genes, whose presence results, for instance, in the reduced amount of pigment present in mice and in guinea pigs quantitatively, without any other visible phenotypic effect.

We noted in last year's report that with the wild-type ($C^+$ $dg^s$), unadapted to use citrate as the sole carbon and energy source, citrate still stimulated growth in the presence of "inhibitory" amounts of glucose. We have recently found that all amino acids tested, both the d and l forms, and versene (tetra sodium salt of ethylene diamine tetra acetic acid) produced a similar effect. Since amino acids, citrate, and versene have in common the ability to bind metal ions, the stimulatory effect of these compounds is probably due to their ability to remove some toxic ion from the medium. Employing a versene-balanced mineral medium containing about 15 gamma of versene per ml, the growth rate of typhimurium with glucose as sole carbon and energy source was equal to that achieved with the addition of 100 gamma/ml of sodium citrate. Aside from this effect, the other characteristics of the variants as previously described were unchanged.

Employing the versene mineral medium, some very interesting results were obtained with glucose-6-phosphate, gluconate, and glucuronate in growth experiments. All three compounds yield a diauxie-type of growth with the wild-type as previously mentioned, are not used as sole carbon and energy sources by $C^-$ $dg^s$, but exhibit a diauxie type of growth with this mutant, with $C^-$ $dg^{r-1}$, and $C^+$ $dg^{r-1}$. All three compounds, therefore, effect a completely different response than glucose. Apparently citrate acts as a source of aspartate and glutamate for growth with gluconate, glucuronate or glucose-6-phosphate as major carbon and energy sources and with the utilization of the latter the cells go through a lag during which time they are induced to use citrate as the sole carbon and energy source. Thus glucose prevents (1) the utilization of citrate at the relatively low rates characteristic for synthesis of aspartate and glutamate required for growth of $C^-$ strains, and (2) the rapid utilization of citrate required when this compound is used as sole carbon and energy source; compounds such as gluconate, glucuronate, and glucose-6-phos-

phate, when accompanied by citrate, affect a diauxie type of growth with $C^-$ variants, apparently allowing reaction 1 to proceed while they inhibit reaction 2. These results indicate that the act of penetration of a substrate and subsequent concentration required for enzymatic induction (Rickenberg, et al.) may be two separate phenomena (see further discussion of this below).

It has been previously shown by Barrett, Larson and Kallio with Pseudomonas fluorescens that cell extracts prepared from citrate-induced and non-induced cells oxidize citrate at the same rate, and on the basis of this and other evidence these workers concluded that the induction of citrate utilization involves in this organism the synthesis of some carrier involved in the transport of citrate to the site of enzyme activity. Similar conclusions were reached by Green and Davis with Aerobacter aerogenes. On the basis of growth and manometric experiments as reported last year, a similar situation appeared to exist in Salmonella typhimurium. This problem was investigated on an enzymatic level during this past summer in collaboration with Dr. N. Entner, assisted by Joan Kent. It was found that aconitase, isocitric dehydrogenase activity, as well as the overall rate of citrate oxidation of extracts prepared from citrate and fumarate-grown cells, were the same. Furthermore, no significant citratase activity was found in either extract. Evidence, therefore, is in agreement with the interpretations of the above investigators.

The Rhamnose Inhibition or Control Mechanism in Salmonella typhi. As we have previously reported, Salmonella typhi 0901$S^r$ is unable to utilize rhamnose as a carbon or energy source for growth ($R^-$). Growth of this organism on a rhamnose nutrient agar medium is inhibited by rhamnose ($R^s$), and rhamnose resistant papillae arise ($R^r$). These rhamnose-resistant mutants are similar to the wild-type in still not being able to utilize rhamnose as carbon and energy source. This complex of rhamnose "non-utilization", inhibition, and mutation to resistance has been reported earlier by other workers and apparently represents a general characteristic of all typhi strains. In order to understand the nature of rhamnose inhibition and its relationship, if any, to rhamnose utilization, we undertook last year a comparative study of $R^-R^s$ (the wild-type, unable to use rhamnose as carbon source and inhibited by rhamnose), $R^-R^r$ (a rhamnose resistant mutant isolated from $R^-R^s$, unable to use rhamnose as a carbon source but resistant to rhamnose inhibition and no longer giving rise to papillae on rhamnose) and $R^+R^s$ (a rhamnose utilizing transductant). The latter was produced by transducing rhamnose utilization from Salmonella typhimurium ($R^+$) to Salmonella typhi, employing phage PLT 22 previously grown on typhimurium. Under optimum conditions of multiplicity of infection, employing casein hydrolysate mineral glucose grown cells and Endo rhamnose agar medium, frequencies of transduction of $R^-R^s$ to $R^+R^s$ of $1.3 \times 10^{-7}$ were achieved. Similarly, it was possible to transduce rhamnose resistant mutants to rhamnose utilization at about $\frac{1}{3}$ that frequency. These relatively low frequencies of transduction are

not due to the ineptness of the phage, since it was shown that in similar experiments the same phage transduces tryptophane independence in this same strain with a frequency of $1 \times 10^{-6}$.

Enzymatic analysis of these three strains, $R^-R^s$, $R^-R^r$, $R^+R^s$, as previously reported, revealed that the wild-type ($R^-R^s$), when grown in a caseine hydrolysate mineral rhamnose medium, contained the first two enzymes involved in rhamnose utilization as previously described in P. pestis, a rhamnose isomerase and a kinase which probably phosphorylates rhamnulose. Further, analysis of the supernatant subsequent to the growth of various strains of typhi, demonstrated that all strains of typhi tested produced rhamnulose, as shown by the Dische carbazole test and paper chromatographic analysis. So it would appear that $R^-R^s$, unable to use rhamnose as a carbon source, has a deficiency in rhamnose metabolism subsequent to the formation of rhamnulose phosphate. Both the isomerase and kinase are present in the $R^+$ transductant, as would be expected. Thus $R^+$ has gained the ability to convert rhamnulose phosphate into a utilizable intermediate. Both enzymes are inducible, in that they are absent in $R^-R^s$ and $R^+R^s$ when these strains are grown in the absence of rhamnose. Most interesting of all, however, was the finding that neither of these enzymes could be detected in $R^-R^r$. On the basis of the sensitivity of the test, we can conclude that the isomerase activity of $R^-R^r$ (if there is any) is less than $2.9 \times 10^{-4}$ of the activity of $R^-R^s$, and all attempts to force induction were unsuccessful.

In last year's report it was indicated that a casein hydrolysate (CH) rhamnose (R) medium had been employed in a study of the nature of the rhamnose inhibition. This medium has since been improved and now also provides an excellent means for demonstrating the differences between the three variants. All three exhibited exactly the same growth characteristics in CH (without rhamnose). Growth of $R^-R^s$ in CHR follows that of growth in CH alone for about 4 hours and then ceases at about ½ the total growth produced in the absence of rhamnose. The initial growth of $R^+R^s$ is stimulated by rhamnose, goes through a lag at approximately the optical density given by growth on CH alone, and then adapts to further exponential growth (a typical diauxie-growth curve). Growth of $R^-R^r$ is the same whether rhamnose is present or not. This is what one would expect from the plate experiments and enzyme assays. Since $R^-R^s$ can be induced to convert rhamnose into rhamnulose and phosphorylate the latter, one might anticipate that the four-hour lag in inhibition involves time required for induction and for production of an inhibitory intermediate. This, however, is not the case. $R^-R^s$, grown in 0.05% CHR until inhibition sets in, is able to utilize additional CH added at this point to the same extent as the original casein hydrolysate. We postulated in the last report, on the basis of similar experiments, that there must be at least two compounds (A and B) in the CH which can be used by $R^-R^s$ as carbon and energy sources in the absence of rhamnose. In a CHR medium, growth occurs on compound A. After A is completely utilized, $R^-R^s$ adapts to rhamnose conversion, which simultane-

ously results in preventing the utilization of compound B. We have since been able to identify these compounds. It was found that 5 amino acids are present in the CH which typhi is able to use as carbon sources: serine, threonine, proline, aspartate, and glutamate. Growth experiments in which the CH medium is supplemented with these amino acids, in the absence or presence of rhamnose, revealed that growth with serine is not inhibited by rhamnose, growth with threonine is partially inhibited, while growth with aspartate, glutamate, or proline is completely inhibited by rhamnose. Furthermore, as with CH, the addition of serine to a CHR $R^-R^s$ culture subsequent to the occurrence of inhibition, results in another increment of growth. Thus the delayed inhibitory effect of rhamnose on the growth of $R^-R^s$ in CH can be explained on the basis that growth first proceeds with serine as carbon and energy source, and to a slight extent with threonine (compounds A). During growth on serine (and threonine) $R^-R^s$ is induced to rhamnose conversion. Apparently simultaneously with this induction process, rhamnose, a product of rhamnose metabolism, or the inductive process itself inhibits the ability of the cells from utilizing aspartate, glutamate, and proline (compounds B) as carbon sources. Since rhamnose is only incompletely attacked by $R^-R^s$ if an intermediate is the inhibitory agent, it may be either rhamnulose or rhamnulose phosphate. Although we have found rhamnulose to be non-inhibitory in growth experiments similar to those using rhamnose, this far from eliminates rhamnulose or rhamnulose phosphate as the internal inhibitory agents. Induction to rhamnulose utilization does not occur until all the utilizable carbon sources in the casein hydrolysate are consumed, and rhamnulose does not stimulate the initial growth on CH as does rhamnose, so that external rhamnulose may be totally excluded from the cell until complete utilization of available carbon in the CH occurs.

Rickenberg, et al. have proposed on the basis of their work with the cryptic $\beta$ galactosidase mutants of E. coli that microbial cells possess certain specific factors which are involved in controlling the penetration and concentration of external substrates within the cell. In coli the factor $\beta$ galactoside permease, as it is called, is inducible and is required for induction of the $\beta$ galactosidase. Evidence from experiments which we have performed have led us to propose that the phenomenon of penetration and concentration may be two separate processes. The stimulatory effect of rhamnose on the initial growth of $R^+R^s$ on CHR followed by a lag and finally exponential growth with rhamnose as carbon source, and a similar phenomenon with regard to citrate stimulation with gluconate, glucuronate, or glucose-6-phosphate as substrates (as we have previously mentioned) are difficult to reconcile with a single factor controlling both penetration and concentration. On the basis of the latter hypothesis, the fact that citrate and rhamnose stimulate growth should preclude the need for any further induction, which, however, does occur. As a working model, we feel justified in proposing the following: that besides an element required to concentrate external substrates there is another factor, a

penetration factor, which acts before the permease and controls the actual penetration of these compounds into the cell.

In the case of $R^-R^r$, since it is not possible to detect any isomerase or kinase activity in this mutant grown under conditions in which these enzymes are induced in the $R^+$ strain, and since it is not possible to force this induction in $R^-R^r$, it would appear that $R^-R^r$ is not just permease deficient, since the $\beta$ galactoside permease deficient mutants so far studied always have slight $\beta$ galactosidase activity and induction of both permease and $\beta$ galactosidase can be forced. Mutation to rhamnose resistance, therefore, may involve the loss or inactivation of a penetrating factor or loss of rhamnose isomerase and perhaps the kinase as well, although the latter could not be put to a critical test because of the small quantities of rhamnulose available for study.

In any case, $R^-R^r$ has two deficiencies: (1) the deficiency of the wild-type ($R^-$) and (2) the deficiency brought about by mutation to rhamnose resistance (no isomerase, etc.). Since it has been possible to transduce $R^-R^r$ to rhamnose utilization and therefore to $R^-R^s$, it must be concluded that the $R^r$ and the $R^+$ markers are closely linked.

# GENETICS OF ESCHERICHIA COLI

P. D. Skaar

This report is based upon four months of work supported by a grant from the National Science Foundation. Most of the work consisted in amplifying data concerned with the tryptophan gene cluster in Escherichia coli. Current information is summarized below.

Eleven independent and revertible tryptophan-requiring mutants have been studied. Ten of the mutations occurred within a small segment of the bacterial genome. The one exception is a mutation which blocks some early step in tryptophan synthesis (before shikimic acid). The ten clustered mutations are resolvable into five functional classes. Four of these are comparable to those studied by Demerec in Salmonella. These are tryA, B, C, and D, which block the successive steps in the synthetic sequence: Shikimic acid to anthranilic acid to indole-glycerol-phosphate to indole to tryptophan. Mutations of the fifth class (here called tryCD) appear to block the last two steps. Mutations within each functional class are distinguishable from one another by the degree of feeding of earlier blocks, response to feeding, and reversion frequency. They are also separable by recombination. The locations of mutations within the cluster are non-random. Two-point tests give data consistent with the following order: (A,A)-(B)-C,C,C,CD,CD,CD,D, where order within parentheses is indeterminate. Abortive transduction appears to occur in E. coli as in Salmonella, and hence allows for testing of the cistrans position effect. Reciprocal transductions of mutants belonging to diverse functional classes have been associated with abortive transductions with one exception. They have not been observed where temperate phage grown on tryCD mutants are plated on tryD mutants, nor vice versa.

A locus concerned with sensitivity to phage T1 is situated to the right of the (C,CD,D) region. The independent mutation of this gene is much less frequent than what appears to be a deletion encompassing not only the sensitivity locus, but also the entire adjoining tryptophan cluster.

# GENETIC STUDIES WITH MAMMALIAN CELLS GROWN IN TISSUE CULTURES

Hermann Moser and Keiko Tomizawa

In recent years somatic cell variation has become an attractive and important field of research because of the development of new, powerful methods of mammalian tissue culture and of cytological analysis of mammalian somatic cells. In combination with cytogenetic, biochemical, biophysical or immunological methods, modern tissue-culture techniques are now widely used to study the origin and dynamics of somatic cell variation in mammalian development, that is, to elucidate the genetic and physiological factors and mechanisms which control differentiation and fate of somatic cells and tissues in man and other mammals.

This study made desirable the availability of a haploid or diploid somatic cell line which has the capacity for fast and unlimited growth in vitro and is genetically stable. Unfortunately, however, we have not yet been able to obtain a cell strain which satisfies these criteria.

Because of their genetic instability in vitro, diploid cell strains derived from primary explants grow slowly and have a limited life span. When grown under ordinary conditions of tissue culture in flasks or tubes, diploid cell lines, after several passages (culture transplants) undergo a process known as "heteroploid cell transformation." During this process alterations of the chromosomes both in number and morphology occur spontaneously, concurrent with a rapid decrease in the growth rate of the cell population and with an increase in cell mortality. In general, after several months of growth and maintenance of a primary culture, a new cell strain with an unlimited capacity to grow in vitro develops from some of the descendants of the diploid parent strain (established cell strain). Cytological examination of a large number of established cell strains derived from human somatic tissues reveals that they are all polyploids with large aneuploid variations. Furthermore, clonal derivatives of the established cell lines do not necessarily yield material of greater genetic uniformity as expected in genetically stable systems.

While our efforts are directed toward creating experimental conditions which inhibit heteroploid cell transformation, i.e., permit the indefinite maintenance and growth of diploid somatic cells in vitro, specific research work is carried out in our laboratory with established (aneuploid) cell strains of human origin.

During the past year we have acquired experience in the methods for rapid plating of human somatic cells developed by Puck, and based upon this experience we have devised an analytical technique for the determination of specific survival fractions of single human somatic cells in vitro. We carried out experiments which were designed to detect, among the survivors of large populations of ultraviolet irradiated human cells, induced mutations affecting biochemical cell functions. We also studied the effects of ultraviolet irradiation on the fate of single somatic cells, and determined and analyzed the survival curves of ultraviolet irradiated single cells in a number of different established human cell lines.

## Clonal Growth of Human Somatic Cells in Vitro

During the past year we also gained considerable experience in the techniques developed by Puck for rapid plating of single human cells. This experience now enables us to study quantitatively and on a large scale, the effects of radiations, radiomimetic chemicals, drugs developed for potential use in cancer therapy, and other agents, on the survival of single cells in vitro, and on the production of aberrant cell types; and to study certain aspects of the nutritional requirements of widely separated human somatic cells.

The application of these plating techniques to the study of the behavior of single somatic cells is limited, however, to heteroploid material obtained from established cell strains. Our attempts—in cooperation with Dr. M. Bender of Johns Hopkins University—to grow normal diploid somatic cells, isolated from human kidney cortex, into macroscopic colonies in vitro, have not yet been successful.

Growth Media for Clonal Growth. Eagle and his associates have shown that established somatic cell lines of human origin require 12 amino acids, 1 amide, 8 or 9 vitamins, a carbon source (sugar), 5 inorganic ionic species, and serum protein for sustained growth and multiplication in vitro and in mass culture. On the basis of this information we found it logical to use for clonal growth of established cell lines, Eagle's basal medium (EBM) plus the supplement(s) necessary for sustained multiplication of widely isolated cells on plane glass surfaces. The composition of EBM, as it is used in our laboratory for clonal growth, is given in Table 1.

Table 1.

Composition of Eagle's Basal Medium used in our Laboratory

| | | | | | |
|---|---|---|---|---|---|
| **Essential amino acids** | | | **Essential amides** | | |
| L-arginine | 0.021 | g/L | L-glutamine | 0.300 | g/L |
| L-cystine | 0.012 | | **Essential vitamins** | | |
| L-histidine | 0.008 | | Biotin | 1.000 | mg/L |
| L-isoleucine | 0.026 | | Choline | 1.000 | |
| L-leucine | 0.026 | | Folic acid | 1.000 | |
| L-lysine | 0.026 | | Nicotinamide | 1.000 | |
| L-methionine | 0.008 | | Pantothenic acid | 1.000 | |
| L-phenylalanine | 0.016 | | Pyridoxal | 1.000 | |
| L-threonine | 0.024 | | Thiamin | 1.000 | |
| L-tryptophan | 0.004 | | Riboflavin | 0.100 | |
| L-tyrosine | 0.018 | | Myoinositol | 1.000 | |
| L-valine | 0.024 | | **Accessory (not growth promoting) factors** | | |
| **Carbon source** | | | $NaHCO_3$ | 1.68 | g/L |
| Glucose | 1. | g/L | Penicillin G | 63. | mg/L |
| **Salts** | | | Streptomycin sulfate | 100. | mg/L |
| NaCl | 6.8 | g/L | Phenolred | 0.010 | g/L |
| KCl | 0.4 | | | | |
| $NaH_2PO_4.H_2O$ | 0.14 | | | | |
| $CaCl_2.2H_2O$ | 0.265 | | | | |
| $MgCl_2.6H_2O$ | 0.175 | | | | |

Growth medium TCM-1, i.e., $E(D_{24}HoS)_{10\%}$, has the composition of EBM and is supplemented with 10% serum protein, that is, 10% dialyzed horse serum, obtained by continuous-flow dialysis of serum against NaCl solution for 24 hours at 4° C. If further supplemented with specific but nonessential growth factors, this medium supports the formation of macroscopic clones by single and widely separated cells in vitro.

Growth medium TCM-2, i.e., $E\ HoS_{10\%}$, is composed of EBM and supplemented with 10% complete horse serum. It is the standard growth medium used in our laboratory for the clonal growth of established cell lines. It contains, in addition to all the essential growth factors and the accessory factors of TCM-1, such nonessential growth factors of the protein-free ultrafiltrate of horse serum as fatty acid derivatives, coenzymes, precursors and derivatives of nucleic acids, conjugated amino acids, and hormones. It is important to note that both TCM-1 and TCM-2 do contain fewer synthetic compounds than the nutrient media employed by Puck and associates.

The Preparation of the Cell Suspensions. Our standard plating technique is similar to that method developed by Puck which does not employ a feeder-layer system. Confluent monolayers of cells grown on the glass surface of culture bottles are detached from the glass and broken up by a static treatment with 0.25% pancreatin solution at 37° C for 5-7 minutes. Subsequently the fluid with the tissue material is agitated for 5 minutes in a Dubnoff metabolic shaking incubator at 37° C and at a frequency of 40 RPM, to break up the cell clumps. Immediately after agitation an equal amount of TCM-2 with a lowered concentration of sodium bicarbonate (0.35 g/L $NaHCO_3$, pH 7.2) is added to the cell suspension to inhibit further action of the proteolytic enzymes of the pancreatin. The cell suspension is then freed of the remaining large cell clumps by filtration through sterile cheesecloth into small prescription bottles. This cell suspension, $S(10^0)$, is then ready for the assay, for the preparation of suitable dilutions $S(10^{-1})$, $S(10^{-2})$, or for direct plating of aliquots.

The Assay of the Cell Suspension. The assay is done rapidly and immediately after the preparation of the suspension $S(10^0)$. It comprises (1) the determination of the mean cell density of the suspension $S(10^0)$; (2) the determination of the mean density of aggregation units (units composed of single cells or clumps) in the suspension $S(10^0)$. In special cases the assay also involves the determination of the frequency distribution of cell aggregation in the undiluted cell suspension, i.e., the estimate of the frequencies of single cells, and aggregates composed of two or more cells in $S(10^0)$.

The Preparation of a Dilution Series, $S(10^{-1})$, $S(10^{-2})$, $S(10^{-3})$. According to the design of the experiment the cell suspension $S(10^0)$ is diluted at room temperature with TCM-2 having a low concentration of $NaHCO_3$. In order to minimize cell clumping or alkalinization of the liquids, the dilutions must be prepared concurrently with the assay of the

undiluted suspension and in rapid succession. Hence a minimum of two persons must be engaged in this part of the plating procedures.

The Inoculation of the Plates. The dishes used in our laboratory for the plating of single human somatic cells are either Petri dishes or a special tissue-culture dish. Several hours before the actual platings, growth medium (10 ml for Petri dishes with 90 mm bottom diameter) is introduced into the culture dishes and the pH adjusted or maintained at 7.4-7.8 by placing the dishes into the $CO_2$/air cabinet. The actual platings of the cell suspension samples are performed following, without delay, the preparation of the dilutions at room temperature, and in ordinary atmosphere. The inoculated plates are gently agitated before being placed on a strictly horizontal surface in the $CO_2$/air cabinet to secure a random distribution of the dispersed cells in the liquid.

The Incubation of the Plates. The plates are incubated at 38° C in a humidified atmosphere containing 10% $CO_2$ in air for a period of between 12 and 16 days. Every third day of the incubation period the nutrient is withdrawn from the culture dish and an equal amount of fresh nutrient is introduced. Periodic media changes were found to improve the formation of macroscopic clones, and to reduce the rate of detachment of single cells from aging colonies. Detached cells may reattach to unoccupied areas or to sister clones. Thus, media changes tend to reduce the chance of production of secondary colonies on the plates and the chance of genetic contamination of primary clones.

The Inspection of the Plates. For inspection and analysis, the nutrient fluid is drained from the culture dishes, the plates are washed with phosphate buffered saline (PBS) twice, and the colonies on the dish bottom fixed with Bouin, stained with Haematoxylin, and dried at room temperature. For finer analysis of clone formation, a new method for preservation of the fixed and stained plates is being developed in our laboratory. The inspection of the stained plates involves colony counts or cell counts. Cell counts are performed with a Bausch and Lomb Tri-Simplex Micro-Projector.

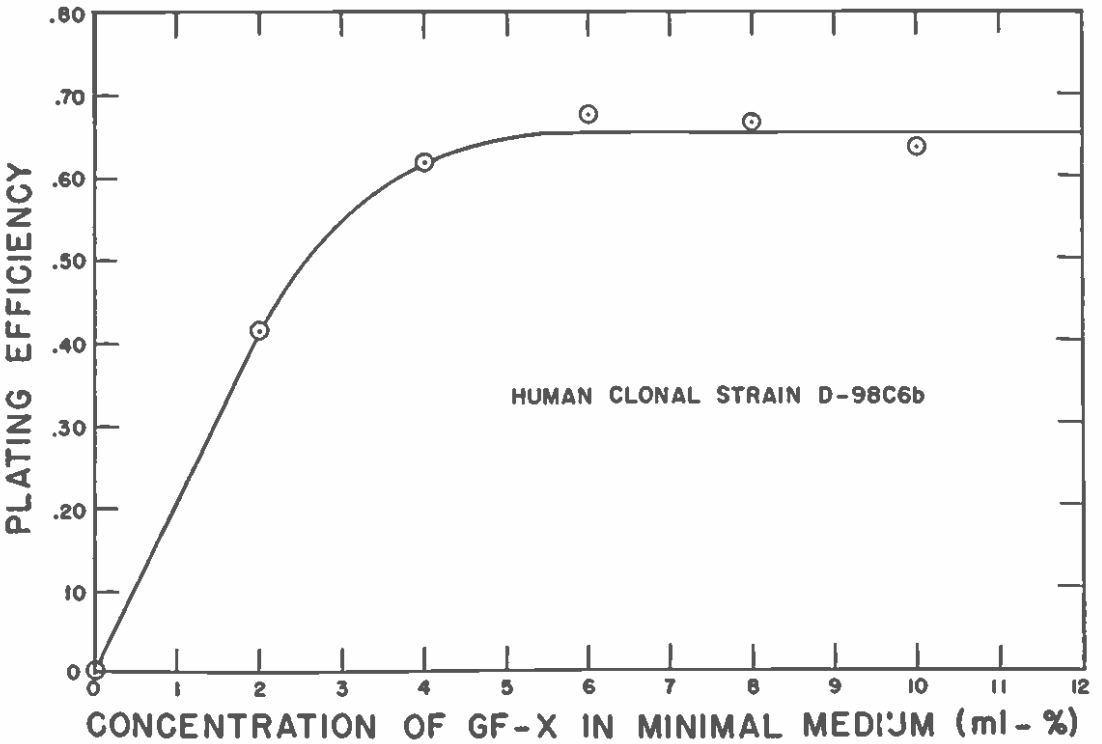
The Determination of the Plating Efficiency. The plating efficiency, E, is defined by the equation $E = C/N$, in which C stands for the mean colony count per plate and N for the mean number of cells plated. Maximum plating efficiency, $E = 1$, is possible only if (a) every trypsinized cell plated is viable and capable of forming a macroscopic clone, (b) if every plated cell does attach to the glass surface of the culture dish, and (c) if all glass-attached cells are single, separated units at the time of onset of cell multiplication. Puck et al. on several occasions reported the observation of plating efficiences of 100% in human somatic cell line HeLa. In contrast to this we were never able to obtain maximum plating efficiency in our laboratory although we used mild trypsinization procedures for the dispersion of cells, and tested a number of different established cell lines. The plating efficiencies of trypsinized but otherwise untreated somatic cells (controls) observed in our laboratory vary from 37% (Human Amnion strain, Hayflick-Fernandez) to 61% (clonal derivative, C6b, of Bone Marrow strain D-98). (Table 2.)

Table 2.

Plating Efficiencies of Established Cell Strains
(growth medium, TCM-2)

| Human strain | Experiment number | Incubation time, days | N | C* | C | E (%) |
|---|---|---|---|---|---|---|
| D-98 | 1 | 14 | 219 | 106<br>119 | 113 | 51.6 |
| D-98C6b | 1 | 15 | 209 | 100<br>135 | 118 | 56.6 |
| " | 2 | 16 | 369 | 277<br>225<br>193<br>185<br>248 | 226 | 61.3 |
| " | 3 | 15 | 242 | 125<br>152 | 139 | 57.5 |
| D-189 | 1 | 16 | 142 | 122<br>87<br>82 | 97 | 68.3 |
| Human Amnion (Hayflick-Fernandez) | 1 | 16 | 265 | 120<br>90<br>80 | 97 | 36.6 |

Figure 2.




Plating efficiency as a function of concentration of unidentified growth factor of horse serum, GF-X (Strain D-98C6b; growth medium, TCM-1)

Cell Aggregation During the Plating of Somatic Cells. During the past year we have consistently observed the occurrence of cell clumping during the platings of somatic human cells. First, complete cell separation by trypsin-dispersion of monolayer cultures is rarely obtained; second, active cell aggregation (agglutination) takes place during the period between plate inoculation and the onset of cell divisions of the glass-attached cells (24-hour period following plate inoculation); third, the extent of cell clumping is a function of the cell density on the plates.

Tables 3 and 4 represent the results of an analysis of cell clumping on the plates in a dilution series. It can be seen that cell aggregation during the plating procedures is increasing as a function of cell density. At low densities (70 to 700 cells plated per ml liquid medium and 64 $cm^2$ plating area) approximately 75% of the cells appear as singles, the remaining 25% as pairs of (two) cells after attachment to the glass surface. At the higher cell densities only about 50% of the cells appear as singles, the other 50% being clumps of two or more cells. This dependence of the frequency distribution of cell aggregation on the cell density has to be taken into consideration when the design of the experiment demands the preparation and plating of a dilution series.

Table 3.

Frequency distribution of cell aggregation on glass surface, 24 hours after platings. (Strain D-98C6b; media, TCM-2; x, number of cells per aggregation unit; N, number of cells plated, per 10 ml growth medium.)

| | Frequency, F(x) | | | | |
|---|---|---|---|---|---|
| x | $N=7.38\cdot10^5$ | $N=2.46\cdot10^5$ | $N=7.38\cdot10^4$ | $N=7.38\cdot10^3$ | $N=7.38\cdot10^2$ |
| 1 | 0.50000 | 0.4730 | 0.4670 | 0.7430 | 0.750 |
| 2 | 0.29800 | 0.3732 | 0.3730 | 0.2570 | 0.250 |
| 3 | 0.08630 | 0.1053 | 0.0933 | 0 | 0 |
| 4 | 0.04970 | 0.0467 | 0.0533 | 0 | 0 |
| 5 | 0.04310 | 0 | 0 | 0 | 0 |
| 6 | 0.00995 | 0 | 0.0133 | 0 | 0 |
| 7 | 0.00662 | 0 | 0 | 0 | 0 |
| 8 | 0 | 0 | 0 | 0 | 0 |
| 9 | 0.00331 | 0 | 0 | 0 | 0 |
| 10 | 0 | 0 | 0 | 0 | 0 |
| 11 | 0 | 0 | 0 | 0 | 0 |
| 12 | 0.00331 | 0 | 0 | 0 | 0 |
| 13 | 0 | 0 | 0 | 0 | 0 |

Table 4.

Reaggregation of trypsin-dispersed cells curing the platings. (Strain, D-98C6b; media, TCM-2; $x'$ = mean number of cells per aggregation unit in suspension sample plated; $x''$ = mean number of cells per aggregation unit after attachment of the cells to the glass surface of the plates; N = number of cells plated.)

| N | $x'$ | $x''$ |
|---|---|---|
| $7.38\times10^2$ | 1.145 | 1.250 |
| $7.38\times10^3$ | 1.145 | 1.255 |
| $7.38\times10^4$ | 1.145 | 1.788 |
| $2.46\times10^5$ | 1.145 | 1.727 |
| $7.38\times10^5$ | 1.145 | 1.997 |

The Determination of the Survival Fraction of Somatic Cells. One of the most important applications of the plating techniques developed for mammalian cells is the determination of the survival fraction (survival probability) of single cells, i.e., the frequency of cells in a very large population of trypsin-dispersed cells which are able to form macroscopic clones in vitro.

The estimation of the survival fraction of mammalian somatic cells directly from the value of the plating efficiency, that is, according to S = E = C/N, is strictly correct only if all the cells plated attach to the glass surface of the plate and if all the glass-attached cells exist as single separated units at the time cell multiplication begins. If, however, a fraction of the cells exist in the form of cell aggregates, as is observed in most cases, the survival fraction or probability has to be calculated.

During the past year we derived an equation expressing the plating efficiency, E, as a function of (a) the survival fraction or probability of trypsin-dispersed cells, S, (b) the frequency distribution of cell aggregation of glass-attached cells prior to the onset of clone formation, F(x), and (d) the probability of trypinized cells attaching to the glass surface of the plates, p. The survival fraction can be calculated by solving the equation, E = f[F(x), p, S], for S.

In order to calculate the survival fraction or probability from this equation, the experimental values of E, p, and F(x) have to be determined. The value of the parameter E is obtained in the usual way from the mean colony count per plate, C, and the mean number of cells plated, N.

The empirical values of p and F(x) on the other hand are determined by microscopic inspection of plates which are fixed and stained 24 hours after the platings (at a time when all the viable cells have attached to the glass surface and at which time these cells have not undergone any divisions).

The value of p can be determined from the mean number of cells plated and the mean number of cells counted on the total glass-surface of the dish bottom 24 hours after the platings. If the estimated value of p happens to exceed unity we have either an indication of an error in our plating procedures, or an indication that some cells have undergone cell division during the 24-hour period following the platings.

By application of the methods described above it is possible to determine the specific survival fraction for a given Dose, D, of radiation or of a chemical with lethal actions, S(D). If S(O) stands for the survival fraction at Dose D = O (survival fraction of controls), S(D) for the survival fraction at Dose D, the specific survival fraction for D is defined as $S_D = S(D)/S(O)$. Then for the same Dose the specific kill is obviously given by the equation $K_D = 1—[S(D)/S(O)]$.

## The Lethal and Semilethal Effects of Ultraviolet Irradiation on Heteroploid Human Somatic Cells

As part of our genetics program we engaged during the past year in a study of the effects of ultraviolet irradiation on human somatic cells. So far these studies were conducted only with established cell strains derived from normal somatic tissues.

Preparations for the UV Treatment. Exactly 24 hours prior to the irradiations, cell suspension samples are plated on Corning No. 3162 Petri dishes with 10 ml TCM-2 culture medium. The culture dish used for UV experiments has a flat-surface bottom and a rimless side which does not cast a shadow on the dish bottom. During the 24-hour period between plating and irradiation the trypsinized cells attach to the glass but do not undergo cell division. One hour before the actual irradiation procedures the UV lamp is set in operation. Immediately before the radiation treatment of the cells the plates are drained with a special pumping device under aseptic conditions and the cells washed once with 10 ml phosphate buffered saline (PBS). For the actual irradiation with UV the cells are covered with a PBS layer of 1.94 mm (corresponding to 10 ml PBS per plate). The UV irradiation is performed in the dark, in a cabinet with a shutter, and the dish covers removed. The radiation source is a 15-Watt General Electric Germicidal Lamp operating at a target-object distance of 21″. The UV irradiation hits the glass-attached cells directly through the PBS layer on the horizontal plane of the culture dish bottom. The incident radiation intensity obtained with this system was kindly calibrated by Dr. Jun-ichi Tomizawa of the Department of Genetics of the Carnegie Institution of Washington, using phage T2 suspended in PBS as the test material. The intensity at 21″ target-object distance was found to be close to 18 ergs $sec^{-1}$ $mm^{-2}$. The irradiations are performed at 37° C. Immedi-

ately after the UV treatment the plates are drained and incubated in the dark with 10 ml TCM-2.

Incubation of UV Plates and Control Plates. The treated and untreated plates (controls) are incubated at 38° C in the dark and $CO_2$/air cabinet for exactly 16 days. During the incubation period the media are renewed every third day.

The Fate of UV-Treated Human Somatic Cells. The fate of single heteroploid cells of human origin which have been irradiated with UV is similar to the fate of cells treated with ionizing radiation, described by Puck and Marcus. A treated cell may form a macroscopic colony consisting of several hundred or several thousand cells. It may form an abortive clone, that is, it may undergo a limited number of cell divisions to form either a self-sustaining microcolony of rarely more than 50 cells or to form a microcolony which disintegrates upon prolonged incubation. Those cells which fail to divide at all may either grow into a single giant cell or simply disintegrate without leaving a trace on the plate. Experiments have been designed to determine the quantitative proportions of the various clonal types produced by UV as a function of UV-dose and of corresponding X-ray dose.

UV Survival Curves. During the past year we determined the survival curves of UV treated single cells of the following established cell strains: Human Bone Marrow, D-98; D-98C6b (clonal derivative of D-98); Human Foreskin, D-189; Human Amnion (Hayflick-Fernandez). Figure 1 represents curves of strains D-98 and D-98C6b in which the specific survival fraction, $S_D$, is ploted on a logarithmic scale against the incident dose of ultraviolet irradiation, measured in units of mW-sec $cm^{-2}$ (1 mW-sec $cm^{-2}$ = 100 ergs $mm^{-2}$).

When plotted in this fashion, the UV survival curves of single heteroploid somatic cells exhibit two distinct sections, a log-linear portion with an initial shoulder, and a non-log-linear section manifesting itself at high UV doses. The existence of an initial shoulder in all the UV survival curves indicates a multiple-hit-killing mechanism of UV irradiation in heteroploid human somatic cells. The first portion of the survival curves fits the equation $S_D = 1 - [1 - \exp(-kD)]^n$ where D is the UV dose, k a constant measuring the radiation sensitivity, and n the hit number. The hit number is determined by extrapolation of the straight line of the log-linear portion of the curve to its intercept on the $S_D$ axis, $S_0^* = n$. The value of k is determined from the slope of the log-linear portion of the curve. The constant k is related to the inactivation dose, $D_0$, i.e., the dose which reduces, in the long-linear portion of the curve, the survival fraction to $e^{-1}$ (by 67%).

Our analysis of the UV survival curves yields hit numbers varying from 1.5 (Human Amnion) to 8.8 (D-98) and inactivation doses, $D_0$, varying from 78 (D-98) to 107 (Human Amnion) ergs $mm^{-2}$ (Table 5).

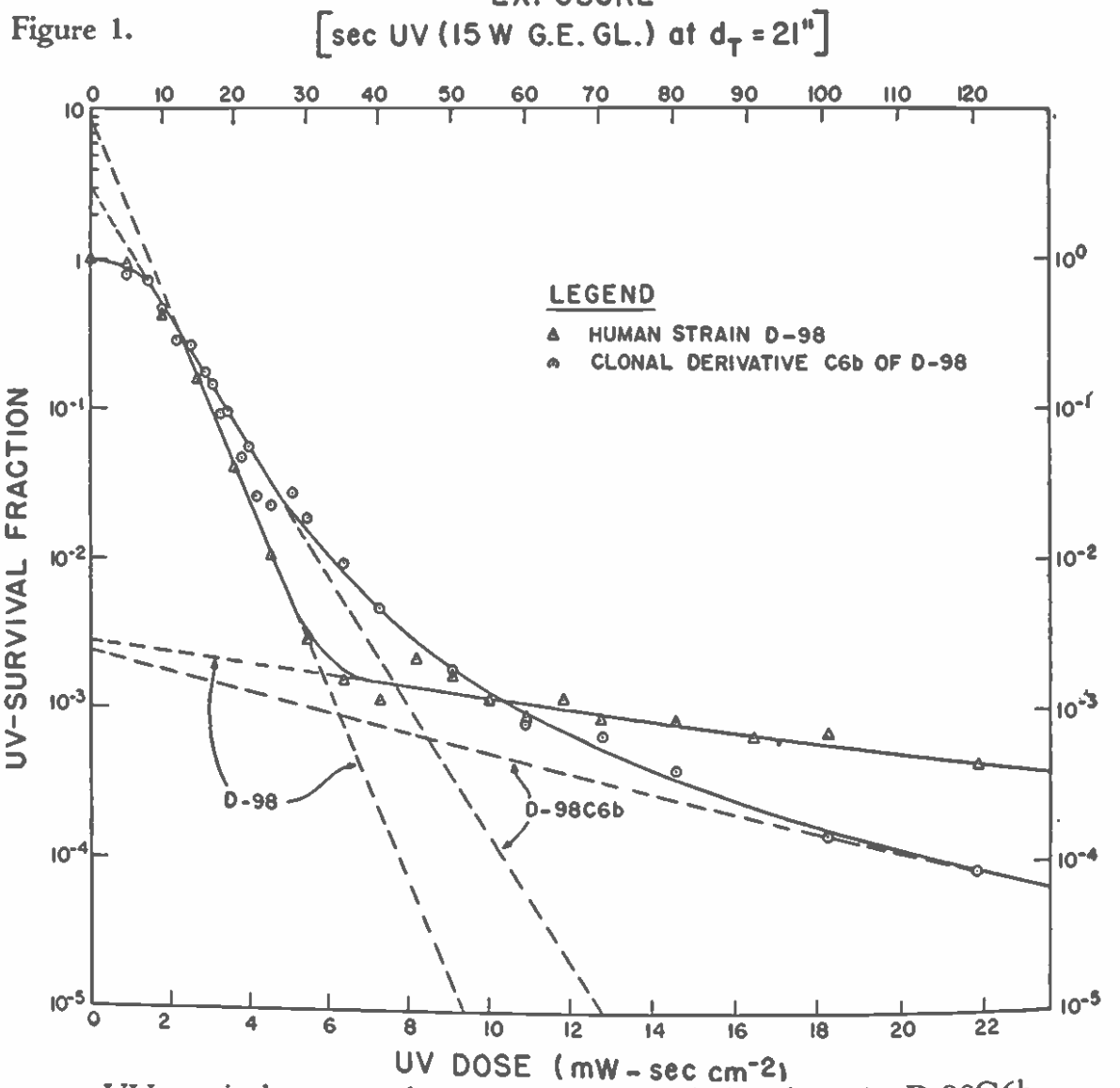
Table 5.
Parameters of UV Survival Curves

| Strain | n | $D_o$ (mW-sec cm$^{-2}$) | $D_x$ (mW-sec cm$^{-2}$) | $S_x$ |
|---|---|---|---|---|
| D-189 | 2.5 | 0.775 | 5.94 | $1.0 \times 10^{-3}$ |
| Human Amnion | 1.5 | 1.070 | 4.95 | $1.4 \times 10^{-2}$ |
| D-98 | 8.5 | 0.684 | 5.40 | $3.0 \times 10^{-3}$ |
| D-98C6b (clonal strain) | 3.0 | 1.000 | 4.95 | $2.0 \times 10^{-2}$ |

At a critical UV dose, $D_x$, which corresponds to a specific survival fraction, $S_x$, the radiation sensitivity begins to change as a function of UV dose. The dose at which radiation resistance "develops" in UV-irradiated heteroploid somatic cells depends on the cell strain tested, and varies in our case from 500 to 600 ergs mm$^{-2}$.

Although the survival curves as such lend themselves only to a limited understanding of the UV-killing mechanism, the strain-specificity of their parameter values indicates the existence of differences in the genetic apparatus of the cells belonging to different heterploid strains. Cytological observations support this conclusion.

Figure 1.




UV-survival curves of strain D-98 and of clonal strain D-98C6b

## Attempts to Induce and Select Nutritional Mutants in Large Populations of Human Somatic Cells

The 1957-58 research program's most ambitious part consisted in the development of a rapid method for the selection of somatic cell mutants which have the ability to multiply in the absence of an essential amino acid in the nutrient medium (dominant myotrophic cell mutants). Large cell populations of a clonal derivative of bone marrow strain D-98 (strain D-98C6b) were exposed to UV irradiation, and the surviving cells permitted to pass through several cell generations in complete growth medium (TCM-2). Then the descendants of the survivors from radiation treatment were subjected, in large numbers, to a nutrient medium (TCM-1 minus an essential amino acid) which—according to Eagle—would support the growth only of mutant cells which have the ability to synthesize an amino acid which cannot be made by the wild-type cells. On a very large number of plates no mutant colonies developed.

This negative result, however, did not disillusion us of the possibility of solving the problem by a more precautious approach. First we had to ascertain the effectiveness of our selective technique. Our selective technique employs Eagle's basal medium supplemented with dialyzed horse serum (growth medium TCM-1) and made deficient in an essential amino acid. If this nutrient medium is adequate in permitting the growth into macroscopic colonies of a few single mutant cells which are surrounded by a large number of non-dividing or dying wild-type cells, then complete minimal medium (TCM-1) must certainly support colony formation of widely isolated single wild-type cells. In order to test this we plated various dilutions of a cell suspension prepared from strain D-98C6b with standard TCM-1. On the plates (area, 51 $cm^2$) which were seeded with $1.86 \times 10^4$ cells, a large number of clones developed which finally fused, producing a confluent monolayer. On the plates which were seeded with 1860 or less cells only, no visible colonies appeared after an incubation period of 15 days. Microscopic inspection of these plates, however, revealed the formation of microcolonies (abortive clones). Hence, while Eagle's basal medium, supplemented with dialyzed horse serum, supports the massive growth of dense cell populations, this medium does not support the growth of widely separated single cells into macroscopic colonies (dilution effect).

Colony formation of widely isolated cells of D-98 plated with TCM-1 can, however, be affected by the addition to TCM-1 of small amounts of complete horse serum, or of protein-free horse serum ultrafiltrate. Thus, the dialyzate or the ultrafiltrate of horse serum contains a yet unidentified growth factor, GF-X, which is necessary for the production of macroscopic colonies by widely separated single cells of strain D-98. Addition of this growth factor to TCM-1, either permanently at a concentration of 5 ml-% serum equivalent (see fig. 2), or only for the first six days of incubation at a concentration of 10 ml-% serum equivalent, restores the plating efficiency to its normal value of 60-65%.

The "dilution effect" observed when D-98 cells are plated with Eagle's basal medium plus dialyzed horse serum (TCM-1), suggests that the factor GF-X can be synthesized by the D-98 cell, thus is not, in the true sense of the word, an essential growth factor for D-98 cells such as, for example, tryptophan or folic acid; but it must be assumed that this compound is synthesized by the D-98 cell only very slowly, at a rate too slow to overcome the excessive leakage from the trypsin-dispersed cells into a growth medium (TCM-1) deficient in the molecule GF-X, when the population density is low and the cells widely separated from each other. A physico-chemical model of this "dilution effect" has been proposed, and we hope to test its validity by kinetic studies. Dr. W. Earle's early work on "cell conditioning" and Dr. Puck's work on the nutritional requirements of single mammalian cells, support our interpretation of the "dilution effect" in somatic cells. Dr. Puck's results further indicate the existence in strain HeLa of genetic variants (leakage mutants) which are, at low cell densities, leaking specific compounds such as i-inositol (strain HeLa-S1) or cholesterol (strain HeLa-S3).

At present we are trying to identify the unknown growth factor GF-X in the protein-free ultrafiltrate of horse serum. If identified and purified this factor can be incorporated into the minimal medium made deficient in one of the essential amino acids. Such an improved selective medium thus would permit the growth into macroscopic colonies of only rare and widely isolated meiotrophic mutant cells and suppress the growth of the abundant nonmutant cells.

The work reported here was done under contract AT(30-1)-1944, U. S. Atomic Energy Commission.

# INVESTIGATIONS OF FLOUR BEETLES AND THE HORSESHOE CRAB

A. Sokoloff

For some thirty years, two species of flour beetles of the family Tenebrionidae, Tribolium castaneum Herbst and T. confusum Duval, have been used in various problems in the field of population ecology. In recent years, a third species in another genus of the same family, Latheticus oryzae Waterhouse, has been the subject of investigations of a similar nature. The chief advantage in using these beetles lies in the fact that the medium which they inhabit (wheat flour) is a solid medium broken up into very small particles. Hence, all the stages of development of these beetles (eggs, larvae, pupae, and adults), can be separated from the medium by sifting, and counted. After counting, all these stages can be returned to the same medium, since it has undergone no change by the treatment. In this respect, flour beetles are superior to Drosophila. Fruit flies require a highly moist, semi-solid medium on which to feed and lay their eggs. Since larvae burrow into the medium, accurate counts of these early stages of development cannot be made without disturbing the culture medium.

From time to time, mutations have appeared spontaneously in Tribolium and they have been reported by several investigators. In T. confusum a recessive (ebony), and a semidominant mutation (McGill black), have been described. Both of these mutations affect body color. In T. castaneum, three mutants have been reported: "pearl" is a recessive mutation affecting the development of the compound eye: in the normal beetle, all the facets making up the eye appear black; in the mutant, only the facets in the outer margin appear black, and the central facets have a pearly appearance. "Paddle" is a recessive sex-linked mutation which results in a fusion of the terminal segments in the antennae of both sexes, and in the male the number of antennal segments is reduced. A third, unchristened, mutation is the result of a semidominant gene also affecting body color: the homozygous recessive is black, the heterozygote is intermediate (reddish black), and the normal, or homozygous dominant, is dark brown or chestnut.

Two new mutations have appeared in flour beetles: one, in T. castaneum, affects body color. It has been called charcoal to avoid confusion with ebony and McGill black. The other, in Latheticus, is so similar to the pearl-eye mutation reported for T. castaneum, that it has been given the same name. In collaboration with Professor Thomas Park and Dr. Amelia Polnik, the mode of inheritance of these mutations has been investigated. Investigations of such an elementary character seem justified, since little is known about the genetics of Coleoptera, one of the largest orders of insects, and since investigators are becoming aware of the usefulness of flour beetles as a tool of research in problems in the field of population genetics.

The data of breeding experiments in the case of the Tribolium mutant are consistent with the hypothesis that the charcoal body color is the result of the action of a semidominant gene: when the normal (+/+) is mated to charcoal (c/c), the progeny (+/c) are intermediate or "purplish". Mating the heterozygotes (+/c × +/c) yields 1 normal (+/+): 2 intermediate (×/c): 1 chestnut (c/c). If the heterozygotes are mated to the normal (+/c × +/+), equal numbers of normal (+/+) and purplish (+/c) beetles are obtained. If the heterozygotes are mated to the charcoal (+/c × c/c), half the progeny will be intermediate (+/c), and half charcoal (c/c).

In the case of the Latheticus-eye mutant, the data are consistent with the assumption that the pearl-eye is the result of an autosomal recessive gene: mating normal-eyed (+/+) with pearl-eyed (p/p) beetles, the resulting progeny will be heterozygous (+/p) for the character, but all possess fully pigmented eyes. Mating the heterozygotes among themselves (+/p × +/p) will yield a generation consisting of three normal-eyed beetles to every pearl-eyed beetle. If the heterozygote (+/p) is mated to the normal (+/+), the progeny should be normal in eye color. The experimental data are consistent with this expectation. If the heterozygote (+/p) is mated to the pearl (p/p) mutant, half the progeny are expected to be heterozygous for the character (hence normal-eyed), and half pearl-eyed. Again, the findings are consistent with the theoretical values.

Dissection of the compound eye of normal, and pearl-eyed beetles has revealed the nature of pearl mutation both in Tribolium and in Latheticus: underlying the facets which make up the compound eye is a black membrane (retinula) which extends from one end of the eye to the other in the normal beetle, but is pierced by an oval window in the mutant. Since the facets of the compound eye are transparent, the individual facets of the eye are "pigmented" only to the extent of development of the retinula: in the normal eye the retinula is complete and all the facets of the eye appear black. In the mutant, only that part of the retinula under the marginal facets develops, giving them a pigmented appearance. The central facets, lacking a retinular background, appear unpigmented, or pearl.

While investigating the reason for lack of pigmentation of the central facets, it was noted that the aperture in the retinula seemed to vary in size: pearl beetles resulting from the test cross (+/p × p/p) appeared to possess a retinular opening much smaller than the pearl beetles from the mutant stock. The following matings were performed to find out the reason for such a variation:

| | | | | | |
|---|---|---|---|---|---|
| Heterozygous | (+/p) | Male | × | pearl (p/p) | Female |
| Heterozygous | (+/p) | Female | × | pearl (p/p) | Male |
| Pearl | (p/p) | Male | × | pearl (p/p) | Female |
| Pearl | (p/p) | Female | × | pearl (p/p) | Male |

The pearl progeny of these crosses were decapitated, and the right eye removed from the head. The area of the aperture and the area of the retinula were determined, and a ratio of the first area over the second computed for each type of mating. The ratios indicate that the size of the aperture is much larger when the pearl parent is a female than when the pearl parent is a male. This means that an egg laid by a heterozygous female contains in its cytoplasm a greater quantity of a substance that contributes to the formation of the retinula than an egg laid by a pearl female. Such cases of a maternal effect have been demonstrated for many characters in various organisms, but this appears to be the first to be described for flour beetles.

These observations give an insight on the mode of formation of the retinula in flour beetles. Apparently, in the mutant, the amount of substance allocated to the formation of the retinula during the development of the beetle is limited. The retinula begins its development from the outside margin to the center of the eye, and it will continue to form only as long as there is material available for its formation. In pearl beetles whose mothers were heterozygous, there is a greater amount of this substance. Consequently, the aperture of the retinula becomes smaller. Finally, in the normal homo-, or heterozygous beetle, a sufficient amount of substance is provided, and the retinula is able to complete its development.

The work on the eye mutant in Latheticus has led to an investigation of a somewhat different nature, using the horseshoe crab, Xiphosura (Limulus) polyphemus. This animal is quite abundant at Cold Spring Harbor in the spring and summer. Late in the 1956 season, walking along the sandspit, I examined the compound eyes of a mating pair of horseshoe crabs. A difference in the eyes of the two individuals was immediately apparent: the male possessed black eyes, whereas the female's eyes had no pigment whatsoever. During the summer of 1957, a census of the horseshoe crab population in regard to eye color was carried out. In order to have a sizable sample and to prevent repetition, every horseshoe crab whose eye color was recorded was marked with a thumbtack. A total of 652 horseshoe crabs were so marked between May 31 and June 19. The first group (108 individuals) was marked with white plastic coated tacks. The second (249 individuals) with yellow plastic coated tacks, and the third (300 horseshoe crabs) with tacks covered with blue plastic. Each of the tacks had a number. Whenever a "mating" pair was found, the male and the female were given tags with successive numbers, and the event so recorded. The method of tagging seems to be painless to the crabs: females engaged in egg-laying continue to do so as if nothing had happened. Males attached to the females also seem to be unaware of the tagging procedure. They only struggle to retain their hold on the female.

A more careful examination of the eyes revealed that the eye color was quite variable. Some crabs had uniformly black eyes, and others had pigmentless eyes. But the majority had eyes which varied from an almost pigmentless eye (with a few small pigmented spots) to an eye which was

almost completely black, but in which light areas could be detected. Furthermore, the amount and distribution of the pigment on the left and right eyes was not equal. For this reason, it was necessary to examine both eyes to make sure that a "pigmentless" eyed individual was indeed pigmentless, and a black-eyed crab was bilaterally black eyed. With these precautions, the crabs were classified into three categories: 1) pigmentless —possessing no pigmented spots in either eye; 2) black—eyes lacking light spots; 3) variegated—possessing any amount of pigment, but not completely black-eyed. The number of animals within each of these categories in the three marking sessions is represented in the following table:

| Tagging period | Black | Variegated | Pigmentless | Total |
|---|---|---|---|---|
| I | 18 | 84 | 5 | 107 |
| II | 30 | 200 | 18 | 248 |
| III | 61 | 195 | 41 | 297 |
| Total | 109 | 479 | 64 | 652 |

It is evident that in the Cold Spring Harbor population about 73% of the horseshoe crabs have variegated eyes, about 17% have black, and about 10% pigmentedless eyes.

On July 24, a sample of horseshoe crabs kept in a cage at the Marine Biological Laboratory, Woods Hole, Mass., was classified according to eye color. Of 360 horseshoe crabs examined 37 (10%) black-eyed, 301 (83.6%) variegated-eyed and 22 (6%) pigmentless individuals were found. Statistical methods show that the populations of Xiphosura of Woods Hole and Cold Spring Harbor are phenotypically distinct as to eye color distribution. Apparently Long Island Sound acts as an effective barrier, allowing the development of phenotypically distinct populations.

The method of tagging horseshoe crabs has made it possible to learn other interesting facts about their natural history:

1. The size of the adult population, estimated by tagging and counting both tagged and unmarked animals in a strip 500 meters long between the high tide mark and a three foot depth, has been found to be between 2,550 and 16,700 adults.

2. The sex-ratio of the adult population in this area is 4 males: 1 female.

3. Xiphosura males apparently locate the females by sight. But their sense of vision must be poor, since they sometimes congregate around submerged rocks, mistaking them for females. At times a male may be seen attached to another male. But mistakes in identifying individuals of the opposite sex occur very seldom.

4. Tagging mating pairs with consecutive numbers has shown that, although the members of a pair separate after the tide recedes, occasionally

a male remains attached to the female for a long time. One pair was found in which the male had remained attached to the same female for 72 hours. And another pair, bearing consecutive numbers, was found as a pair nine days after tagging! It is doubtful that members of these pairs had separated at all during that period of time.

Other information unrelated to the former observations but worth while reporting:

With the deepening of the channel leading to the inner harbor, females and their mates find it more difficult to find their way to the southern edge of the sandspit. The few pairs that manage to make their way to this site (no mean accomplishment for the female, since she must drag herself and the male through the reed grass) find an area in which the females can oviposit with the minimum of disturbance. Japanese investigators have reported that Tachypleus tridentatus, an oriental relative of Xiphosura (Limulus) polyphemus, lays as many as 1,000 eggs in one spawn, and a female may spawn twelve times within a single favorable tide. Time did not permit following single females and collecting their eggs for counting. However, there is some evidence that, if a female is not disturbed, she may lay all her eggs for that day in a single nest: A nest was found on the southern edge of the sandspit. A six inch cube of sand and its contents was removed, and eggs, embryos and larvae (called trilobite larvae because of their resemblance to the extinct Trilobites) separated from the sand. As soon as the trilobite larvae were freed from the sand and introduced in water, they began to swim on their backs.

In the laboratory, counts were made of all the developmental stages of the horseshoe crab. In that sample, there were 1025 eggs, 137 embryos, and 9879 trilobite larvae. The latter figure is probably somewhat inaccurate, since some larvae may have been left behind in the nest, and others escaped while washing off the sand. Nevertheless, these counts are comparable to those reported by the Japanese investigators.

The counts of the larvae were greatly facilitated by the fact that, when they were placed in a glass dish with just a few drops of sea water, they began to move away from the source of light. Animals behaving in this manner are said to be negatively phototactic, i.e., if they have a choice, they will move towards the dark. This behavior may explain why trilobite larvae, which are already capable of swimming and crawling movements, do not escape from their nests. If they did, they would fall prey to the many fish swimming in the harbor at this time.

After the first moult, the trilobite larvae acquire the appearance of the adult horseshoe crab: they develop a tail, and the body shape is generally like that of the adult. However, the sexual dimorphism in the appendages is acquired only at the last moult, from which sexually mature

horseshoe crabs emerge. It is said that adult horseshoe crabs continue moulting. Tagging methods will enable us to confirm or deny this statement.

After the first moult, the young horseshoe crabs abandon their nests. At low tide, thousands of the young Xiphosura can be found in shallow pools on the beach, feeding on small organisms found in the sand.

Some abnormal individuals, probably arising by mutation, have been observed in the Cold Spring Harbor population. One, a castoff shell about 8 cm in length, had an obviously short telson or tail. In normal specimens, the tail is about the length of the body (prosoma and opisthosoma combined). In the mutant, the tail was about half the length of the body. There was no evidence that this condition was the result of an accident. Mutants bearing such a short tail probably do not survive to adults, since this appendage is necessary to the animal to right itself, should it accidentally find itself on its back. The short-tailed animal would not be able to turn itself over. The other abnormality is a less serious one: two male specimens were found lacking ocelli (the single eyes located on each side of the anterior medial spine) and the anterior medial spine.

The writer would appreciate it very much if members of the Laboratory or of the Long Island Biological Association would report to him the detection of any marked horseshoe crabs. By now, owing to the action of the salt water, the head of the thumbtack must be quite rusty. If it is present at all, the tack will be found about an inch to the left of the right eye.

# THE SECOND EDITION OF "THE MUTANTS OF DROSOPHILA MELANOGASTER" by C. B. Bridges and K. S. Brehme

Katherine Brehme-Warren

This reference book for research workers in Drosophila genetics was originally published in 1944, five years after the death of C. B. Bridges. It was written from brief memoranda prepared by Bridges and greatly amplified by Brehme from the literature and from correspondence. The second edition, which is now being written, constitutes an extensive revision of the earlier publication. The first (1944) and a reprint (1950) edition totaled 2250 copies and are almost completely out of print. The Department of Publications of the Carnegie Institution of Washington therefore plans a considerably larger printing of the work now in production.

## Methods

The author is collecting descriptions of new mutants and additions to those of mutants included in the 1944 edition in the following ways: 1). A form letter calling for material was sent in March, 1957 to all Drosophila melanogaster workers throughout the world. There was a good response to the letter, and many mutant descriptions which are as yet unpublished or which appear in obscure journals were accumulated in this way. 2). A great deal of correspondence has been carried on by the author with investigators to obtain specific information. 3). Conferences with investigators in their own laboratories have brought to light facts which they had not thought of reporting, information on the loss or continued existence of mutants, and bodies of data which shed new light on certain problems, such as the evaluation of linkage date. Such conferences have necessitated travel to the University of California at Berkeley and at Los Angeles, California Institute of Technology, Stanford University, Johns Hopkins University, the Lankenau Institute, Harvard University, Oak Ridge National Laboratory and Indiana University. 4). The main source of material, as one would expect, is the literature. The author is systematically covering the genetical journals from the year, 1943, when she left off in the earlier work. The amount of new material published since the end of the second world war is enormous, and can be indicated by that derived from a single paper, the report of Ward and Alexander in the January, 1957 issue of "Genetics", which contains full descriptions of 26 new rearrangements and cytological data on 8 old loci. Whenever a large number of new or rewritten descriptions are prepared from the work of an individual or a laboratory, a copy is sent to the responsible investigator for confirmation and permission to publish.

## Results

Authorship and introductory matter: The joint authorship will be maintained as C. B. Bridges and K. Brehme-Warren. The Foreword by the late T. H. Morgan will be retained. In addition there will be a biographical sketch of C. B. Bridges by Jack Schultz.

Nomenclature and symbolism: The International Committee on Ge-

netical Nomenclature which met in Zurich in August, 1957, recommended only one change from the symbolism used in the 1944 edition, the use of En- or en- as the symbol for enhancers, rather than E- or e-, which was previously used. As this involves very few mutants, the recommendation will be followed. The discovery of pseudoalleles in 1952 presents a new problem in nomeclature; after consultation with E. B. Lewis and others, it has been decided to use the original mutant name (for example, bithorax, Contrabithorax, Ultrabithorax) and to indicate the pseudoallelic relationship in the mutant description. A third problem has been presented by the out-of-date criteria for ranking the mutants with respect to their usefulness in genetic research; at a seminar in February, 1958 at Indiana University, Professor H. J. Muller and his staff helped the author to devise a new series of definitions of Rank.

Mutant descriptions: It has been decided to include descriptions of mutants which were well described in the 1944 edition but which have been lost or discarded; such information will, however, be indented to the right, so that the reader can run his eye down a page and see at once which mutants are available for current research. The author has ascertained the existence of mutants by examination of stock lists at the major laboratories and by personal inquiry. There are on record at least 850 lost mutants. Some of these were not well described and located, or are of no special interest; these will merely be listed. Some, however, were thoroughly studied (for example, the Austin translocations and the Demerec aberrations); these descriptions will be included in the new edition. So far, the author has written or received 500 new descriptions; many more will be written as the journals are covered. About 1200 mutants included in the 1944 edition are still in existence; descriptions of these will be republished in their original form or rewritten.

Wild stocks: As most of the wild stocks described in the 1944 publication are no longer in existence, it has been decided to republish the descriptions of the 6 stocks established and analyzed by C. B. Bridges and still in use (Swedish-b, Oregon-R, Florida, Canton-S, Urbana and Lausanne). As one other, Samarkand, is in widespread use, a description of this stock has been prepared by P. T. Ives.

Chromosome maps: The salivary chromosome maps of C. B. Bridges will be republished, with the addition of the cytological loci of the mutants, where these are known. The linkage maps will be reorganized and greatly increased.

Illustrations: Most of the original illustrations will appear again. Many unpublished drawings by the best of all genetical illustrators, Miss Edith M. Wallace, have been lent to the author by E. B. Lewis, and these will be included in the new edition. Many other drawings from journal papers will be added, and of the numerous photographs which have been submitted, a few will be grouped in plates and reproduced.

It is estimated that the second edition of this book will be considerably longer than the first, with an increase of about 25 per cent.

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# PSYCHOBIOLOGY SECTION

H. A. Abramson, B. Sklarofsky, M. O. Baron, H. H. Gettner, B. Weiss, N. Fremont-Smith, M. P. Hewitt, G. J. Neviackas, W. J. Turner, S. Merlis, and H. Lennard

Experiments have been continued to study the nature of the psychotic process in man, as well as the effect of animal tissue extracts on the blocking of the LSD reaction in Siamese fighting fish and man.

Production of Tolerance to Psychosis-Producing Doses of Lysergic Acid Diethylamide. The effect of MLD-41 on man was obtained by giving it to a group of five nonpsychotic test subjects who have been used in the study of LSD-25 and its derivatives for the past three years. Both LSD-25 and MLD-41 were administered orally in distilled water or tap water with no essential differences observed between the two. Development of tolerance to LSD-25 was achieved by administering MLD-41 for five or six days in increasing doses, starting with 100 mcgm on the first day and reaching 350 mcgm on the fifth day. Since the threshold to MLD-41 is approximately 70 mcgm orally, tolerance to MLD-41 itself was developed rapidly. It appears that approximately 1,000 mcgm of MLD-41 administered in this way protects against approximately 80 to 100 mcgm of LSD-25 taken orally eight hours after the last dose of MLD-41.

A similar experiment in which BOL-148 was substituted for MLD-41 resulted in 21 positive responses to the questionnaire (June 21, 1957). The 21 positive responses obtained represent the equivalent of at least a 25 mcgm response to the LSD-25 administered. The subject himself estimated that he experienced a 35 mcgm LSD response.

The fact that a substance like MLD-41, which is less toxic than LSD-25, can produce a marked tolerance to LSD-25 lends hope to the possibility that if the schizophrenias are produced by a disturbance in biochemical mechanisms analogous to that resulting from the administration of mescaline, LSD-25, and similar substances, there is good reason to believe that comparatively non-toxic molecules might be administered to produce a similar tolerance to the chemicals that originate the schizophrenic state.

Tissue Extracts. Several beef tissue extracts were prepared and experimented with to observe blocking. In all the experiments the procedure was as follows:

| | | |
|---|---|---|
| Jar I | 2 mg/cc extract | 1 mcgm/cc LSD |
| Jar II | 0.2 mg/cc extract | 1 mcgm/cc LSD |
| Jar III | 2 mg/cc extract | (extract control) |
| Jar IV | 1 mcgm/cc LSD | (LSD control) |
| Jar V | Water control | |

The jars contained a total volume of 100 cc and only distilled water was used. The jars were kept in a water bath at approximately 80° F. Ten Bettas were added to each jar for the experiment. Readings were taken and recorded for nose-up, tail-down position, kink-in-tail, top-of-

the-water, and bottom-of-the-water. The following beef extracts were prepared and tested: cerebrum, cerebellum, gray matter, white matter, spinal cord, heart muscle, striated muscle, liver, pancreas, spleen, and whole-brain. Semi-quantitative data are available on the quantity of LSD blocking substance found in these tissues.

Simplification of Procedure in the Preparation of Tissue Extract Blocking the LSD Reaction in Siamese Fighting Fish. One step in the procedure previously employed was the dialysis, for 48 hours, of the boiled and minced tissues. An attempt was made to eliminate this time-consuming step. By filtering the boiled, minced tissue and following the procedure as before on the filtrate, we have obtained an extract from beef spleen and beef brain that blocks the action of LSD in Siamese fighting fish. This extract was found to be as effective as the dialyzed extract. It is anticipated that large quantities of the LSD blocking substance will be presently available for chemical and pharmacological study.

Cyanide Experiments and the Mechanism of Action on Oxidase Systems of LSD. With concentrations of potassium cyanide analogous to those used to obtain readily observable LSD-25 effects, i.e., one mcgm per ml in the outside liquid, the fish, in general, act as if they were under a slightly lower concentration of LSD-25, rising to the surface and assuming a nose-up, tail-down position. The cartesian-diver position is often assumed, although some of the motor characteristics of the LSD-25 effect are different. Casual inspection of fish under LSD-25 and potassium cyanide might not reveal an important difference in the response. However, certain differences in the response are present and a trained observer can usually distinguish between the effect of cyanide and the effect of LSD-25, especially by the absence of the kinking of the tail. Apparently, the KCN is utilized more rapidly than LSD-25 by the fish, because if the fish are transferred to fresh water, they recover faster from the KCN than they do from LSD-25.

Hydrogen sulfide at high concentrations (100 mcgm per ml) was lethal, but the surviving fish assumed a nose-up, tail-down position at the surface. The effect of hydroxylamine hydrochloride up to concentrations of 100 mcgm per ml as well as hydrazine sulfate to 10 mcgm per ml has thus far been negative. Most interesting was the effect of azide. Although sodium azide is more toxic than potassium cyanide, an effect analogous to LSD-25 is readily observed at concentrations of sodium azide similar to those observed for LSD-25.

Preliminary data indicate that a decreased oxygen supply with simultaneous prevention of accumulation of carbon dioxide resulted in the nose-up, tail-down position similar to LSD-25 in several hours—the fish remaining alive. When carbon dioxide was permitted to accumulate in the absence of renewed oxygen, all the fish assumed the nose-up, tail-down position in several hours. The fish rose rapidly to the surface when disturbed. Recovery was rapid from both anoxia and asphyxia. Experiments are in progress with improved techniques of observation.

A concentration of methylene blue of 100 mcgm per ml produced a suggestive LSD-25 reaction. Bindschedler's green affected the fish, producing a noticeable excitatory period; 20 mcgm per ml produced the nose-up, tail-down position with no kink in the tail. In a comparative experiment 20 mcgm per ml of Bindshedler's green was more effective than one mcgm of potassium cyanide. It is of interest that the effect of methylene blue increased with time. Fifty mcgm per ml of toluylene blue and indigo disulfonate were without an important effect. Fifty mcgm per ml of each, produced a nose-up, tail-down position in more than half benzine indophenol was effective, the effect increasing with the concentration up to 50 mcgm per ml, where an effect equivalent to one mcgm per ml of LSD-25 was observed. Although 50 mcgm per ml of gentian violet and methyl violet killed the fish, lower concentrations, 20 mcgm per ml of each produced a nose-up, tail-down position in more than half of the fish at the end of 4 hours.

It has often been observed that human subjects under the influence of comparatively small doses of LSD-25 have the confusion and other symptoms that are associated with anoxia. The effects of LSD-25 in man, therefore, are not incompatible with the assumption that LSD-25 in the fish and in man acts by poisoning some parts of the enzymatic processes connected with oxidation. Although at the time that this report was made we had not had access to the data dealing with the effects of nonlethal doses of potassium cyanide on the mental state of man, the available data in the literature indicate that an exogenous psychosis with loss of reality sense is produced with nonlethal doses. Indeed, potassium cyanide has been used in the therapy of schizophrenia, apparently to stimulate the respiratory enzymes. It is worthwhile on the basis of these data to explore the possibility that not only the LSD-25 psychosis in man may be connected with the poisoning of special oxidative enzymes, but also that the schizophrenic process may be connected with a similar process where special respiratory enzymes of the brain are not functioning adequately.

The Effect of Asphyxia on Siamese Fighting Fish. Asphyxia was studied with low concentrations of LSD and without LSD. Progressive asphyxia was achieved by placing on the water in the jars a thin layer of melted paraffin. Aged distilled water was used. A total of 135 fish were studied, 70 in jars with the air sealed off and 65 in jars with water surface open to the air. Observations were made at 15 minute intervals until all the fish in one jar had died. During the course of four experiments a total of 45 observations were made.

Four points were noted:

1. Five fish sealed in 100 ml of water kept at 80° F, or ten fish sealed in the same volume kept at 75° F, would survive for from two to four hours, whereas ten fish sealed in 100 ml of water kept at 80° F would survive for no longer than an hour.

2. When the air was sealed off, fish in jars containing 0.1 mcgm/ml LSD survived on an average of one-half hour longer than those in jars with no LSD.

3. Thirty-five fish in jars in which the air was sealed off and which contained 0.1 mcgm/ml LSD showed a total of 122 typical responses (nose up, tail down at the surface) in 45 observations. An equal number of fish in jars with the air sealed off, but containing no LSD, showed a total of 57 of these typical LSD responses in 45 observations. In the unsealed control containers, 35 fish in water containing 0.1 mcgm/ml LSD showed 25 of these typical LSD responses in 45 observations and in the water controls without LSD, 30 fish showed a total of only five of these LSD responses in 45 observations.

4. The excitatory phase which rarely occurs in concentrations below 0.5 mcgm/ml of LSD was observed in many of the jars in which the air was sealed off whether or not they contained LSD. This violent activity was usually noted about a half-hour after the onset of the typical LSD response described in (3) and would precede death by about a half-hour. This can be contrasted to the reaction of fish in jars in which the water surface was open to the air to 1.0 mcgm/ml LSD, in which the excitatory phase precedes or is simultaneous with the onset of the typical LSD response and which is followed by semi-stupor at the surface in the nose-up, tail-down position rather than death at the bottom of the jar. It is planned to continue these experiments with special emphasis on the effect of temperature and LSD.

Experiments on Man. 1. Non-psychotic Test group. During the past year experiments on the non-psychotic test group have been continued. Five normal subjects who have become quite expert on assaying the effects of LSD-25 have been given: (a) derivatives of LSD-25 (b) derivatives of LSD-25 in a study of cross-tolerance to LSD-25.

(a) During the year we have succeeded in getting data on LSD derivatives like 1-methyl lysergic acid diethylamide (MLD), 1-acetyl lysergic acid diethylamide (ALD), oxymethyl lysergic acid diethlyamide, lysergic acid dimethylamide (DAM), and other derivatives, by comparing the ratio $N \div d$, where N is the number of dose positive responses to the standard questionnaire, and d, the dose expressed in mcgm. We have developed a new numerical method, the Response Index, a value useful in comparing various psychoticomimetic drugs especially sensitive at threshold doses. On the basis of LSD-25 taken as 100, we may list some of the compounds studied as follows:

| | |
|---|---|
| LSD | 100 |
| ALD | 73 |
| Oxymethyl LSD | 45 |
| MLD | 33 |
| DAM | 9 |
| LAE | 1.8 |

In this way it has been possible to get a clearer picture of the order of effectiveness of these drugs and to obtain the relationship between structure and psychic activity.

(b) Most important in the past year has been the observation that MLD, although less effective than LSD in producing psychic effects, is the most effective of all the drugs studied thus far in producing tolerance to LSD.

By administering MLD for six days in advance with increasing doses we have produced tolerance to very high doses of LSD in non-psychotic subjects. Thus, one of our subjects has taken 150 mcgm of LSD without any symptoms, after being suitably prepared by previous administration of MLD. These experiments, of course, point the way to the possibility of protecting schizophrenic patients against a possible chemical cause of schizophrenia by administration of non-toxic substances. Cross-tolerance experiments with LAE (containing only one ethyl group in the amide link) show that little, if any, tolerance is produced by LAE.

2. Psychotic Subjects. Mrs. Hewitt and Mrs. Neviackas have continued their work studying the effect of these drugs at the State Hospital at Central Islip. These experiments have repeatedly shown that the schizophrenic patients studied respond to LSD-25 even in small doses, as low as 50 mcgm. In addition, four schizophrenic subjects to whom MLD was administered developed a tolerance to 250 mcgm of LSD-25 in the same way that non-psychotic subjects do. A schizophrenic individual, therefore, has the power to develop tolerance to the psychoticomimetic drug, LSD-25. The entire staff agrees that a somewhat unexpected result of administering MLD to the group of schizophrenic patients just mentioned was the consistent observation that this group of four subjects improved considerably during the period that MLD was administered. The severity of the psychotic process diminished, and the patients themselves became more communicative, developing a certain amount of insight.

If these experiments are confirmed, using double-blind precautions, we may have discovered a new approach to the therapy of schizophrenia by the production of tolerance to a psychosis-producing drug by the administration of another closely related drug.

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——— The average effect of radiation-induced mutations on viability in Drosophila melanogaster. Evolution (In press)

——— Studies on inbred strains of Drosophila melanogaster (In manuscript) (1958)

——— Studies of the relative fitness of experimental populations of Drosophila melanogaster (In manuscript) (1958)

——— The role of heterozygosity in Drosophila populations (In manuscript) (1958)

Bernheimer, Alan W., and Lois Schwartz, New York University College of Medicine, New York, N.Y.—We have been interested in finding specific inhibitors of diphosphopyridine nucleotidase (DPNase) and have searched for such agents among naturally occurring materials. Extracts prepared from 40 species of plants growing at Cold Spring Harbor were tested and several were found to inhibit the enzyme in high dilution. Extracts of the leaves of three species, Cornus, Ailanthus, and Myrica were prepared in amounts sufficient for further study. The inhibitors in all three were thermostable dialyzable substances. The crude extracts precipitate with a variety of proteins and give a dark blue color with ferric chloride, indicating the presence of tannins. It is not clear at present whether the agents responsible for DPNase-inhibition are tannins or specific substances separable from tannin.

Calef, E., University of Illinois, Urbana, Ill.—Integrated Genetics of Lambda Prophage and Coli K-12. As a test of linearity of the lambda prophage chromosome a program of crosses between K-12 strains carrying marked prophages was carried on.

Previously crosses involving the lambda markers h (Appleyard) and co (Kaiser) gave results in agreement with linearity of that portion of the prophage and the remaining bacterial chromosome. The crosses carried out in the summer attempted to investigate that portion of the prophage chromosome which corresponds in the vegetative phage to the segment between co and mi (Kaiser). The standard condition used in the experiments involved the simultaneous replica of a large number of purified recombinants on suitable indicator strains. The use of two different Hfr (C) & (H) did not affect the frequency of segregation of lambda markers, though one of them, Hfr (C), showed a linked segregation and could be located between Gal and T6, while the other was unmappable.

In these crosses over 2000 purified recombinants were scored; out of these only 350 were scorable for mi. In this group four only were segregant for mi, making any conclusion on the position of this gene impossible. The residual group was scored for co & h and the sequence h co Gal Hfr T6 was verified.

Calef, Nina Schwarz, University of Illinois, Urbana, Ill.—Studies on Spontaneous Mutants of Salmonella phage $P_{22}$. Experiments were done to investigate the production of spontaneous mutants of phage by changing conditions of the host. Starved or aged bacteria were found not to affect the mutation rate of the phage. Experiments were then done with irradiated bacteria. The irradiation time was chosen to give a bacterial survival of $5 \times 10^{-6}$. The phage stock used, $P_{22}c^{+}hm^{+}$, contained mutants at the following concentration: $h^{+}$, $8 \times 10^{-4}$; c, $3 \times 10^{-4}$; m, $8 \times 10^{-5}$.

In one experiment which gave useful information, 96 tubes were prepared, each containing 50 bacteria infected at a multiplicity of 2. Unabsorbed phage was inactivated by serum. After plating such tubes, the plates with mutants fell into 5 groups:

A) Fourteen plates with 1 mutated plaque each (2 $h^+$; 11 c; 1 m).
B) Sixteen plates with more than 1 and less than 18 mutated plaques of single type (4 $h^+$; 9 c; 3 m).
C) Five plates with more than 18 mutated plaques of 1 type (3 $h^+$; 2 c; 0m).
D) Eleven plates with various amounts of 2 types (9 $h^+$ and c; 1$h^+$ and m; 2 c and m).
E) Four plates with multiple mutants, alone or together with other mutants (1 plate with a single c m; 1 plate with a single $h^+$ m, 1 c plaque and 1 $h^+$ plaque; 1 plate with 205 $h^+$ m plus 2 c plaques; 1 plate with 61 $h^+$ m plaques).

These data show an increased production of mutants of phage grown on irradiated bacteria. No evidence of coincidental production of mutants in the same cell was found.

Evans, Audrey H., The Rockefeller Institute for Medical Research, New York, N.Y.—Experiments were conducted to determine quantitatively the ability of several strains of pneumococci to synthesize folic acid in the presence of sulfanilamide and other closely related drugs. Folic acid was measured by a new endogenous assay developed by Dr. R. D. Hotchkiss, a collaborator in these studies. A wild-type strain and several resistant mutants derived from it through the mechanism of DNA-mediated transformation were compared in this respect. It had been demonstrated in previous experiments that this particular series of mutations is present in closely linked units within a single region of a DNA molecule affecting an unidentified protein which leads to the formation of folic acid. The purpose of these experiments was to relate these gene modifications to identifiable modifications in the affinity of this protein for para-aminobenzoic acid and various sulfonamides of known structure. The drugs tested included sulfanilamide, sulfathiazole, sulfadiazine, sulfaguanidine, and para-aminosalicylic acid.

Granick, S., The Rockefeller Institute for Medical Research, New York, N.Y.—The summer was spent in preparing two review papers: one on "Iron Metabolism in Plants and Animals"; the other on "Chloroplasts: Structure, Function and Inheritance."

Hamilton, L. D., Sloan-Kettering Institute, New York, N.Y.—In collaboration with Dr. M. L. Errera, preliminary experiments were continued on the incorporation in vitro of thymidine-$H^3$ by rat thymus cells and thymus nuclei isolated in sucrose, and by teased rat-lymph node preparations. Satisfactory labelling of thymus and lymph node cells was obtained by incubation at 37° in a medium consisting of 1 part Eagle's medium and 1 part autologous rat serum; nuclei were incubated in Allfrey's medium (J. Gen. Physiol. 40, 451:1957). These experiments were designed eventually to provide a dependable source of labelled nuclei for studies of the incorporation of nuclei by lymphocyte precursor cells.

Hotchkiss, Rollin D., The Rockefeller Institute for Medical Research, New York, N.Y.—Growth rates of pneumococcal strains were studied in

the presence of metabolic inhibitors of several kinds, including the sulfonamides mentioned in Miss Evans' report. The purpose was to seek evidence for certain metabolic pathways and to recover mutants altered in these pathways. Several mutant strains were isolated and preserved for later, more detailed, study.

Luria, S. E., and Sylvia Smith, University of Illinois, Urbana, Ill.—Studies on T*2 Phage. A reinvestigation of the T*2 phage produced by bacteria E. coli B/4. (Luria and Human, J. Bact. 64, 557, 1952) was initiated. Phage T*2 grows on Sh. dysenteriae Sh but not on E. coli B or K-12. Phage $T2r_{22}$, a mutant in the $r_{II}$ region, does not grow in cells of K-12 (λ), because of the Benzer effect. Mixed infection of K-12 (λ) with $T2r_{22}$ and $T^*2r^+$ did not give any significant help in either direction. Since a functional $r^+$ particle can fully supplement the $r_{II}$ particles and permit their growth, even when the $r^+$ locus belongs to a UV-inactivated particle and is not itself cross reactivated (Kreig, Ph. D. thesis, Univ. of Rochester, 1957), we conclude that the T*2 particles are blocked before the stage at which the $r_{II}^+$ locus becomes functional. Since a non-star particle can help T*2 to participate in the phage yield, we conclude that the $r_{II}$ particles, in the bacteria where they do not grow, do not carry out whatever reaction is needed to help T*2. Further experiments are in progress to test whether the T*2 phage does inject its DNA into cells where it cannot grow.

S. E. Luria wrote a chapter of "The Replication of Viruses" for "The Viruses" (Burnet and Stanley, Eds.).

Maramorosch, Karl, The Rockefeller Institute for Medical Research, New York, N.Y.—The summer was devoted to a new experimental approach to plant-virus chemotherapy. A benzimidazole riboside, DRB, that was shown by Mirsky et al. to inhibit ribonucleic acid synthesis and by Tamm, et al. to inhibit influenza-virus multiplication in tissue culture was tested in vivo against 4 plant viruses in their respective vectors. Aster yellows, curly-top, corn-stunt, or wound-tumor virus was acquired by leafhopper vectors feeding on diseased plants. A suspension of 5 mg/ml ca. 0.001 ml per insect, was injected after virus acquisition. An increase in the length of virus incubation periods or an inhibition in virus transmission was used as the criterion of inhibition of virus multiplication. The results of extensive tests indicated that DRB in the concentration used had no effect on the multiplication of the 4 plant viruses in leafhoppers. This work was supported by a summer research fellowship of The Lalor Foundation.

Melechen, Norman E. and Leonard Mindich, Saint Louis University School of Medicine, Saint Louis, Mo., and The Rockefeller Institute for Medical Research, New York, N.Y.—The initiation of bacteriophage $T_2$-DNA synthesis requires a chloramphenicol-sensitive process (most likely protein synthesis). Once initiated, DNA synthesis continues in the presence of chloramphenicol. These observations raise the following questions: (1) Does the chloramphenicol sensitive process involve the formation of

material with genetic specificity, e.g., does it act as a template for progeny-DNA synthesis? (2) Is the DNA made in the presence of chloramphenicol genetic material? To answer the first question, a test was made of the capacity of the initiating material synthesized in response to infection by one bacteriophage ($T_2hr$) to support the formation of DNA of different genetic specificity, $T_2H$ (wild-type, i.e., $h^+r^+$).

Cultures of E. coli H in a tris buffered-glucose-minimal salts medium (growth medium) were infected with $T_2hr$ at a multiplicity of 5/bacterium. Chloramphenicol, at a final concentration of 20 $\mu$g/ml., was added at 7 minutes after infection. At 9 minutes the culture was superinfected with 200 wild type phage/bacterium. After 5 minutes for adsorption (at $t = 14'$), samples of the culture were diluted: (1) into growth medium + anti-$T_2$ serum = culture 1, (2) into growth medium + anti-$T_2$ serum + 20 $\mu$g/ml. chloramphenicol = culture 2. Culture 1 was diluted out of the antiserum, at $t = 19'$. At intervals, beginning at $t = 20'$, culture 1 was artificially lysed by diluting into cyanide broth. Culture 2 was allowed to remain in chloramphenicol until $t = 60'$ and then diluted into growth medium, i.e., out of both the chloramphenicol and antiserum. It was then similarly lysed at intervals.

Culture 1 constitutes a control culture for assaying the DNA made with concomitant protein synthesis (without chloramphenicol). Except for the four minute period from $t = 10 — 14$ (DNA synthesis starts at about 10 minutes after infection in this medium) all of the DNA in culture 1 is made in absence of chloramphenicol.

Culture 2 served as an assay of phage made with DNA formed without concurrent protein synthesis. Chloramphenicol being present from 7 minutes to 60 minutes, all of the DNA which accumulates has been made in the presence of chloramphenicol. Only that DNA synthesis taking place after removal of the chloramphenicol has been accompanied by protein synthesis. It had been shown previously that both kinds of DNA enter the same precursor pool.

The phage in the two sets of lysates was then tested for the four possible genotypes. The experiments allow some qualitative observations to be made:

(1) Among the first progeny phage (less than an average of 1 per bacterium) were found both parental types as well as recombinants. The presence of superinfecting phage (and especially recombinants) among the first phage would indicate that the initiating protein material was not genetically specific. This conclusion is, of course, justified only if it is assumed that the DNA synthesized in the presence of chloramphenicol is genetically specific, i.e., that the answer to the second question mentioned previously is affirmative.

(2) Among the early progeny the proportion of superinfecting to primary parental types is somewhat higher in culture 2 than in culture 1. The proportion recombinants/parental types is higher in 1 than in 2. These differences seem to disappear with time, i.e., with the increase in

the number of phage/bacteria. The implications of these observations in relation to the process of recombination are being studied.

This work was supported by a grant from The National Foundation for Infantile Paralysis.

Novick, Aaron, The University of Chicago, Chicago, Ill.—An investigation was begun of the appearance of the enzymes involved in the utilization of galactase following the introduction of the capacity to form these enzymes into genetically incompetent bacteria. For this purpose suitable stocks of the K-12 strain of E. coli bacteria as well as appropriate stocks of the transducing phage lambda were prepared. Preliminary experiments were performed to determine the conditions required for the transduction of the maximum fraction of the treated population. Some experiments, all inconclusive, were made in an attempt to understand the nature of several rare reversions in the E. coli strain B/i,t. Also some time was spent preparing several manuscripts.

Ting, R. C., University of Illinois, Urbana, Ill.—Curing of Phage Infection in Salmonella by Chloramphenicol. When cells of Salmonella typhimurium LT2 in the logarithmic phase of growth in broth are infected with bacteriophage P22 and exposed within a few minutes to bacteriostatic concentrations of chloramphenicol, many infected cells are cured and can give rise to normal colonies upon removal of the antibiotic. This effect is observed with phage P22 and with some of its mutants, but not with other mutants. The phenomenon is illustrated for the phages $P22c_1$ and $P22c_2$. In controls without chloramphenicol, all cells infected with these phages are lysed. When chloramphenicol (50 $\mu$g/ml) is added within 10 minutes after infection with phage $c_2$, allowed to act for 30 minutes, and removed by dilution, about 90% of the infected cells, instead of being lysed, survive. If plated these give rise to colonies of phage-sensitive cells. With the phage mutant $c_1$, instead, chloramphenicol rescues only about 30% of the cells if added immediately after infection. If added 10 minutes later, no curing occurs and, upon removal of the antibiotic, all cells lyse and produce phage.

The phages $c_1$ and $c_2$, in mixed infection, can cooperate to prevent cell lysis. Many cells survive and become lysogenic for phage $c_1$. Thus, phage $c_2$ helps $c_1$ to become prophage (Levine, Virology 3:22, 1957). If cells are mixedly infected with $c_1$ and $c_2$ and treated with chloramphenicol, some cells are lysed and produce only phage $c_1$. Up to 50% of the cells survive and become lysogenic for phage $c_1$. Thus, chloramphenicol treatments specifically eliminates phage $c_2$ but does not suppress its function of helping $c_1$ to establish lysogeny.

Uetake, H., and S. E. Luria, University of Illinois, Urbana, Ill.—Conversion of Antigens by Phage in Group E Salmonella. Antigen 15 appears within a few minutes in Salmonella group $E_1$ cells infected with phage $\varepsilon^{15}$, while antigen 10 disappears. Nonlysogenic segregants reacquire antigen 10 and lose antigen 15. In the summer, using phage C, which adsorbs only to bacteria with antigen 10, and phage $\varepsilon^{34}$ vir, which only

attacks bacteria with antigen 15, we studied the evolution of these properties following infection and during segregation. The results showed that following infection the "complex 10" (antigen 10 and receptors for C) disappears more rapidly than by dilution of preexisting amounts among progeny cells. Complex 15 (antigen 15 and receptors for $\varepsilon^{34}$ vir) reaches full development in 5 generations. The nonlysogenic segregants lose complex 15 by simple dilution among progeny cells. An article on this subject was prepared and submitted to "Virology."

Defective lysogeny for phage $\varepsilon^{15}$ was investigated. One type of defective cell loses both phage-producing ability and sensitivity to UV (inducibility) by mutation, but retains antigen 15. Another type of defective produces no phage, but still has antigen 15 and is UV-sensitive. A third mutant bacterium has lost UV-inducibility but retains phage $\varepsilon^{15}$ and antigen 15; the phage from this defective gives rise to inducible lysogenic cells. The relation of the antigen-controlling factor to phage-production genes is being investigated.

# COURSE ON BACTERIOPHAGES

June 17-July 6, 1957

Instructors: G. Streisinger, Carnegie Institution of Washington; S. E. Luria, University of Illinois.

Assistants: Sylvia Smith, University of Illinois; Robert Ting, University of Illinois.

The intensive three weeks' course on bacteriophages was given for the thirteenth time in the summer of 1957. As in the previous two summers, the course followed a stepped-up schedule which permitted covering some of the recent developments in the field. In addition to the intensive laboratory sessions, five seminars were presented by investigators active in phage research. The speakers and their topics were:

A. D. Hershey, Carnegie Institution—DNA as genetic material.

J. D. Mandell and E. Burgi, Carnegie Institution—Fractionation of T2 DNA.

J. Tomizawa, Carnegie Institution—Phage DNA synthesis in the presence of Chloramphenicol.

G. Streisinger, Carnegie Institution—Partial exclusion and sweetness in T2 and T4.

S. E. Luria, University of Illinois—Lysogeny.

The eighteen students and one auditor who enrolled in the course are listed below:

T. Amano, Ph.D., Rockefeller Institute for Medical Research, New York, N.Y.

J. Bruce Ames, Ph.D., National Institutes of Health, Public Health Service, Bethesda, Md.

Helen V. Clugston, Grad. Stud., University of Rochester, Rochester, N.Y.

G. Fermi, M.A., Princeton University, Princeton, N.J.

William Firshein, Grad Stud., Rutgers University, New Brunswick, N.J.

Forest J. Funk, B.S., Biochemical Research Foundation, Newark, Del.

Allan Granoff, Ph.D., The Public Health Research Institute of the City of New York, New York, N.Y.

Samuel H. Love, Ph.D., Bowman Gray School of Medicine, Winston Salem, N.C.

Lewis N. Lukens, PhD., Massachusetts Institute of Technology, Cambridge, Mass.

William Ogata, Grad. Stud., Dartmouth College, Hanover, N.H.

Elmer Pferfferkorn, Grad. Stud., Harvard University, Cambridge, Mass.

Frank Rothman, Ph.D., University of Wisconsin, Madison, Wisc.

Hassan Rouhandeh, Grad. Stud., Kansas State College, Manhattan, Kan.

Norman P. Salzman, Ph.D., National Inst. of Allergy & Infectious Diseases, Bethesda, Md.
Melvin Santer, Ph.D., Haverford College, Haverford, Penna.
Alexandra E. Shedlovsky, Grad. Stud., Harvard Medical School, Cambridge, Mass.
D. V. Siva Sankar, Ph.D., Adelphi College, Garden City, N.Y.
T. Watanabe, Ph.D., Keio University School of Medicine, Tokyo, Japan.

Auditor

Richard S. Schneider, Undergrad., Dartmouth College, Hanover, N.H.

# COURSE ON GENETICS OF FILAMENTOUS FUNGI

July 8-August 4, 1957

Instructors: R. W. Barratt, Dept. of Botany, Dartmouth College, and E. Käfer, Dept. of Genetics, McGill University.

This year the course was extended from three to four weeks and expanded to include both Aspergillus and Neurospora. The exercises were directed toward a comparison of the genetics of a homothallic and heterothallic fungus; the induction, detection, selection, and isolation of mutant strains; the analysis of the parasexual cycle in Aspergillus; linkage detection and mapping via single strand analysis, tetrad analysis, and the parasexual cycle; the use of heterocaryons in testing allelism; the control of heterocaryons by incompatibility factors; and the analysis of nuclear ratios in heterocaryons.

In addition to the intensive laboratory sessions, eight seminars were presented by investigators active in phage research. The speakers and their topics were:

W. T. Ebersold, Harvard University—Crossing over in Chlamydomonas reinhardi.

John R. Raper, Harvard University—Clouds of Witnesses—or, the genetics of incompatibility in Schizophyllum.

R. W. Barratt, Dartmouth College—Studies on the glutamic acid dihydrogenase locus in Neurospora.

N. H. Giles, Yale University—Recent evidence on gene conversion in Neurospora.

Patricia St. Lawrence, Yale University—Current work on gene conversion in Neurospora.

Etta Käfer, McGill University—Mitotic recombination in Aspergillus.

W. Szybalski, Rutgers University—Recombination in Streptomyces.

A. J. H. Carr, Columbia University—Genetics of Sordaria fimicola.

The nine students and one auditor who attended the course were as follows:

Helen V. Clugston, Grad. Stud., University of Rochester, Rochester, N.Y.

Murray W. Coulter, Grad. Stud., University of California, Los Angeles, Calif.

Jen-yah Hsie, Ph.D., Des Moins Still College of Osteopathy and Surgery, Des Moines, Iowa

Eugene P. Goldschmidt, Ph.D., Fort Detrick, Frederick, Md.

Paula Gottdenker, Rutgers University, Newark, N.J.

Minna B. Rotheim, M.A., University of Rochester, Rochester, N.Y.

Hassan Rouhandeh, Grad. Stud., Kansas State College, Manhattan, Kan.

T. Watanabe, Ph.D., Keio University School of Medicine, Tokyo, Japan

Dow O. Woodward, Grad. Stud., Yale University, New Haven, Conn.

Auditor

Richard S. Schneider, Undergrad., Dartmouth College, Hanover, N.H.

# COURSE ON BACTERIAL GENETICS

August 5-24, 1957

Instructors: M. Demerec, V. Bryson, E. M. Witkin, P. D. Skaar, in collaboration with E. L. Lahr, H. Moser, W. D. Cannon, I. Goldman, and H. Ozeki

In 1957 this course on selected methods in bacterial genetics research was offered for the eighth time to a group of advanced graduate and postgraduate students. The course emphasized current methods used in the study of bacterial heredity, and some of the important results of recent work in this field. The following students were enrolled:

Warren F. Carey, Grad. Stud., Walter Reed Army Institute of Research, Washington, D.C.
Mary H. Costa, M.S., Adelphi College, Garden City, N.Y.
Robert J. Doyle, Grad. Stud., Windsor, Ontario, Canada
Nathan Entner, Ph.D., New York University Medical School, New York, N.Y.
Julian Gross, Grad. Stud., Institute of Animal Genetics, Edinburgh, Scotland
Henry J. Hearn, Ph.D., Biological Warfare Laboratories, Fort Detrick, Frederick, Md.
Ann E. Heuer, Grad. Stud., Rutgers University, New Brunswick, N.J.
L. Joan McGowan, Grad. Stud., Acadia University, Nova Scotia, Canada
William Ogata, Grad. Stud., Dartmouth College, Hanover, N.H.
Hassan Rouhandeh, Grad. Stud., Kansas State College, Manhattan, Kan.
Andrew G. Smith, Ph.D., University of Maryland School of Medicine, Baltimore, Md.
P. R. Srinivasan, Ph.D., College of Physicians and Surgeons, New York, N.Y.
Barnet M. Sultzer, Grad. Stud., Michigan State University, East Lansing, Mich.
Jesse S. Tucker, M.S., Chas. Pfizer and Co., Brooklyn, N.Y.
Tiiu Vaharu, Grad. Stud., Syracuse University, Syracuse, N.Y.
Tsutomu Watanabe, Ph.D., Keio University School of Medicine, Tokyo, Japan
John A. Wohlhieter, Grad. Stud., University of Pittsburgh, Pittsburgh, Penna.
Walter A. Zygmunt, Ph.D., Mead Johnson and Company, Evansville, Ind.

Auditor

Richard S. Schneider, Undergrad., Dartmouth College, Hanover, N.H.

In connection with the course, the following special lectures and seminars were given by instructors and by other research scientists in the field:

M. Demerec, Carnegie Institution—Transduction and its uses in genetic studies of Salmonella.

S. E. Luria, University of Illinois—Mating between Escherichia coli and Shigella.

P. D. Skaar, Biological Laboratory—Tryptophan gene cluster in E. coli.

Werner Braun, Rutgers University—Effect of breaddown products on bacterial population changes.

Hermann Moser, Biological Laboratory—Genetic studies with mammalian somatic cells.

Aaron Novick, University of Chicago—Mutations in bacterial populations.

E. Calef, University of Illinois—Integrated genetics of prophage and bacteria in K-12 of E. coli.

Ellis Englesberg, Biological Laboratory—Mutation to diauxie resistance in Salmonella.

Evelyn M. Witkin, State University of New York—Postirradiation metabolism and mutagenesis in bacteria.

Stephen Zamenhof, College of Physicians and Surgeons—The transforming phenomenon.

R. Thomas, University of Brussels—Reaction of DNA with bacteria.

H. J. Vogel, Yale University—Comparative biochemistry of microorganisms.

P. E. Hartman, Johns Hopkins University—Transduction.

R. D. Hotchkiss, Rockefeller Institute for Medical Research—Mechanism of transformation.

# NATURE STUDY COURSE

July 1-August 2, 1957
August 5-23, 1957

Instructors: Marvin J. Rosenberg, Department of Science, Northport High School, Northport, N.Y.
Jill A. Lamoureux, Port Washington, N.Y.
Otto Heck, Department of Science, Island Trees High School, Levittown, N.Y.

Assistants: Donna Granick, New York, N.Y.
Gail Geraghty, Northport, N.Y.
Elizabeth Pierce, Huntington, N.Y.

This summer the Nature Study Course for children met during the five week period from July 1 to August 2. This course is given to a group of young people who want to learn more about the environment surrounding them. One of the functions of the course is to stimulate an interest in nature by observation and study of flora, fauna and geology of the area around Cold Spring Harbor, N.Y.

The enrollment this year mounted to 137 children. Each class, except the seniors, met twice a week on alternate days for a two-hour period. The seniors met for four hours each Friday, which allowed for trips to areas difficult to reach and explore adequately during a shorter time. Hours of the classes were arranged in a manner which permitted families with several children participating in different age groups to attend simultaneously. This schedule was received quite favorably. Pupils were classified according to age and/or experience in nature study as Beginners I and II (6 and 7 years), Intermediates I and II (8 and 9 years), Juniors I and II (10 and 11 years) and Seniors (12 to 14 years).

Wawepex Laboratory again served as the home base for the classes. Due to the unusually large enrollment, two additional rooms were converted for use, one for a classroom and the second as a museum room. Additional insulation and two large window fans made it pleasant even on very warm days. Another small room on the lowest floor was equipped as an aquarium room where all the large fresh and salt water aquaria were centrally located. Additional closet space was provided. New equipment included a new Bausch and Lomb stereoscopic microscope and lamp, dip nets, insect nets, insect preserving materials, sea buckets and miscellaneous art supplies. A set of Collier's Encyclopedias received on loan was used many times by the children.

Mr. Heck, new to the staff, brought with him a reptile collection which included a boa constrictor, a king snake, a black snake, several milk snakes and several fence swift lizards. The last mentioned lizards successfully hatched a clutch of eggs. The children never tired of watching the snakes being fed.

The two film showings on Tuesday evenings were very well attended. This year the Walt Disney nature films were featured and those shown

included Nature's Half Acre, Seal Island, Beaver Valley, and the Olympic Elk. Kodachrome slides and film strips were used as part of the rainy-day programs.

During the last week of the course each class took an "all-day trip" to various spots on the Island. These trips, which conclude the course, are always awaited eagerly. Trips were taken to Jones Beach, Mill Neck, Bayville Beach, Tackapausha Preserve and Shelter Rock Pines. Many thanks are due the deserving mothers who volunteer to drive classes to the various spots.

The first session of the Nature Study Course closed on August 2 with an Open House in Wawepex. The collections, special projects and other work of the children were exhibited. Refreshments were served on the lawn beneath the apple tree.

An additional session was offered this year for those who wished to remain and for those who could not enter the first session because of closed classes. These classes met on Mondays, Wednesdays and Fridays for a three-hour period from August 5 to 23. There were two sections, taught by Mr. Rosenberg and Mr. Heck, with a total of 31 youngsters.

The following students were enrolled in the Nature Study Course:

July 1-August 2

Abramowitz, Elizabeth
Abramowitz, Joel William
Alston, Kathleen H.
Anselmini, Ludwig
Baer, Melinda C.
Barmon, Ward
Baron, Richard
Bartlett, Edmund, III
Berman, Mark Edward
Bernst, Sydney E.
Bernstein, Elizabeth
Bernstein, Theodore
Billman, Christopher W.
Blauman, Beth Ann
Blecher, Robin
Bulpitt, William S.
Burns, Claudia
Butler, Thorne
Colyer, Barent
Colyer, Josephine
Cook, Kenneth A.
Curry, Ora, Jr.
Davis, Deborah
Dropkin, Gregory
Drosin, Larry A.
Dunn, William M.
Dupont, Pierre W.
Eastment, Jeffrey T.
Egan, Wesley W., Jr.
Elliott, Robert E.
Fieldgate, David J.
Flashenberg, Robert J.
Flett, David M.
Foster, Barrett W.
Foster, Elon, III
Galehouse, Shelley
Gould, Arnold
Griffiths, Mark
Hanson, Mark
Harmon, John E.
Harris, Jean
Heiberg, Bruce Kindsley
Herskovitz, Stephen R.
Higgs, Gregory D.
Hillman, Charles
Hollander, Jane
Howe, Nancy
Jayne, Richard
Joyce, Kevin
Kafka, Robert M., Jr.
Kane, Robert S.
Kanter, Judith

Kapelas, William S.
Karpen, Joseph
Karpen, Seth
Klips, Stephen A.
Knowles, Kenneth E. III
Korwan, Mark
Korwan, Mark
Korwan, William S.
Kraemer, Kerry A.
Kronenberg, Fredi
Krumenauer, Jimmy
Krumenauer, Susan
Lederberg, Michael
Levin, Stephanie
Levine, Daniel A.
Lionel, Danny
Luria, Daniel D.
Mack, Stephen
Mankin, Eric
Mankin, Joan
Marasco, Frank
Marks, David
McGuire, Leslie S.
Maramorosch, Lydia
Mitchell, Janet E.
Muller, Eugene A., Jr.
Muren, Peter L.
Murray, Charlotte
Murray, Neil
Nash, Nicholas
Nichols, Clayton W., III
Novak, Michael
Noyes, James
Nunez, Adeline T.
Olson, Gregory
O'Neill, George, Jr.
Peck, Charles S.
Pepper, Francis
Pepper, Richard
Pittis, Milicent
Porterfield, Mary
Porterfield, Sarah
Powers, Francis C., Jr.,
Rankin, Bruce A.
Rankin, Douglas C.
Raymon, Elyn
Raymon, Mark
Read, Barbara
Read, Curtis S., Jr.
Rink, Douglas
Robertson, John
Robertson, William
Roosevelt, Susan
Rousmaniere, David
Schaeffer, Colin
Schaeffer, David
Schaeffer, Deborah
Seavers, Jon
Seitz, Karl
Seitz, Ronald
Simons, Lavinia
Sinclair, Thomas
Sloane, David J.
Smith, Linda
Spahn, David
Springer, Richard N.
Steinberg, Alan
Stier, Frances
Sundgaard, Jeremy
Takami, Bruce C.
Tatz, Vicki
von Glahn, Richard G., Jr.
Vuckovic, Jill
Wagner, Gay
Wagner, Glenn A.
Wallace, Mary Louise
Wallace, Peter
Warren, Contance
Warren, Virginia
Waters, Dennis P.
Wechsler, Douglas
Weissman, Jo
Witkin, Joseph
Youngwirth, Stephen A.
Zimmerman, Alan
Zoller, Thomas R.

## August 5-23

Baker, Deborah
*Blecher, Robin
Bourdelle, Stephanie R.
Bourdelle, Peter A.
Bryan, Bobby
Bushell, Gordon J.
Cook, Frank
Du Brul, Karen
*Elliott, Robert
Gauvin, Alan
*Gould, Arnold
Greene, Peter
Gulick, William
*Harris, Jean
Houghton, E. David
*Kane, Robert
Karpen, Daniel N.
*Knowles, Kenneth E.,III
Laine, Donald
Nash, Nicholas
*O'Neill, George, Jr.
Robinson, Jac W.
Robinson, Jon R.
Rowe, David
Schapiro, Jon
Seaholm, Carl G., Jr.
Tuttle, Christopher N.
*Warren, Constance
*Warren, Virginia G.
*Wechsler, Douglas
*Witkin, Joseph

*Repeaters from July course.

# WORKSHOP IN NATURE STUDY

June 24-July 5, 1957

Instructors: Marvin J. Rosenberg, Department of Science, Northport High School, Northport, N.Y.

Jill A. Lamoureux, Port Washington, N.Y.

The Workshop in Nature Study was offered for the second year in 1957. It is designed primarily for teachers of the elementary grades, and, upon successful completetion of the course students are awarded two in-service credits, as authorized by The State Education Department, Division of Teacher Education.

The Workshop was conducted for the two weeks from June 24 to July 5—Monday through Friday, with sessions from 9:00 a.m. to 12:00 noon, and from 1:00 to 3:00 p.m. There was one early morning bird walk. The morning sessions were devoted to field trips to the various types of natural habitats on Long Island, and the afternoon sessions to lectures, demonstrations, discussions, examination of field collections and project work.

The chief objective of the Workshop is to acquaint the teachers with the natural environment of Long Island, and to give background information and experience in nature study to help them in their teaching careers. The principal method which was used to attain this objective was to visit and thoroughly explore a variety of ecological habitat situations. In each situation the teachers collected specimens of animals and plants for identification and study. Various techniques for incorporating nature study in the curriculum were emphasized. Information on organization of field trips for class groups was presented. Each student kept a nature log for the duration of the course and prepared a term project. Project reports and demonstrations were given on the last day of the course.

An all-day trip was taken to Jones Beach, the Bird Sanctuary at Tobay Beach and Captree Beach. Certificates were awarded on the fifth of July to the following fourteen teachers who were enrolled in the course:

Beam, Mildred E.
Briggs, Florence E.
Butz, Patricia J.
Cady, Kenneth
Diggory, Marjorie B.
Gehde, Janet Reid
Gerard, Carol
Izzo, Theresa
Myles, Doris M.
Orlando, Rose T.
Ricciotti, Joseph A.
Richards, Marion E.
Rustin, Helen M.
Skinner, Mildred C.

# SYMPOSIA PUBLICATIONS

| | | |
|---|---|---|
| *Vol. I | (1933) | Surface Phenomena, xii + 239 pp. |
| *Vol. II | (1934) | Growth, xii + 284 pp. |
| *Vol. III | (1935) | Photochemical Reactions, xvi + 359 pp. |
| *Vol. IV | (1936) | Excitations, xii + 376 pp. |
| *Vol. V | (1937) | Internal Secretions, xvi + 433 pp. |
| *Vol. VI | (1938) | Protein Chemistry, xiv + 395 pp. |
| *Vol. VII | (1939) | Biological Oxidations, xiv +463 pp. |
| *Vol. VIII | (1940) | Permeability and the Nature of Cell Membranes, xii + 283 pp. |
| Vol. IX | (1941) | Genes and Chromosomes, x + 315 pp. |
| *Vol. X | (1942) | The Relation of Hormones to Development, xii + 167 pp. |
| *Vol. XI | (1946) | Heredity and Variation in Microorganisms, xii + 314 pp. |
| *Vol. XII | (1947) | Nucleic Acids and Nucleoproteins, xii + 279 pp. |
| Vol. XIII | (1948) | Biological Applications of Tracer Elements, xii + 222 pp. |
| Vol. XIV | (1949) | Amino Acids and Proteins, xii + 217 pp. |
| Vol. XV | (1950) | Origin and Evolution of Man, xii + 425 pp. |
| Vol. XVI | (1951) | Genes and Mutations, xvi + 521 pp. |
| Vol. XVII | (1952) | The Neuron, xiv + 323 pp. |
| Vol. XVIII | (1953) | Viruses, xvi + 301 pp. |
| Vol. XIX | (1954) | The Mammalian Fetus: physiological aspects of development, xii + 225 pp. |
| Vol. XX | (1955) | Population Genetics: the nature and causes of genetic variability in populations, xvi + 346 pp. |
| Vol. XXI | (1956) | Genetic Mechanisms: structure and function, xviii + 392 pp. |
| Vol. XXII | (1957) | Population Studies: animal ecology and demography, xiv + 437 pp. |
| Vol. XXIII | (1958) | Exchange of Genetic Material: mechanisms and consequences (in press) |

*Out of print.

# LABORATORY PERSONNEL

Abramson, Harold A.—Research Psychiatrist
Baron, Myrna—Research Assistant
*Barratt, Raymond—Instructor, Fungi Course
*Beaubian, Wendell—Dining Hall Manager
†Bennett, James, Jr.—Maintenance Man
†Borsching, Arlene—Typist
†Branton, Geneva—Technical Assistant
*Burtch, Ethel—Typist
†Colistra, Claudia—Technical Assistant
Coyne, Mary—Technical Assistant
Demerec, M.—Director
*De Seta, Maxine—Technical Assistant
Diglio, Dominick—Gardener
†Englesberg, Ellis—Bacteriologist
Franzese, Eleanor—Business Manager
*Frazzetta, Thomas—Research Assistant
Fremont-Smith, Nicholas—Research Assistant
†Friedman, Sheila—Research Assistant
Gardner, Henry—Maintenance Man
Gillies, Gloria—Research Assistant
Grein, Patricia—Stenographer
†Hadden, Joanna—Research Assistant
*Heck, Otto—Nature Study Course Instructor
Hollely, Dorothy—Research Assistant
*Hotchkiss, Paul—Technical Assistant
*Huckman, Michael—Technical Assistant
Hyde, Olive—Administrative Assistant
†Ingraham, Laura—Research Assistant
†Isenberg, Alice—Technical Assistant
*Käfer, Etta—Instructor, Fungi Course
†Kennard, John F.—Gardener
*Kent, Joan—Research Assistant
King, James C.—Geneticist
*Lamoureux, Jill—Nature Study Course Instructor
*Luria, S. E.—Collaborator, Bacterial Viruses Course
Madden, Carol V.—Research Assistant
Matson, Joan A.—Stenographer
McMullen, Ellen—Research Assistant
Meissner, Richard—Superintendent of Buildings and Grounds
Moser, Hermann—Geneticist
Neviackas, Gwendolyn—Stenographer
*Page, Gilbert—Maintenance Man
Reddy, William F.—Maintenance Man
Rolfe, William—Research Assistant

*Rosenberg, Marvin—Nature Study Course Instructor
*Ross, Richard—Technical Assistant
Sams, Joan—Research Assistant
*Skaar, Palmer D.—Geneticist
Sklarofsky, Bernard—Psychobiologist
Sokoloff, Alexander—Geneticist
†Sokoloff, Olga—Research Assistant
Stache, Jean—Secretary
*Streisinger, George—Instructor, Bacterial Viruses Course
†Thurston, Robert K.—Superintendent of Buildings and Grounds
Tomizawa, Keiko—Research Assistant
Wallace, Bruce—Geneticist; Assistant Director
Warren, Katherine Brehme—Executive Editor of Symposia; author "Mutants of D. melanogaster."
*Wickersham, Cornelius W., III—Research Assistant
Zerfass, Arthur—Mechanic
*Zots, Barbara—Technical Assistant

*Summer or temporary.

†Resigned during the year.

# SUMMER RESEARCH INVESTIGATORS

Bernheimer, Alan W.—New York University College of Medicine, New York, N.Y.
Calef, Enrico—Instituto di Genetica, Pavia, Italy and University of Illinois, Urbana, Ill.
Entner, Nathan—New York University College of Medicine, New York, N.Y.
Evans, Audrey H.—The Rockefeller Institute for Medical Research, New York, N.Y.
Fraser, Dorothy K.—University of Illinois, Urbana, Ill.
Granick, Sam—The Rockefeller Institute for Medical Research, New York, N.Y.
Hamilton, Leonard—Sloan-Kettering Institute for Cancer Research, New York, N.Y.
Hotchkiss, Rollin—The Rockefeller Institute for Medical Research, New York, N.Y.
Luria, S. E.—University of Illinois, Urbana, Ill.
Maramorosch, Karl—The Rockefeller Institute for Medical Research, New York, N.Y.
Melechen, Norman—St. Louis University School of Medicine, St. Louis, Mo.
Novick, Aaron—University of Chicago, Chicago, Ill.
Schwarz, Nina—University of Illinois, Urbana, Ill.
Ting, Robert—University of Illinois, Urbana, Ill.
Uetake, H.—University of Illinois, Urbana, Ill.
Wasserman, F. E.—New York University College of Medicine, New York, N.Y.

# REPORT OF THE SECRETARY

A meeting of the Executive Committee was held on Nov. 2, 1957 in the office of Dr. Demerec, with Dr. Demerec and five members of the committee present. The meeting was called by President Ames to consider the future policy of the Association. After full discussion, it was agreed that a special committee of the Board should be formed to meet with Dr. Caryl P. Haskins of the Carnegie Institution in order to formulate and activate the future policy of the Association.

The 34th annual meeting of the Long Island Biological Association was held in the Lecture Hall at Cold Spring Harbor on June 25, 1957. President Ames presided. The Secretary reviewed the chief acts of the Association during the previous year. The report of the Laboratory Director as published in the Annual Report and summarized by Dr. Demerec, and the reports of the Secretary, as given, were approved and filed. The Treasurer stated that his report would be in the forthcoming Annual Report. Mr. Ames presented the names of candidates for Board membership, on behalf of the Nominating Committee which consisted of Mr. Ames, Mrs. Page and Mr. Morris, as follows:

To serve until 1961: Dr. H. A. Abramson, Mr. Hoyt Ammidon, Mr. Duncan B. Cox, Dr. M. Demerec, Mr. Nevil Ford, Dr. Stuart Mudd and Dr. Robert Cushman Murphy. All were duly elected to the office.

At the 75th meeting of the Board of Directors, called on Nov. 3, 1957, President Ames and 14 other members of the Board were present. Dr. Demerec reported the acquisition of a new electron microscope through a grant received from the Public Health Service. He mentioned in particular the work of Dr. Sokoloff, and Dr. C. Oshima, comparative newcomers to our scientific staff; and spoke of the plans for the 1958 Symposium and the courses which the Laboratory will give during the summer of that year. In conclusion, he expressed the opinion that the Laboratory is well organized and well integrated, but that its financial structure and future policies should be reviewed and reevaluated. After serious discussion, following a recommendation of the Executive Committee, a committee of nine was named to formulate the future policy of the Biological Laboratory, as follows: Mrs. Franklin, Nevil Ford, Howard Curtis, Lloyd Berkner, William Nichols, Arthur Page, Grinnell Morris, M. Demerec and Amyas Ames.

The 76th meeting of the Board of Directors was held on March 19, 1958, in New York City, with Mr. Walter H. Page and 14 members of the Board present. President Ames presented the report of the special Policy Committee which had been appointed at the November meeting of the Board. Discussion which followed disclosed no adverse opinion. The resignations of Amyas Ames as president, and Jane N. Page and Arthur W. Page, as directors, were accepted with regret. Mr. Walter H. Page

was elected as a director to fill the vacancy in the Class of 1962; and as President of the Association, and ex-officio, as Chairman of the Board or Directors and of the Executive Committee.

The office of Chairman of the Association as defined in the Executive Committee report was created; and Mr. Nevil Ford elected to that position. Mr. Ford and Dr. H. J. Curtis were elected to the Executive Committee.

A meeting of the Executive Committee was held on March 31, 1958, in the Lecture Hall, with Dr. Demerec and five members of the Committee present. The purpose of the meeting was to discuss a proposed new laboratory building. Dr. Demerec estimated the required size of such a building. The Executive Committee authorized the expenditure of such funds for architects' fees as might be required for preliminary sketches, and the names of several architectural firms were suggested for consideration. The budget for the forthcoming year was discussed and approved. The Finance Committee for the year was appointed as follows: Grinnell Morris, Chairman, Hoyt Ammidon and Walter Page.

E. C. MacDowell, Secretary
Long Island Biological Association

# REPORT OF THE TREASURER

## AUDIT CERTIFICATE

MAIN AND COMPANY
Certified Public Accountants
233 Broadway
New York 7, N.Y.

Long Island Biological Association,
Cold Spring Harbor, L.I., N.Y.

Gentlemen:

We have examined the balance sheet of the Long Island Biological Association as of April 30, 1958 and the related statements of income and expense and net worth for the year then ended. Our examination was made in accordance with generally accepted auditing standards, and accordingly included such tests of the accounting records and such other auditing procedures as we considered necessary in the circumstances.

In our opinion, the accompanying balance sheet, statements of income and expense and net worth, and supporting schedules present fairly the position of the Long Island Biological Association at April 30, 1958 and the results of its operations for the year ended on that date in conformity with generally accepted accounting principles applied on a basis consistent with that of the preceding year.

MAIN AND COMPANY
Certified Public Accountants

New York, N.Y.
May 29, 1958

# BALANCE SHEET

## April 30, 1958

## ASSETS

### General and Endowment Fund

| | | | | |
|---|---|---|---|---|
| Cash | | | $ 31,121.09 | |
| Investments (market value $108,038.27) | | | 104,347.37 | |
| Accounts receivable: | | | | |
| On grants and contracts for special research | | $5,407.94 | | |
| Other | $6,542.03 | | | |
| Less: Reserve for uncollectible accounts | 1,180.29 | 5,361.74 | 10,769.68 | |
| Inventory of books | | | 16,674.15 | |
| Deferred expenses | | | 1,016.66 | |
| Land, buildings and equipment | | | 283,050.30 | $446,979.25 |

### Special Funds

| | | | | |
|---|---|---|---|---|
| Cash in bank | | | $ 1,537.02 | |
| Investments (market value $15,470.26) | | | 15,708.00 | 17,245.02 |
| Total | | | | $464,224.27 |

## LIABILITIES AND NET WORTH

| | | | |
|---|---|---|---|
| **General and Endowment Fund** | | | |
| Liabilities: | | | |
| Accounts payable | | $ 3,781.47 | |
| Accured payroll and taxes | | 1,524.48 | |
| Grants and contracts for special research | | 84,212.75 | |
| **Total liabilities** | | $ 89,518.70 | |
| Deferred income | | 1,260.00 | |
| Reserve for scientific research | | 20,000.00 | |
| Endowment Fund: | | | |
| Dr. William J. Matheson Bequest | | 20,000.00 | |
| Net worth | | 316,200.55 | $446,979.25 |
| **Special Funds** | | | |
| Blackford Memorial Fund: | | | |
| Principal | | $ 5,000.00 | |
| Charles Benedict Davenport Memorial Fund: | | | |
| Principal | $4,934.75 | | |
| Unexpended income | 1,339.93 | 6,274.68 | |
| Charles Benedict Davenport, Junior, Fund: | | | |
| Principal | | 1,037.12 | |
| Temple Prime Scholarship Fund: | | | |
| Principal | $2,500.00 | | |
| Unexpended income | 73.20 | 2,573.20 | |
| Dorothy Frances Rice Fund: | | | |
| Principal | $2,314.96 | | |
| Unexpended income | 45.06 | 2,360.02 | 17,245.02 |
| **Total** | | | $464,224.27 |

## STATEMENT OF NET WORTH
For the Year Ended April 30, 1958

| | | |
|---|---|---|
| Balance, May 1, 1957 | | $318,939.92 |
| **Add:** Excess of income over expense for year ended April 30, 1958 | | 8,848.69 |
| | | $327,788.61 |
| **Deduct:** Depreciation on equipment for year | | 11,588.06 |
| Balance, April 30, 1958 | | $316,200.55 |

# STATEMENT OF GENERAL FUND INVESTMENTS

April 30, 1958

| No. of Shares or Par Value | | Book Value | Market or Redemption Value |
|---|---|---|---|
| 12 | American Telephone and Telegraph Company | $ 1,759.19 | $ 2,109.00 |
| $ 2,000 | Dow Chemical Company Convertible Debenture Bonds, 3% due 7/1/82 | 2,062.50 | 2,600.00 |
| 22 | General Motors Corporation | 995.50 | 825.00 |
| 50 | General Motors Corporation, $5.00 Preferred Stock | 5,012.50 | 5,812.50 |
| $ 5,000 | General Motors Acceptance Corporation Debentures, 3½% due 3/15/72 | 4,937.50 | 4,931.25 |
| $ 5,000 | General Telephone Corporation, 15-Year Convertible Debentures, 4% due 5/1/ 71 | 5,287.50 | 5,337.50 |
| 21 | Merck and Company, Inc. $4.00 Second Cumulative Convertible Preferred Stock | 2,443.88 | 2,982.00 |
| 124 | Phillips Petroleum Company | 4,420.49 | 4,851.50 |
| $ 5,000 | Phillips Petroleum Company, Convertible Subordinated Debentures, 4¼% due 2/15/87 | 5,412.50 | 5,525.00 |
| 30 | Tennessee Gas Transmission Company | 709.85 | 840.00 |
| $ 5,000 | Twelve Federal Land Banks Consolidated Federal Farm Loan Bonds, 2¾% due 5/1/58 | 4,937.50 | 5,000.00 |
| $ 5,000 | Twelve Federal Land Banks Consolidated Federal Farm Loan Bonds, 2¼% due 5/1/59 | 4,834.38 | 5,028.12 |
| 37 | United States Steel Corporation | 2,048.88 | 2,247.75 |
| $30,000 | United States Treasury Bills, due 5/1/58 | 29,832.90 | 30,000.00 |
| $10,000 | United States Treasury Bills, due 6/5/58 | 9,965.80 | 9,988.50 |
| $ 5,000 | United States Treasury Bonds, 2¼% due 12/15/62-59 | 4,906.25 | 4,987.50 |
| $ 5,000 | United States Treasury Bonds, 2½% due 6/15/72-67 | 5,018.75 | 4,800.00 |
| $10,000 | United States Treasury Bonds, 2½% due 11/15/61 | 9,687.50 | 10,081.25 |
| $ 100* | United States Savings Bond, Series F, due 9/1/60 | 74.00 | 91.40 |
| | Totals | 104,347.37 | 108,038.27 |

*Maturity Value

## STATEMENT OF SPECIAL FUND INVESTMENTS

April 30, 1958

| Maturity Value | | Book Value | Market or Redemption Value |
|---|---|---|---|
| $ 500* | United States Treasury Bond, 2½%, due 3/15/70-65 | $ 500.00 | $ 481.56 |
| 100 | United States Savings Bond, Series J, due 9/1/68 | 72.00 | 73.50 |
| 1,400 | United States Savings Bond, Series F, due 9/1/60 | 1,036.00 | 1,279.60 |
| 14,000 | United States Savings Bonds, Series K, 2.76%, due 9/1/66 | 14,000.00 | 13,538.00 |
| 100 | United States Savings Bond, 2½% Series G, due 9/1/60 | 100.00 | 97.60 |
| | **Totals** | $15,708.00 | $15,470.26 |

*Par Value

## LAND, BUILDINGS AND EQUIPMENT
April 30, 1958

| | | | |
|---|---|---|---|
| Land: | | | |
| Purchased with funds raised through public subscription | | $52,198.22 | |
| Land purchased from Estate of Mary E. Jones | | 15,674.99 | |
| Henry W. de Forest land | | 12,000.00 | |
| Airslie land | | 5,000.00 | $ 84,873.21 |
| Improvements to land: | | | |
| Pipe line | | $ 1,860.39 | |
| Road | | 746.64 | |
| Light and telephone poles | | 290.98 | 2,898.01 |
| Buildings: | | | |
| Airslie building | | $ 5,000.00 | |
| Blackford Hall* | | 19,000.00 | |
| Cole Cottage | | 3,851.75 | |
| Davenport Laboratory | | 8,500.00 | |
| Henry W. de Forest building | | 15,000.00 | |
| Reginald G. Harris House | | 8,500.00 | |
| Dr. Walter B. James Laboratory | | 13,500.00 | |
| George L. Nichols Memorial Laboratory | | 13,700.00 | |
| Arthur W. Page building | | 18,186.55 | |
| Williams House | | 11,300.00 | |
| Urey Cottage | | 2,660.00 | |
| Machine shop and garage | | 3,204.64 | 122,402.94 |
| Land and buildings leased from Wawepex Society under lease expiring in 1979: | | | |
| Land | | $13,500.00 | |
| Buildings: | | | |
| Hooper House | $13,200.00 | | |
| Jones Laboratory | 10,000.00 | | |
| Osterhout Cottage | 5,500.00 | | |
| Wawepex Laboratory | 7,500.00 | 36,200.00 | 49,700.00 |
| Equipment: | | | |
| General | | $38,577.27 | |
| Biophysics | | 16,849.90 | |
| Physiology | | 2,513.15 | |
| | | $57,940.32 | |
| Less: Reserve for depreciation of equipment | | 34,764.18 | 23,176.14 |
| **Total** | | | $283,050.30 |

*Built on land leased from Wawepex Society.

## STATEMENT OF INCOME AND EXPENSE
For the Year Ended April 30, 1958

| | | |
|---|---|---|
| **Income:** | | |
| Grant. contracts and research fees | | $205,610.64 |
| Contributions: | | |
| Dues | $ 8,840.41 | |
| Wawepex Society | 2,100.00 | |
| John D. Jones Scholarship | 900.00 | |
| Walter B. James Fund | 180.00 | 12,020.41 |
| Book sales—Symposium of Quantitative Biology | | 19,304.00 |
| Dining Hall | | 13,989.55 |
| Rooms and apartments | | 17,073.13 |
| Summer activities | | 11,069.50 |
| Registration fees for annual symposium | | 121.00 |
| Interest and dividends on investments | | 2,629.76 |
| Miscellaneous | | 90.00 |
| **Total income** | | $281,907.99 |
| **Expense:** | | |
| Expenditures directly chargeable against grants and contracts for special research: | | |
| Salaries | $93,699.00 | |
| Supplies and equipment | 50,497.79 | |
| Expense of participants in annual symposium and related costs | 27,582.02 | |
| Portion of cost of printing book on annual symposium | 5,117.98 | |
| Other expenses | 11,892.75 | $188,789.54 |
| Cost of books sold and publication expense—Symposium of Quantitative Biology | | 5,586.97 |
| Dining hall | | 16,013.13 |
| Rooms and apartments | | 6,328.07 |
| Research expense | | 1,081.25 |
| Summer activities | | 7,077.19 |
| Distribution of John D. Jones Scholarship | | 920.00 |
| Buildings and grounds maintenance: | | |
| Salaries | $14,123.12 | |
| Materials and supplies | 7,539.32 | |
| Heat, light and water | 5,323.10 | 26,985.54 |
| General and administrative: | | |
| Salaries | $ 7,063.45 | |
| Insurance | 2,118.47 | |
| Printing and stationery | 956.67 | |
| Telephone, telegraph and postage | 826.55 | |
| Other | 2,908.57 | 13,873.71 |
| Loss on sale of securities | | 1,554.35 |
| Provision for obsolescence of inventory of books | | 4,849.55 |
| **Total expense** | | $273,059.30 |
| Excess of income over expense | | $ 8,848.69 |

# STATEMENT OF UNEXPENDED GRANTS AND CONTRACTS FOR SPECIAL RESEARCH

April 30, 1958

| | Authorized Amount | Amount Expended to April 30, 1958 (Including fees to Association) | Unexpended Balance April 30, 1958 |
|---|---|---|---|
| Josiah Macy, Jr. Foundation | | | |
| Grants expiring June 30, 1958 | $ 52,800.00 | $ 36,378.93 | $ 16,421.07 |
| Josiah Macy, Jr. Foundation | | | |
| Three year grant which expired September 1955 | 2,090.00 | 704.75 | 1,385.25 |
| National Science Foundation | | | |
| Grant for a period of two years expiring September 1957 | 19,800.00 | 18,417.20 | 1,382.80 |
| Eighteen month grant expiring July 31, 1958 | 12,500.00 | 10,316.64 | 2,183.36 |
| Grant for a period of two years expiring May 31, 1959 | 23,500.00 | 10,048.79 | 13,451.21 |
| Grant for a period of two years expiring September 30, 1959 | 9,500.00 | 1,861.03 | 7,638.97 |
| Special summer research grant for three years to June 30, 1958 | 15,000.00 | 14,512.13 | 487.87 |
| United States Atomic Energy Commission | | | |
| Two year contract expiring June 14, 1958 | 50,934.00 | 43,189.00 | 7,745.00 |
| One year contract expiring February 28, 1958 | 38,610.00 | 37,896.31 | 713.69 |
| Six month contract expiring August 31, 1958 | 9,450.00 | 3,486.10 | 5,963.90 |
| United States Public Health Service | | | |
| One year grant expiring August 31, 1958 | 34,247.00 | 32,149.78 | 2,097.22 |
| One year grant expiring December 31, 1958 | 18,070.00 | | 18,070.00 |
| Grants for annual symposia: | | | |
| Carnegie Corporation | 15,000.00 | 4,200.00 | 10,800.00 |
| The Rockefeller Foundation—Period of five years to January 31, 1963 | 25,000.00 | | 25,000.00 |
| United States Public Health Service | 5,750.00 | | 5,750.00 |
| National Science Foundation | 6,500.00 | | 6,500.00 |
| | $338,751.00 | $213,160.66 | $125,590.34 |

# ORGANIZATION OF THE ASSOCIATION

## OFFICERS

President
Walter H. Page

Chairman
Nevil Ford

Secretary
E. C. McDowell

Vice-President & Treasurer
Grinnell Morris

Assistant Secretary
B. P. Kaufman

Laboratory Director: M. Demerec

## BOARD OF DIRECTORS

To serve until 1962

Amyas Ames ..... Cold Spring Harbor, N.Y.
Th. Dobzhansky ..... Columbia University
Rollin D. Hotchkiss ..... Rockefeller Institute
Mrs. David Ingraham ..... Cold Spring Harbor, N.Y.
Walter H. Page ..... Cold Spring Harbor, N.Y.
Gerald Piel ..... New York, N.Y.
Richard Storrs ..... Oyster Bay, N.Y.

To serve until 1961

H. A. Abramson ..... Cold Spring Harbor, N.Y.
Hoyt Ammidon ..... Cold Spring Harbor, N.Y.
Duncan B. Cox ..... Oyster Bay, N.Y.
M. Demerec ..... Carnegie Institution
Nevil Ford ..... Cold Spring Harbor, N.Y.
Stuart Mudd ..... University of Pennsylvania
Robert Cushman Murphy ..... Setauket, N.Y.

To serve until 1960

Vernon Bryson ..... Rutgers University
Crispin Cooke ..... Huntington, N.Y.
Mrs. George S. Franklin ..... Cold Spring Harbor, N.Y.
E. C. MacDowell ..... Cold Spring Harbor, N.Y.
William B. Nichols ..... Syosset, N.Y.
Mrs. William S. Smoot ..... Syosset, N.Y.
B. H. Willier ..... Johns Hopkins University

To serve until 1959

Lloyd V. Berkner ..... Associated Universities
H. J. Curtis ..... Brookhaven National Laboratory
B. P. Kaufmann ..... Carnegie Institution
Jesse Knight, Jr. ..... Cold Spring Harbor, N.Y.
Robert V. Lindsay ..... Syosset, N.Y.
Grinnell Morris ..... Oyster Bay, N.Y.
Franz Schneider ..... Oyster Bay, N.Y.

Members Emeriti

George W. Corner ..... Rockefeller Institute
Ross G. Harrison ..... Yale University
R. C. Leffingwell ..... Oyster Bay, N.Y.

## EXECUTIVE COMMITTEE

Walter H. Page, Chairman

H. J. Curtis
Nevil Ford
Mrs. G. S. Franklin
E. C. MacDowell
Grinnell Morris
William B. Nichols

## FINANCE COMMITTEE

Grinnell Morris, Chairman

Hoyt Ammidon
Walter H. Page

## BUILDINGS AND GROUNDS

William B. Nichols, Chairman

B. P. Kaufmann
Franz Schneider

## SCIENTIFIC ADVISORY COMMITTEE

E. Caspari
L. C. Dunn
Edwin J. Grace
Alexander Hollander
Alfred E. Mirsky
E. C. McDowell

# FORMER PRESIDENTS, LABORATORY DIRECTORS, AND BOARD MEMBERS

## Presidents

Blackford, Eugene 1890-1904
Matheson, Wm. J. 1905-23
Blum, Edward C. 1923
Williams, T. S. 1924-26
James, Walter B. 1926-27
Page, Arthur W. 1927-40
Murphy, Robert Cushman 1940-52
Ames, Amyas 1953-58

## Laboratory Directors

Dean, Bashford 1880
Conn, Herbert W. 1891-98
Davenport, C. B. 1898-1924
Harris, Reginald 1924-36
Ponder, Eric 1936-40

## Directors

Abbott, Lyman 1896-1901
Adams, Mark H. 1951-56
Atkins, C. D. 1915-23
Ayer, J. C. 1930-33
Ayres, H. M. 1892-1900
Backus, T. J. 1890-1901
Blackford, Eugene 1890-1904
Blackford, Mrs. Eugene 1906-17
Bleecker, C. M. 1926-45
Bleecker, T. B. 1946-51
Blum, E. C. 1923
Boody, D. A. 1890-1917
Brackett, G. C. 1904-08
Brower, G. V. 1899-1917
Brown, Addison 1890-1913
Brown, J. S. 1908-17
Bumpus, H. C. 1903-12; 1927-30
Butler, N. M. 1903-17
Chambers, Robert 1932-54
Cochran, D. H. 1890-1902
Cole, K. S. 1940-43
Cole, W. H. 1934-52
Coombs, W. J. 1890-1910
Crittenden, W. H. 1922-23
Crozier, W. J. 1928-44
Davenport, C. B. 1903-44
Davenport, W. B. 1916-17
de Forest, H. W. 1912-17; 1924-25
de Forest, R. W. 1902-17
Denbigh, J. H. 1923
Detwiler, S. R. 1928-42
Doubleday, F. N. 1908-11
Draper, George 1924-43
Edey, Mrs. Maitland A. 1951-58
Field, Marshall 1924-47
Fisher, G. C. 1924
Fisk, H. D. 1924
Flinsch, Rudolph 1909-17
Francis, Mrs. L. W. 1923
Frick, Childs 1924-29
Gager, C. S. 1915-17
Hall, C. H. 1890-95
Harris, R. G. 1930-36
Harrison, R. G. 1926-51
Haskins, Caryl P. 1946-55
Healy, A. A. 1896-1921
Heckscher, August 1902-17
Hendrix, Joseph 1890-97
Hicks, Henry 1924-53
Hoagland, C. N. 1890-98
Hooper, F. W. 1890-1914
Hoyt, Colgate 1902-17
Hulst, G. D. 1894-1900
Huntington, L. D. 1894-1900
James, O. B. 1926-41
James, W. B. 1902-17; 1924-27

Jennings, H. S. 1924-27
Jennings, Walter 1906-17; 1924-33
Johnson, D. C. 1924
Jones, F. S. 1899-1909
Jones, J. D. 1890-95
Jones, O. L. 1890-1913
Jones, Mrs. O. L. 1907
Jones, W. E. 1903-06
Kahn, Mrs. O. H. 1924
Leffingwell, R. C. 1924-32
Levermore, C. H. 1896
Lloyd-Smith, Wilton 1928-40
Low, Seth 1890-1902
Lucas, F. A. 1905-17
Lusk, Graham 1909-17
MacCracken, H. M. 1890-1905
Mather, Frederic 1890-1900
Matheson, W. J. 1901-22
Mayr, Ernst 1950-58
Mayer, A. G. 1903-17
Merle-Smith, Mrs. Van S. 1931-50
Mickleborough, John 1890-1917
Mills, D. H. 1926-52
Montant, A. P. 1902-09
Morgan, T. H. 1924-28
Newberry, J. S. 1890-93
Nichols, Acosta 1927-45
Nichols, J. W. T. 1910-17
Noyes, H. F. 1902-21
Osterhout, W. J. V. 1927-41
Overton, Frank 1924
Page, Arthur W. 1924-58
Page, Mrs. Walter H. 1950-58
Palmer, L. M. 1899-1913
Parshley, H. M. 1924-33
Peabody, Julian 1911-17
Perkins, A. C. 1890-92
Ponder, Eric 1937-41
Pratt, H. I. 1929-30
Prime, Cornelia 1909-17
Raymond, J. H. 1890-1900
Roosevelt, John K. 1927-1956
Rumsey, Mary H. 1924
Schiff, J. M. 1931-50
Schiff, M. L. 1924-31
Scott, Donald 1911-17
Seamans, C. W. 1906-15
Shapley, Harlow 1943-51
Smith, H. C. 1913-17
Stewart, J. H. J. 1893-1917; 1924-26
Stimson, H. L. 1925-36
Stockard, C. R. 1924-39
Stoddard, Howland B. 1951-55
Stratford, William 1890-1917
Straubenmuller, Gustav 1911-17
Strauss, Albert 1914-17
Stutzer, Herman 1911-23
Swingle, W. W. 1924-44
Taylor, H. C. 1926-42
Thompson, Edward 1903-17
Tiffany, L. C. 1892-1917
Urey, H. C. 1934-49
Vanderbilt, W. K. 1924-43
Walter, H. E. 1924-43
Webb, Alexander 1890-1902
Weld, F. M. 1914-17
Wetmore, C. W. 1902-07
White, Mrs. Alexander M., Jr. 1951-58
White, S. V. 1890-1905
Williams, T. S. 1910-30
Wilson, E. B. 1903-17
Wood, Willis D. 1926-52
Woodbridge, C. L. 1894-1901
Woodward, J. B. 1890-96
Woodward, R. B. 1890-1914

## FOUNDERS

Contributions of at least $5,000 in money or property

Carnegie Corporation
Mrs. Ethel Clyde
Mrs. Henry W. de Forest
Mrs. Leonard Elmhirst
Marshall Field
Russell C. Leffingwell
John & Mary Markle Foundation
Mrs. Van Santvoord Merle-Smith
Arthur W. Page
Rockefeller Foundation
John M. Schiff
Wawepex Society

## PATRONS

Contributions of at least $500

Amyas Ames
Miss Rosina Boardman
W. R. Coe
John W. Davis
W. E. Erhart
Mrs. George S. Franklin
Childs Frick
Hugo Fricke
Princess Andrew Gagarin
E. J. Grace
Mr. and Mrs. R. Graham Heiner
Alfred Ephriam Kornfeld
Gerald M. Livingston
Mrs. Wilton Lloyd-Smith
Mrs. George Nichols
Herbert L. Pratt
Victor Rakowsky
John K. Roosevelt
Walter J. Salmon
Carl J. Schmidlapp
Franz Schneider
Donald Scott
Howard C. Smith
Henry C. Taylor
William C. Whitney Foundation
George Whitney
Mrs. Willis D. Wood

## SUSTAINING MEMBERS

H. A. Abramson
W. H. Alston
Amyas Ames
Charles E. Ames
Mrs. Charles E. Ames
Hoyt Ammidon
Mrs. Hoyt Ammidon
Mrs. Henry H. Anderson
Donald Arthur, Jr.
Mrs. Donald Arthur, Jr.
Mrs. Paul Atkins
Richard F. Babcock
Mrs. Daniel Bacon
Edmund Bartlett
Mrs. Edmund Bartlett
E. Farrar Bateson
Mrs. E. Farrar Bateson
Lloyd V. Berkner
Frederick Bernheim
Alan Bernheimer
Sydney Bevin
Nicholas Biddle
Mrs. Nicholas Biddle
Russell W. Billman
Edward S. Blagden
Mrs. Edward S. Blagden
B. DeWitt Bleecker
Mrs. B. DeWitt Bleecker
Bache Bleecker
Lyman C. Bleecker
Mrs. T. Bache Bleecker
Kenneth Boardman
Mrs. Kenneth Boardman
Rosina C. Boardman
Dietrich Bodenstein
Mrs. Herbert Bodman
L. H. Bonn
George T. Bowdoin
Mrs. George E. Brower
Vernon Bryson
Louis H. Buck
Mrs. Louis H. Buck
Dean Burk
Frank Bursi
Mrs. Trowbridge Callaway
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E. W. Caspari
Frank L. Clough
Mrs. Henry E. Coe
William Rodgers Coe, Jr.
Mrs. William Rodgers Coe, Jr.
Robert B. Colgate
Crispin Cooke
George W. Corner
Duncan Cox
Mrs. Duncan Cox
John L. Cox, 2nd
Clinton H. Crane
Miner D. Crary, Jr.
Mrs. Miner D. Crary, Jr.
Arthur M. Crocker
George A. Crocker
R. L. Cummings, Jr.
Howard J. Curtis
Paul Cushman
Mrs. Paul Cushman
F. Trubee Davison
Mrs. F. Trubee Davison
Henry P. Davison, Jr.
Mrs. Henry P. Davison
Raymond de Clairville
Mrs. Raymond de Clairville
Mrs. Henry W. de Forest
Robert F. de Graff
M. Demerec
Mrs. M. Demerec
Mrs. William Denby
Dr. Richard Derby
Mrs. Richard Derby
Mrs. Alvin Devereux
Russell E. Duvernoy
Jackson A. Dykman
Mrs. Jackson A. Dykman
Walter K. Earle
Mrs. Walter K. Earle
Ferdinand Eberstadt
Mrs. Ferdinand Eberstadt
Mrs. Maitland A. Edey

Joseph R. Eggert, Jr.
George L. Fair
Mrs. B. Tappen Fairchild
J. Douglas Fairchild
Mrs. J. Douglas Fairchild
Ugo Fano
M. H. Farnham
William M. Farrell
Ernst Fischer
W. Allston Flagg
Mrs. W. Allston Flagg
George H. Fonde
Dr. Alexander Forbes
Nevil Ford
Mrs. Nevil Ford
Mrs. George S. Franklin
George S. Franklin, Jr.
Childs Frick
A. H. Funnell
Clarence E. Galston
Mrs. Clarence E. Galston
Chauncey B. Garver
Mrs. William C. Gay
Mrs. E. S. Gilman
Mrs. Robert N. Gilmore
H. Bentley Glass
Gustave Goldstein
Joseph Gots
H. A. Gottfried
Edwin J. Grace
Charles V. Graham
E. K. Graves
Mrs. E. K. Graves
Margery Guerra
Arthur Gwynne
Mrs. Arthur Gwynne
Mrs. Joanna J. Hadden
Morris Hadley
Bruce Wood Hall
Mrs. Joseph W. Hambuechen
L. D. Hamilton
Paul L. Hammond
Mrs. Paul L. Hammond
Mrs. Montgomery Hare
Henry U. Harris
Augustin Hart, Jr.
Mrs. Augustin S. Hart, Jr.
Dr. Philip Hartman
Mrs. Philip Hartman
Caryl P. Haskins
Mrs. Caryl P. Haskins
Charles F. Havemeyer
Horace Havemeyer, Jr.
Ashton Hawkins
R. W. Hawkins
R. Graham Heiner
J. E. Hellier
Mrs. Robert L. Hoguet, Jr.
Alexander Hollaender
Davenport Hooker
Clarence A. Horn
Mrs. George S. Hornblower
William B. Hornblower
J. Taylor Howell
Mrs. J. Taylor Howell
David Ingraham
Mrs. David Ingraham
Francis I. Jacobs
Mrs. Henry James
Kenneth D. Jamieson
Mrs. Kenneth D. Jamieson
Norman D. Jamieson
Oliver B. Jennings
Mrs. Percy H. Jennings
Everett C. Jessup
Ward L. Johnson, Jr.
Mrs. Ward L. Johnson, Jr.
E. Elizabeth Jones
Robert Kafka
Mrs. Robert Kafka
Morris I. Karpen
B. P. Kaufmann
Francis C. Keil, Jr.
Mrs. Francis C. Keil, Jr.
James C. King
John P. Kipp
Mrs. John P. Kipp
Jesse Knight, Jr.
William A. Korwan
Ernest L. Lahr
Mrs. Ernest L. Lahr
Katherine E. Lascelle
Orin T. Leach
Mrs. Randall J. LeBoeuf, Jr.

Mrs. G. H. T. Le Boutillier
Mrs. Burton J. Lee, Sr.
Russell C. Leffingwell
Mrs. Russell C. Leffingwell
David A. Lindsay
Mrs. David A. Lindsay
George N. Lindsay, Jr.
Mrs. George N. Lindsay, Jr.
Vladimir S. Littauer
Mrs. Vladimir S. Littauer
W. E. Little
John Marshall Lockwood
William H. Long, Jr.
The Long-Islander
S. E. Luria
E. C. MacDowell
John F. MacKay
Karl Maramorosch
John B. Marsh
John M. Martin
William H. Mathers
Ernst Mayr
R. B. McAdoo
R. L. McCollom
Ross McFarland
Mrs. Ross McFarland
Mrs. Van S. Merle-Smith
Leo M. Meyer
Dudley L. Miller
Mrs. Dudley L. Miller
R. H. Mitchell
Mrs. Douglas M. Moffat
Walter V. Moffitt
Mrs. Louis deB. Moore
Grinnell Morris
Mrs. Grinnell Morris
Mrs. Ray Morris
Hermann Moser
Stuart Mudd
John R. Muma
Mrs. John R. Muma
Alfred E. Munier
Mrs. Alfred E. Munier
Robert Cushman Murphy
Winthrop Nelson
Mrs. Winthrop Nelson
H. H. Neumann
Mrs. George Nichols
John Treadwell Nichols
William B. Nichols
John W. Nields
Hawley M. Norins
Juliet Nourse
Aaron Novick
Charles P. Noyes
Mrs. Charles P. Noyes
Mrs. D. Chester Noyes
Mrs. D. Grinnell Noyes
Robert G. Olmsted
George D. O'Neill
Mrs. George D. O'Neill
A. W. Page, Jr.
Arthur W. Page
John H. Page
Mrs. Walter H. Page
Mrs.. Paul G. Pennoyer
Isabel Peters
William C. Pierce
Harold Pivnick
Mrs. Harold Pivnick
Collier Platt
Francis T. P. Plimpton
Mrs. Francis T. P. Plimpton
Mrs. Arthur W. Pope
Keith R. Porter
Mrs. Edward Everett Post
Francis C. Powers
Mrs. Francis C. Powers
Richard Mather Powers
Mrs. Charles Pratt
Mrs. Frederic R. Pratt
H. Irving Pratt
Mrs. H. Irving Pratt
Theodore H. Price
E. Racker
Mrs. Roland L. Redmond
Mrs. Lansing P. Reed
Mrs. Gordon Rentschler
Oscar W. Richards
R. Oliver Rippere
Harry C. Robb
Charles S. Robertson

Mrs. Charles S. Robertson
Archibald B. Roosevelt
Mrs. Archibald B. Roosevelt
George Emlen Roosevelt
John K. Roosevelt
Mrs. Philip J. Roosevelt
Walter N. Rothschild, Jr.
Mrs. Walter N. Rothschild, Jr.
Mrs. Stanley M. Rumbough
John Rutherfurd
Charles E. Saltzman
Mrs. Theodore F. Savage
Mrs. Cooper Schieffelin
John M. Schiff
Richard Schlaugies
Francis O. Schmitt
Franz Schneider
Frederick Schuelke
Mrs. Frederick Schuelke
Donald Scott, Jr.
Donald Seavey
Mrs. Donald Seavey
William S. Smoot
Mrs. William S. Smoot
Mrs. J. Barstow Smull
Edward P. Snyder
Marvin H. Soalt
Peter Solbert
Theodore E. Stebbins
Curt Stern
Mrs. M. Chase Stone
Mrs. Richard Storrs
Joseph S. Stout
Mrs. Joseph S. Stout
Arnold Sundgaard
Eric P. Swenson
Mrs. Eric P. Swenson
Waclaw Szybalski
Mrs. T. C. Takami
Eugene S. Taliaferro
Stanley Tarrant
Mrs. E. P. Taylor, Jr.
Mrs. Henry C. Taylor
Evan W. Thomas, II
Mrs. Evan W. Thomas, II
Norman Thomas
Irving A. Tittler
Mrs. Edmund S. Twining, Jr.
Norman M. Vaughn
Mrs. Martin Victor
T. R. Vohs
Mrs. Philip Wadsworth
Bruce Wallace
William J. Wardall
Charles Wardell, Jr.
Ethelbert Warfield
Bradford A. Warner
Mrs. Bradford A. Warner
Charles O. Warren
Katherine Brehme Warren
J. B. Watkins
Gordon J. Watt
Wawepex Society
Mrs. Percy S. Weeks
David Weld
Mrs. David Weld
Mrs. Francis M. Weld
Thomas C. Wheelock
Taggart Whipple
Mrs. John M. Whitaker
Mrs. Edward Whitcraft
Alexander M. White
Mrs. Alexander M. White
John C. White, Jr.
Mrs. John C. White, Jr.
C. W. Wickersham, Jr.
Alec Wilder
Douglas Williams
Mrs. Douglas Williams
B. H. Willier
William W. Willock, Jr.
Henry S. Wingate
Keyes Winter
David I. Wolsk
Guy W. Wood
W. Wilton Wood, Inc.
Mrs. Willis D. Wood
Charles F. Young
Mrs. Charles F. Young

# CERTIFICATE OF INCORPORATION

We, the undersigned, all being of full age, citizens of the United States and residents of the State of New York, desiring to form a membership corporation for the purposes hereinafter mentioned, pursuant to the Acts of the Legislature of the State of New York, do hereby CERTIFY AND DECLARE:

1. The name of the corporation shall be LONG ISLAND BIOLOGICAL ASSOCIATION, INC.

2. The principal office of the corporation shall be at Cold Spring Harbor, Town of Oyster Bay, in the State of New York; and the territory in which its operations are to be principally conducted is Long Island, New York and vicinity.

3. The purposes of the corporation shall be

(a) To establish and maintain an institution and laboratories for scientific research in biology and other kindred subjects.

(b) To conduct special investigations in agriculture, horticulture, the breeding of domestic animals, the promotion of oyster culture and marine fisheries, the protection and conservation of animal and plant life, zoology, botany and biology; and to diffuse information and give instruction relating thereto.

(c) To furnish specimens, materials and facilities for biological and medical investigation through the agency of other educational institutions, medical schools and hospitals, particularly in Greater New York.

(d) To take, hold and convey real property by grant, lease or otherwise for the purposes of the corporation.

(e) To take, hold and use for the purposes of the corporation moneys, securities or other property received by gift, bequest, membership dues, or otherwise.

(f) To act as Trustee of any Trust created for the carrying out of any or all the purposes of the corporation.

4. The number of Directors of the corporation shall be twenty (20) [amended to read "28", July 26, 1927.]

5. The names and places of residence of the persons who shall be Directors of the corporation until the first annual meeting of the members shall be as follows:

| Names | Residence |
|---|---|
| Charles B. Davenport | Oyster Bay, N.Y. |
| Henry W. De Forest | Oyster Bay, N.Y. |
| George Draper | New York City, N.Y. |
| G. Clyde Fisher | Douglaston, N.Y. |
| Harold D. Fish | Pittsburgh, Pa. |
| Marshall Field | Huntington, N.Y. |
| Henry Hicks | Westbury, N.Y. |
| Walter Jennings | Cold Spring Harbor, N.Y. |
| Walter B. James | Cold Spring Harbor, N.Y. |
| Duncan S. Johnson | Baltimore, Md. |
| Mrs. Addie Wolff Kahn | Cold Spring Harbor, N.Y. |
| Frank Overton | Patchogue, N.Y. |
| Howard M. Parshley | Northampton, Mass. |
| Mary H. Rumsey | Wheatley Hills, N.Y. |
| Wilbur W. Swingle | New Haven, Conn. |
| John H. J. Stewart | Oyster Bay, N.Y. |
| Mortimer L. Schiff | New York City, N.Y. |
| William K. Vanderbilt | New York City, N.Y. |
| Herbert E. Walter | Providence, R.I. |
| Timothy S. Williams | Huntington, N.Y. |

6. At the first annual meeting of the members of the corporation, or as soon thereafter as practicable, Directors shall be elected by the members of the corporation in four classes, of five Directors in each class, as follows: Directors of the first class who shall hold office for the term of one year; those of the second class for two years; those of the third class for three years; and those of the fourth class for four years. As the term of office of the Directors of each class shall expire, their successors shall be elected by the members of the corporation for a term of four years each.

7. A vacancy occurring in the office of any Director before the expiration of his term shall be filled by the remaining Directors for the remainder of the term.

8. The By-Laws of the corporation shall prescribe the qualifications for membership in the corporation, and may provide for the classification of members.

9. The annual meeting of the corporation shall be held at the office of the corporation upon such date as may be fixed by the By-Laws of the corporation (as amended July 28th, 1953).

10. Subject to action by the members, the Directors may adopt and amend the By-Laws of the corporation.

IN TESTIMONY WHEREOF, we have made and signed this Certificate in duplicate this thirteenth day of February, 1924.

| | |
|---|---|
| Walter Jennings | Cold Spring Harbor, N.Y. |
| Marshall Field | Huntington, N.Y. |
| Henry Hicks | Westbury, Nassau Co., N.Y. |
| Phoebe E. Hewlett Willets | Cold Spring Harbor, N.Y. |
| Charles B. Davenport | Cold Spring Harbor, N.Y. |
| Timothy S. Williams | Huntington, N.Y. |
| John L. Wells | Northport, N.Y. |
| A. F. Blakeslee | Cold Spring Harbor, N.Y. |
| John Chase | Cold Spring Harbor, N.Y. |

Approved by Norman S. Dike, a Justice of the Supreme Court of New York, February 15th.

Filing consented to by Frank P. Graves, President of the University of New York and Commissioner of Education, February 19.

Certificate of incorporation filed with the Secretary of State, at Albany, February 19, 1924.

# BY-LAWS

## ARTICLE I

### Members

Sec. 1. Any person may become a member of the Association, subject to ratification by the Board of Directors or Executive Committee, upon his or her enrollment in any one or two of the following classes:

Class I. Founders—consisting of those who by bequest or gift have each contributed to the capital funds of the Association at least $5,000 in money or property.

Class II. Patrons—consisting of those who have each contributed at least $500 to the Association but less than an amount sufficient to qualify them as founders.

Class III. Sustaining Members—those who contribute fixed annual sums for the support of the Association.

Members of all classes shall have equal voting powers.

## ARTICLE II

### Meetings

Sec. 1. The annual meeting of the members of the Association for the election of Directors and such other business as may be specified in the call, or as may be duly brought before the members, shall be held at the Biological Laboratory, Cold Spring Harbor, on the fourth Tuesday of June in each year.

Sec. 2. Special meetings of the members may be called by the Board of Directors, to be held at such time and place, and for such purposes, as shall be stated in the call, provided that at least one week's notice of such meeting be given to the members. A special meeting shall be called by the Board of Directors when requested in writing by at least thirty members.

Sec. 3. At both annual and special meetings ten members shall constitute a quorum. Each member present shall be entitled to one vote.

## ARTICLE III

### Directors

Sec. 1. The Board of Directors shall consist of twenty-eight members, who shall be elected as provided in the Certificate of Incorporation. Nine Directors shall constitute a quorum. The Board of Directors shall have general control and management of the affairs of the Association. It shall elect the officers and, except as otherwise herein provided, appoint all other employees, prescribe their duties and fix their compensation and tenure of employment. It shall choose annually an Executive Committee of seven Directors of whom the President shall be one. The Executive Committee shall have all the powers of the Board between meetings of

the Board and their acts and proceedings shall be reported to the Board at the next meeting thereof. Three members shall constitute a quorum of the Executive Committee.

Sec. 2. Stated meetings of the Board shall be held twice in each year at such place and time as may be stated in the call. Special meetings of the Board, and meetings of the Executive Committee, may be called by the President at any time upon reasonable notice, and shall be called by him upon the written request of any three Directors.

Sec. 3. A vacancy in the office of any Director before the expiration of his term shall be filled by the remaining Directors for the unexpired portion of the term.

Sec. 4. Any Director residing more than 50 miles from New York City shall be entitled to have reimbursed to him from the Association's treasury his necessary railroad fare incurred in attending meetings of the Board of Directors.

Sec. 5. Any Director who is engaged in administration, instruction or research may receive such salary as shall be voted to him from time to time by the Board.

## ARTICLE IV

### Officers

Sec. 1. The officers of the Association shall be: A President, a Chairman, 3 Vice Presidents (all of whom shall be Directors), a Secretary, an Assistant Secretary, a Treasurer, one or more Assistant Treasurers, and a Laboratory Director, each of whom shall be elected by the Board of Directors and shall hold office during the pleasure of the Board. Such additional officers may be appointed as the Directors may deem advisable.

Sec. 2. No officer shall receive compensation for his services except the Laboratory Director, whose salary shall be fixed by the Board of Directors.

## ARTICLE V

### Duties of Officers

Sec. 1. The President shall preside at all meetings of the Board of Directors and Executive Committee, and in the absence of the Chairman, at the annual and special meetings of the Association.

Sec. 2. The Chairman shall preside at all annual and special meetings of the Association and shall perform the duties of the President in case of the absence or inability of the President.

Sec. 3. The Vice Presidents shall perform the duties of President in order of their seniority in case of the absence or inability of the President and Chairman.

Sec. 4. The Secretary shall keep a list of the names and addresses of members, have the custody of the seal, the records and file of the

Association, give due notice of all meetings, keep the minutes of the Board of Directors and Executive Committee, and attach the seal, together with his signature, to all instruments requiring sealing which shall have been executed by the President or Vice President. The Assistant Secretary shall perform the duties of the Secretary in case of the absence or inability of the Secretary.

Sec. 5. The Treasurer shall receive, collect and hold, subject to the order of the Board, all dues, subscriptions, donations and other revenue of the Association. He shall deposit all funds, in the name and to the credit of the Association, in such financial institution or institutions as the Board of Directors may designate. He shall pay all salaries, make deposits to laboratory bank account as authorized in advance by the Board of Directors or Executive Committee, and shall pay all bills of the Association, shall keep proper books of account and shall make such reports from time to time as the Board of Directors or the Executive Committee may require. Drafts upon Association funds shall be by voucher checks bearing the signature of the Treasurer and the Laboratory Director or such other officer as the Board of Directors or the Executive Committee shall designate. Funds not required for current operations shall be invested and reinvested in such manner as the Board of Directors or Executive Committee shall determine.

Sec. 6. The Laboratory Director shall be responsible executive officer of the Association in matters pertaining to the immediate conduct of the Laboratory and such other of the activities of the Association as may be assigned him. He shall prepare and issue the general announcements, after their approval by the Board of Directors, provide for the needs of instructors, investigators and students at the Laboratory. He shall attend the meetings of the Board of Directors and Executive Committee and furnish them, from time to time, with information regarding the operations of the Laboratory and his other activities in connection with the Association. Subject to approval of the Board of Directors or Executive Committee he shall appoint instructors, lecturers, laboratory assistants and other laboratory employees. Prior to the beginning of each fiscal year he shall submit for approval to the Board of Directors or Executive Committee a budget of estimated expenditures and receipts for the coming year; and no expenditures in excess of those approved shall be incurred except with like approval. There shall be established a separate laboratory bank account in the name of the Director of the Laboratory. In this account shall be deposited such amounts as the Board of Directors or the Executive Committee shall determine and such additional moneys as may be received by the Laboratory Director on behalf of the Association. Drafts upon such account shall be made by voucher check signed by the Laboratory Director or, in emergency, by the Treasurer, or such other person as the Board may designate. All revenues of the Association received by the Laboratory Director shall be paid over to the Treasurer of the Association periodically. The Laboratory Director shall make reports

to the Board of Directors, or Executive Committee, from time to time of his receipts and disbursements.

## ARTICLE VI

### Seal

Sec. 1. The seal of the Association shall be in circular form with the name "Long Island Biological Association" in the circumference and the words "Corporate Seal" in the center.

## ARTICLE VII

### Amendments

Sec. 1. These By-Laws may be amended by a majority vote of those present at any regular meeting of the Board of Directors, provided notice of such proposed amendment has been given to each director at least two weeks in advance of such meeting, or without such notice they may be amended at any meeting of the Directors with the unanimous vote of those present. They may also be amended at the annual meeting of the members of the Association or at any special meeting thereof called for such purpose.

## ARTICLE VIII

### Women's Committee

Sec. 1. The Board of Directors of the Executive Committee may appoint annually a Women's Auxiliary Board, with such powers as may be prescribed from time to time. Such Auxiliary Board shall determine its own organization and cooperate with the Board of Directors in all matters relating to the welfare of the Association. Particularly it shall arrange for a visiting day at the Laboratory, for the formation of children's classes for nature study, for increasing the membership and funds of the Association and shall endeavor in all ways to promote the mutual interests of the Laboratory and the community. All funds received by the Auxiliary Board shall be paid over to the Treasurer of the Association.

## ARTICLE IX

### Conveyance of Land

Sec. 1. Pursuant to the provisions of Section 13 of the Membership Corporation Law, the Association may from time to time convey to a member of the Association a portion of any real property owned by the Association for the erection thereupon of a dwelling house.

Sec. 2. The Board of Directors is hereby authorized to convey real property for residence purposes to such members of the Association and to impose such restrictions in the covenants as in the opinion of the Board will result in a residential development attractive to scientists and promote the best interests of the Association.

June—1958

## A BEQUEST FOR THE BIOLOGICAL LABORATORY

The Biological Laboratory of the Long Island Biological Association, whose work and organization is described in this booklet, carries on basic research in biology; conducts annual international symposia on topics lying in interrelated fields of biology, chemistry, physics, and mathematics; makes laboratory facilities available to scientists from other institutions during the summers; offers special courses for research scientists, as well as a nature study course for young people of the community; and arranges technical lectures for scientists and nontechnical lectures for neighbors of the Laboratory.

At present the Association depends on the contributions of over 300 of its friends and neighbors for its primary support. To insure stability in the functioning of the Laboratory, the Association is anxious to build a fund to be used as a guaranty of the salaries of key scientists, and as an endowment for the Laboratory.

A bequest in your will to help us toward this goal would greatly help the work of our Association.

## FORM OF BEQUEST

I give and bequeath to the Long Island Biological Association the sum of ..... dollars to be applied to the uses and purposes of said Association.

SUSAN COOPER