

ANNUAL REPORT

OF

THE BIOLOGICAL LABORATORY

1956 - 1957

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-Seventh Year

1956-1957

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.

## TABLE OF CONTENTS

A Message from the President	5
Report of the Director	6
Reports of Laboratory Staff	16
Bibliography	46
Reports of Summer Investigators	48
Course on Genetics of Filamentous Fungi	56
Course on Bacteriophages	58
Course on Bacterial Genetics	60
Nature Study Course	62
Workshop in Nature Study	65
Symposia Publications	66
Laboratory Personnel	67
Summer Research Investigators	69
Report of the Secretary	70
Report of the Treasurer	72
Organization of the Association	80
Officers	80
Board of Directors	80
Committees	81
Former Officers and Board Members	82
Members	85
Certificate of Incorporation and By-Laws of the Association	89

## A MESSAGE FROM THE PRESIDENT

The work of the Biological Laboratory is focused on some of the most basic problems that face us in the world today.

One of our research groups is investigating the effects of atomic radiation on populations of flies, with particular attention to hereditary changes and the degree to which populations can resist the debilitating effect of these mutations. Eventually it is expected that these studies will have a bearing on problems facing mankind.

Another group is tracing the development and inheritance of resistance on the part of insects to DDT and still others are analyzing inheritance in bacterial populations. This work has already paid important dividends in better understanding not only of how to control pests, but of how medical men can best use the new miracle drugs.

Each year since 1933 we have held an international Symposium. One hundred and twenty-three scientists—thirty-four of them from countries around the world—met this year for ten days to discuss world population problems. Cold Spring Harbor is famous among scientists for this annual Symposium. We could perform no finer function, for, aside from the great scientific value, it is through such meetings of thoughtful men that international understanding and tolerance are furthered.

The Biological Laboratory has two major sources of income. We are backed in part by scientific foundations. But, having no endowment, we depend on the annual support of our neighbors and friends. It is their contributions which help to make this vital work possible.

AMYAS AMES, President

Long Island Biological Association

## REPORT OF THE DIRECTOR

If we wish to preserve our status at the general high level attained through many years of effort, our program must be flexible and readily adaptable to the new situations that continually arise in this changing world. For an institution like our Biological Laboratory, complacency and an unquestioning belief in its own rightness could be the most dangerous of pitfalls. A program of work that is perfect in today's circumstances may be out of place tomorrow, and to maintain our position it is necessary to try new things and follow new leads.

We are guided by this principle of flexibility in our year-to-year activities as well as in our long-term program. Our year-round research, summer research, advanced teaching, and nature study teaching are constantly being adapted to the needs disclosed by new developments. Ten years ago our staff research was limited to genetic studies utilizing microorganisms; today, in addition, we have a vigorous program of population studies. Our summer laboratories, on the other hand, were equipped only for research with *Drosophila* ten years ago, whereas now we have modern equipment available for microbial research. Our advanced teaching was then limited to one course, on methods used in research with bacterial viruses; but the teaching program has since been developed to include two others, on genetics of bacteria and of fungi, and it is closely coordinated with our research program. Ten years ago we had nature study courses for children, but today we have courses for grade school teachers as well. In addition, our Symposium meetings are becoming more and more truly international in scope.

Our long-term program, too, has undergone considerable adaptations during recent years. The collaborative arrangement with the Department of Genetics of the Carnegie Institution of Washington has helped to focus our research program on genetic problems, and to coordinate the summer research and teaching programs with our common research interests. Acquisitions of property along Bungtown Road, first through the generous gift of Mrs. Henry W. de Forest and later through purchases, have assured us of preserving a variety of natural environments for our studies, as well as the degree of seclusion that is important for our efficient functioning. Our relations with members of the community have received our constant attention; and, through activities promoted for members of the Long Island Biological Association, we are continuing to draw the interest of the public to our work and to science generally.

With the deepest regret I record the deaths of three former members of the Board of Directors, all of whom had a prominent part in the activities of our Association. Dr. Mark H. Adams, professor of bacteriology at the New York University College of Medicine, died on October 17, 1956. Dr. Adams came to the Biological Laboratory in the summer of 1946 to take the course on bacterial viruses, which was then being

given for the second year. In 1947 he cooperated in teaching the course, and for six years thereafter he was in full charge of it. He was elected a member of the Board of Directors in 1952. Mr. Marshall Field, who died on November 8, 1956, was a charter member of the Association, and for 23 years (1924-47) he served as vice-president, treasurer, and member of the Board of Directors. Mr. Willis D. Wood, who died on May 6, 1957, was also one of the original members of the Association, and a member of the Board of Directors for 26 years (1926-52).

## RESEARCH

The work of the Laboratory staff is carried on in three sections: Population Genetics, Microbial Genetics, and Psychobiology. During this report year the program of the Microbial Genetics section was expanded to include research in which human cells are grown in tissue cultures and studied like bacterial cells, with the object of developing methods for investigation of certain problems in human genetics. Dr. H. Moser is in charge of the new project, which is supported by a grant from the United States Atomic Energy Commission. Detailed progress reports of the work in each research section are presented later in this Report by the members in charge of the projects. Here I will summarize them briefly.

Bruce Wallace has been attempting to obtain experimental data that will enable him to estimate the importance of hybrid vigor, or heterosis, in determining the genetic structure of populations. He has approached the problem by constructing two alternative theoretical models, one based on the absence of heterosis within populations and the other on an extreme prevalence of heterozygote superiority. Although in many respects these models are similar, they lead to different expectations regarding the effects of new mutations on the viability of individuals that are homozygous for entire chromosomes. In the absence of heterosis, nearly all these new mutations are expected to be deleterious; if heterosis occurs commonly, some portion of the new mutations is expected to improve the viability of homozygous individuals. As described later, in his report, Dr. Wallace finds that a considerable proportion—apparently more than half—of these new mutations improves the viability of his homozygotes. This result indicates a role of heterosis within populations greatly exceeding that which is generally suspected. It indicates that hybrid vigor so commonly used by animal and plant breeders in the improvement of agricultural products is also an important factor within local populations of interbreeding organisms, possibly in human populations as well.

A five-year investigation of the development and inheritance of resistance to DDT in *Drosophila melanogaster* has been completed by James C. King. The study has produced a considerable mass of evidence that resistance in this species is produced by a large complex of different genetic factors. Up until a few months ago most of this evidence was indirect

and inferential; but a rather elaborate genetical experiment, carried out during the past year with two of the resistant lines, is now giving clear and unmistakable proof that the factors for resistance are distributed among all three of the larger chromosomes. It is possible to make twenty-seven different combinations of resistant and nonresistant chromosomes, and all these combinations for both lines have been produced and tested. The experimental work has been completed for both lines, but a factorial analysis of the results has been carried through for only one. This analysis gives very clear-cut answers. Each of the three chromosomes contributes an increment of resistance, but these increments are not equal. The X chromosome contributes least, the second chromosome most, the third an intermediate amount. There is no evidence of any interaction between chromosomes of different pairs. The X, for example, makes its proper contribution no matter what chromosomes constitute the other two pairs. Finally, there is no dominance. The heterozygote is intermediate between the two homozygotes; one chromosome of a pair contributes half as much resistance as two. There can be no question that resistance in the line analyzed is produced by a polygenic system. It remains to be seen whether the evidence will be the same or different for the second line.

Ellis Englesberg, working with bacteria, has continued the study of "gain mutations" in *Pasteurella pestis*, and has evidence of the presence of a feedback control mechanism in which an excess of a metabolite (methionine) acts as a signal, so to speak, to shut off or inhibit the synthesis of this compound by the cell. With *Salmonella typhimurium* he has concentrated on a study of diauxie, a phenomenon whereby a cell, when given two utilizable substrates, uses one of them first and then, when its supply is depleted, adapts to growth on the other. This phenomenon is being analyzed with the aid of recently isolated diauxie-resistant mutants. In collaboration with Dr. L. Baron, of the Walter Reed Army Institute of Research, Dr. Englesberg has undertaken a study of the possible linkage, in *Salmonella typhi*, of genes controlling the transport of rhamnose with those controlling the enzymes involved in rhamnose dissimilation.

P. D. Skaar has continued the analysis of the small region of the bacterial (*Escherichia coli*) chromosome that contains genes concerned with tryptophan synthesis. In some tryptophan mutants, two or more enzyme-producing capacities are lost simultaneously. In certain ones of these, the responsible genetic event appears to be a deletion; in others, it appears to be a revertible mutation. Dr. Skaar, in collaboration with Dr. A. Garen, has also completed a study which indicates that the transfer of genes between mating bacteria is closely paralleled by the transfer of metabolically stable phosphorus.

Recent advances in techniques for culture of mammalian tissues have made it possible to apply to genetical studies of single mammalian cells the same precise experimental approaches that are now used with many microorganisms. During the past year, Hermann Moser has undertaken



an investigation of spontaneous and induced genetic changes in human somatic cells grown in tissue cultures. He has established a tissue-culture laboratory, and has acquired experience in routine techniques of mammalian tissue culture and methods for clonal growth of single mammalian cells. At present he is trying to induce mutations involving biochemical and other cell functions, and to find conditions under which such mutations are phenotypically expressed and therefore detectable.

H. A. Abramson and his collaborators have continued their studies of the biological effects of LSD-25, a compound that affects the nervous system and, when administered in extremely small amounts, can produce in normal people states that resemble schizophrenia. Experiments made during the past year have shown that the effects of LSD-25 on the Siamese fighting fish (a very sensitive test object) can be partially blocked by beef brain extract. An unsuccessful effort has been made to determine what component of the extract is responsible for this action. A number of other substances have been tested for ability to block the LSD-25 reaction.

During the summer of 1956, visiting scientists were particularly active in research with microorganisms (bacterial viruses, bacteria, and fungi), a type of experimentation for which our laboratories are well equipped. A group from the University of Illinois, consisting of S. E. Luria, D. K. Fraser, and G. P. Kalle, studied lysogenization of *S. typhimurium* with phage P22 by isolation of single cells through micromanipulation (Fraser and Luria), and investigated the effect of thymine deficiency on the growth of *S. typhimurium* (Kalle). A. H. Doermann, M. C. Chase, E. I. Kataja, and R. S. Edgar, from the University of Rochester, studied linearity and additivity over very short intervals of the genetic map of phage T4 of *E. coli*, as well as the mechanism of recombinant formation in that organism. F. E. Wassermann, L. Berman, and F. F. Igalsohn, of New York University, investigated the kinetics of phage infection of *E. coli*, and the genetics of enzyme production in various phage mutants. J. D. Watson and R. W. Risebrough, of Harvard University, carried out preliminary experiments with a small phage of *E. coli*, in order to be able to use it for electron microscope studies and genetical analysis. A. Novick, of the University of Chicago, developed techniques for determination of the quantity of the enzyme beta-galactoside in a single *E. coli* bacterium. H. Roman, of the University of Washington, studied the genetics of adenine synthesis in yeast. C. Yanofsky, of Western Reserve University, investigated biochemical blocks in the synthesis of tryptophan by *Neurospora* mutants.

E. W. Caspari and I. Blomstrand, of Wesleyan University, carried on biochemical studies of various pigments formed in the moth *Ephestia* under the influence of certain genes. K. Maramorosch, of The Rockefeller Institute for Medical Research, investigated in maize the protection afforded a plant already infected by a certain strain of virus against infection by another strain. S. Granick, also of The Rockefeller Institute, made

a preliminary study of the fresh-water flagellate *Euglena*, to determine its usefulness for work on chlorophyll synthesis. A. W. Bernheimer, of New York University College of Medicine, tested the effect of streptomycin O on the isolated beating heart of the mouse, and also the response of tadpoles to injection with materials known to be antigenic in mammals.

### SYMPOSIUM

The twenty-second Cold Spring Harbor Symposium on Quantitative Biology was held for ten days, June 3rd to 12th, 1957. It was attended by about 150 scientists interested in the fields of animal ecology and demography, sciences which deal with numbers, age distributions, and sex ratios within animal and human populations. The purpose of the meetings was to bring together outstanding scientists of these two fields as well as geneticists, anthropologists, economists, and statisticians, in order that the experimental and analytical methods and conclusions of each group might be shared by the others.

The program was organized by a committee consisting of L. C. Birch, University of Sydney, Australia; Th. Dobzhansky, Columbia University; Frank Lorimer, American University, Washington, D.C.; and Bruce Wallace, the Biological Laboratory.

About forty of the participants came from foreign countries, including Australia, Finland, France, Great Britain, India, Italy, Japan, Netherlands, and Sweden, as well as Canada and South America. The Laboratory financed the conference with funds obtained from The Population Council, Inc., the Milbank Memorial Fund, the United States Atomic Energy Commission, the United States Public Health Service, the National Science Foundation, and the Carnegie Corporation of New York.

### TEACHING

The Nature Study Course was taught in the summer of 1956 by Dr. John A. Gustafson, of the State University Teachers College, Cortland, New York; Mr. Marvin Rosenberg, of Northport Central High School; and Mrs. Jill A. Lamoureux, of Port Washington. They were assisted by Mrs. Shayna Rosenberg and Miss Donna Granick. This course is designed to stimulate interest in nature among young people of the community, by improving their observation of the many animals and plants in their environment, by teaching them how to find the answers to questions raised by their observations, and by expanding their knowledge of natural phenomena. The children ranged in age from six to thirteen years, and were divided into six groups on the basis of age and experience. At the conclusion of the regular course, a second class was organized for the month of August by special request. In all 99 students attended the course.

A two-week course for elementary school teachers, Workshop in Nature Study, was offered for the first time in 1956. 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 13 teachers who successfully completed the requirements.

The three-week course in techniques and problems of research with bacterial viruses was held for the twelfth consecutive year, with Professor A. H. Doermann, University of Rochester, and Professor S. E. Luria, University of Illinois, as instructors. Eighteen students and one auditor were enrolled.

The course in bacterial genetics, in its seventh year, was conducted by Drs. M. Demerec, E. M. Witkin, V. Bryson, P. E. Hartman, and P. D. Skaar, in collaboration with Mr. E. L. Lahr and Dr. H. Moser. The enrollment in this course also was eighteen students and one auditor.

The same number of students, which represents top capacity for any of the Laboratory's advanced courses, took part in the new course on genetics of fungi, taught by Professor G. Pontecorvo and Dr. E. Käfer, from the University of Glasgow. Like the bacterial viruses and bacterial genetics courses, this one is designed for research workers and advanced graduate students, and it complements them by greatly extending the teaching program in genetics of microorganisms. It is being offered again in the summer of 1957, with Professor R. W. Barratt of Dartmouth College and Dr. E. Käfer, now of McGill University, as instructors.

## LECTURES

As part of the 1956 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 instructors' reports of the courses. In connection with the Nature Study Course, films were shown on several evenings to the students, their parents, and friends. Dr. Morris Schaeffer, of the United States Public Health Service in Washington, gave a travelogue lecture, illustrated with Kodachrome projections, on "Adventures in Bolivia, or Bring the Virus Back Alive."

During the 1957 Symposium, which was attended by scientists interested in population studies, the Laboratory collaborated with the Planned Parenthood Center of North Suffolk in organizing a public panel discussion on "World Population Problems." With Professor George J. Stolnitz of Indiana University as moderator, five panelists opened a discussion in which a number of the Symposium scientists and other members of the audience participated. The panel consisted of Professor L. L. Cavalli-Sforza, University of Parma, Italy; Dr. J. Hajnal, University of Manchester, England; Professor A. Kusakawa, Kyushu University, Japan; Dr. A. J. Nicholson, Commonwealth Scientific and Industrial Research Organization, Australia; and Dr. L. D. Sanghvi, Indian Cancer Research Center, Bombay, India. The meeting was held in the gymnasium of the West Side School, which was better suited than our own lecture hall to accommodate the

audience of more than 200 persons. Mrs. David A. Lindsay, of the Planned Parenthood Center, suggested this meeting and was active in its organization.

Our lecture hall was used by several local organizations during the year. The American Association of University Women sponsored a public lecture on Thursday evening, February 28, 1957, on the subject "Research in Abnormal Child Psychology," and on the afternoon of May 18, 1957 the Long Island Chapter of The Nature Conservancy held a meeting for local groups of Boy Scouts.

### SPECIAL EVENTS

On Sunday, September 23, 1956, 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 "Investment in Science," was given by Dr. B. P. Kaufmann, who described the genetic dangers associated with radiation exposure. The serving of tea and refreshments by members of the Women's Committee was efficiently organized by Mrs. Herbert Glasier.

### SCHOLARSHIPS

The funds available for scholarships in 1956 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—Nine students: Donald F. Bacon, University of Otago, New Zealand; Dr. Jack Nicol Coetzee, University of Pretoria, South Africa; Robert S. Edgar, University of Rochester, Rochester, N.Y.; Dr. Hans Martin, University of Texas, Austin, Texas; William S. Medart, St. Louis, Mo.; Haruo Ozeki, Carnegie Institution, Cold Spring Harbor, N.Y.; Takashi Yura, Yale University, New Haven, Conn.; Tetsuo Iino, University of Wisconsin, Madison, Wisc.; Dr. Etta Käfer, The University, Glasgow, Scotland.

Temple Prime Scholarship—Student: Donald M. Green, University of Rochester, Rochester, N.Y.

Dorothy Frances Rice Fund—Students: Constance Thomas, University of Wisconsin, Madison, Wisc.; Memory P. F. Elvin, University of Pennsylvania, Philadelphia, Penna.

Biological Laboratory Scholarships—Four research workers: Dr. K. Maramorosch, Rockefeller Institute for Medical Research, New York, N.Y.; Dr. Aaron Novick, University of Chicago, Chicago, Ill.; Dr. M.

C. Niu, Rockefeller Institute for Medical Research, New York, N.Y.; Dr. H. Roman, University of Washington, Seattle, Wash. Nine students: Donald F. Bacon, University of Otago, New Zealand; Dr. Jack Nicol Coetzee, University of Pretoria, South Africa; Dr. Sheldon Goldberg, Rutgers University, New Brunswick, N.J.; Fred F. Igalsohn, New York University School of Medicine, New York, N.Y.; Eva Kataja, University of Rochester, Rochester, N.Y.; Dr. R. H. Pritchard, The University, Glasgow, Scotland; Harvard Reiter, New Haven, Conn.; Thomas B. Stim, Biological Warfare Laboratories, Ft. Detrick, Frederick, Md.; Dr. Roger Weinberg, Ft. Detrick, Md.

## BUILDINGS AND GROUNDS

The efficiency of the staff at Blackford Hall has been hampered for some time by out-of-date equipment, and the lack of certain needed facilities. Therefore a complete modernization of the kitchen, with the addition of up-to-date equipment, was authorized by the Executive Board, and undertaken and completed in time for use during the 1957 summer session. Among the equipment installed were a special 40-cubic-foot stainless steel refrigerator, stainless steel table tops and cabinets, sliding doors, backs, bases and shelving of the same material.

A special Women's Committee (see "Acknowledgements") undertook to raise sufficient funds from a small group of ladies, to refurbish and redecorate the dining area and the living room at Blackford. A sound-proof ceiling was installed in the dining hall; both rooms received attractive new floor covering and were completely redecorated. The downstairs area was converted into recreation rooms for guests; new lighting fixtures and furniture were installed.

Wawepex, the laboratory mainly used for Nature Study courses (workshop and children's) was improved by the addition of new benches. The third floor was equipped with insulation and an electric fan. Extensive repairs were made to the basement floor of Hooper House. These included painting and redecorating. Kitchens were added to the two cabins, making them suitable for use as housekeeping apartments. New refrigerators, beds and bedding were installed. The carpenter and maintenance shop was greatly improved by the addition of a new wing.

## 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 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 Office of the Surgeon General of the Army.

For research of the Microbial Genetics section: the Office of Naval Research, 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, and the Geschickter Fund for Medical Research.

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, Milbank Memorial Fund, and The Population Council, Inc.

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

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

I wish to express thanks to the following members of the Long Island Biological Association for entertaining our guests at dinner parties during the Symposium: Mr. and Mrs. Amyas Ames, Mr. and Mrs. Donald Arthur, Mr. and Mrs. Edward S. Blagden, Mr. and Mrs. Bern Budd, Jr., Mr. and Mrs. Duncan Cox, Mr. and Mrs. George Crocker, Mr. and Mrs. Joseph Eggert, Jr., Mr. and Mrs. Richard Emmet, Mr. and Mrs. Nevil Ford, Mrs. George S. Franklin, Mr. and Mrs. R. Graham Heiner, Mr. and Mrs. David Lindsay, Mr. and Mrs. Alfred Loomis, Mr. and Mrs. Richard McAdoo, Mr. and Mrs. Grinnell Morris, Mr. and Mrs. Charles P. Noyes, Mr. and Mrs. Arthur W. Page, Mr. and Mrs. Walter Page, Jr., Mr. and Mrs. H. Irving Pratt, Mr. and Mrs. Charles Root, Mr. and Mrs. Franz Schneider, Mr. and Mrs. Robert Schorn, Mr. and Mrs. Richard Storrs, Mr. and Mrs. Eugene Taliaferro, Mr. and Mrs. Alexander M. White, Jr., and Mr. and Mrs. Cornelius Wickersham.

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.

We wish to acknowledge, too, the generous support in terms of time and effort given the Laboratory by the special committee—Mrs. George S. Franklin, Mrs. B. P. Kaufmann, Mrs. William B. Nichols and

Mrs. Alexander M. White Jr.—which supervised the collection of funds and the decoration and refurbishing of Blackford Hall. To the following ladies who gave to this fund we also express thanks: Mrs. Harold A. Abramson, Mrs. Amyas Ames, Mrs. Hoyt Ammidon, Mrs. Edward Blagden, Mrs. Herbert Bodman, Mrs. H. Schuyler Cammann, Mrs. Ernst Caspari, Mrs. Raymond de Clairville, Mrs. William Denby, Mrs. Richard Derby, Mrs. Maitland A. Edey, Mrs. W. Allston Flag, Mrs. Nevil Ford, Mrs. George S. Franklin, Mrs. A. R. Goldfarb, Mrs. Joseph S. Gots, Mrs. Arthur Gwynne, Mrs. Caryl P. Haskins, Mrs. Ashton Hawkins, Mrs. Rollin D. Hotchkiss, Mrs. J. Taylor Howell, Mrs. Kenneth D. Jamieson, Mrs. Percy H. Jennings, Mrs. B. P. Kaufmann, Mrs. Ernest L. Lahr, Mrs. Russell C. Leffingwell, Mrs. John F. MacKay, Mrs. Robert C. Murphy, Mrs. George Nichols, Mrs. William B. Nichols, Miss Juliet Nourse, Mrs. Walter H. Page, Mrs. H. Irving Pratt, Mrs. G. Hale Pulsifer, Mrs. Roland Redmond, Mrs. Gordon Rentschler, Mrs. Morris Schaefer, Mrs. Franz Schneider, Mrs. J. Barstow Smull, Mrs. Eugene Taliaferro, Mrs. Felix Wasserman, Mrs. David Weld, Mrs. Alexander M. White, Jr., Mrs. C. W. Wickersham, Jr.

We acknowledge with appreciation the use of the auditorium of the West Side School for the panel discussion on World Population Problems which was arranged in cooperation with the Planned Parenthood Group of North Suffolk at the time of our Symposium, and thank the Planned Parenthood Group for their assistance in planning the lecture.

In particular I want to express gratitude to the Wawepex Society for its annual gift, and to our many friends who during the year contributed as members of the Association.

M. DEMEREC

Director of the Laboratory

## REPORTS OF LABORATORY STAFF

### VIABLE MUTATIONS

B. Wallace, C. Madden, G. Gillies, E. McMullen, and O. Sokoloff

During the past several years we have reported upon studies of irradiated *Drosophila* populations; the purpose of these studies was to establish the role of gene mutations in determining the fitness of populations. During this time we have been led to the view that many genes which appear deleterious when they are studied in the homozygous condition are actually retained in populations by natural selection on the basis of heterozygote superiority. Briefly, three types of individuals—"AA" and "aa," the two homozygotes, and "Aa," the heterozygote—can be formed by two different forms of one gene; our data would indicate that frequently the "Aa" individuals are superior in fitness to both "AA" and "aa" individuals. In proceeding with this type of reasoning, we have supposed that selection may act to establish series of many different alleles at a great many gene loci; the greater the number of different alleles ( $a_1, a_2, a_3, a_4, \dots$ ), the smaller the probability becomes that homozygous individuals (for example,  $a_1a_1$ ) will be formed under a system of random mating. An analogous situation is quite common among plant species in which pollen grains carrying certain genes are unable to function on the stigma of another plant carrying the same gene. Within these plant species it is found that these "self-sterility" genes are generally represented by enormous series of alleles and, consequently, sterility is minimized within any one population.

We have made a number of tests of our genetic model and these, too, have been reported previously. One of these concerned the variation one can recover through the recombination of chromosomes. There were two parts to the argument underlying that experiment. First, two chromosomes which produce individuals of the same viabilities when homozygous and whose recombination products do not differ in this respect from either of the two original chromosomes are assumed to be genetically similar. (Let each of the two chromosomes carry the genes "AB," then the crossover products can only be "AB," too. On the other hand, let one chromosome be "Ab" and the other "aB;" recombination in this case will produce two new types of chromosomes, "AB" and "ab.") Second, if each of these two chromosomes, assumed to be genetically similar, is allowed to recombine with a third, the arrays of crossover products should again be similar when tested experimentally; however, the latter did not prove to be the case. Therefore, it was concluded that seemingly identical chromosomes can really differ considerably from one another in gene content.



Much of the past year has been spent on still another experimental approach to this same question: "What is the genetic structure of a population?" Our model of heterozygote superiority, upon close examination, leads to the conclusion that under some circumstances a high proportion of random mutations may be beneficial. In the paragraphs that follow an attempt will be made to show why and under what circumstances random mutations may be beneficial, to set up an experimentally testable hypothesis, to describe briefly experimental procedures, and to present the results obtained until now. In spite of the amount of work which has gone into these experiments, these experimental results must be regarded as preliminary. In its extreme form the model of heterozygote superiority leads to expectations that are contrary to common sense; under these circumstances, experimental results seeming to support the model must be examined carefully before they can be accepted at face value.

The argument. We propose two extreme models of the genetic structure of populations. The first, based on homozygote superiority, leads ideally to populations of genetically identical individuals whose genotype can be represented as "AA BB CC DD EE FF . . . ." Furthermore, it is postulated that under normal circumstances this ideal situation is not attained; gene mutation and inconsistencies in the direction of selection at different times result in the presence of a low frequency of deleterious, usually recessive, genes within the population. Consequently, normal individuals within a population under this model are of a variety of types which can be represented as "AA BB CC Dd EE FF . . . ." It is important to note in this connection, however, that the overall frequency of the deleterious mutations among the various loci is extremely low according to this model.

The second extreme model is based on heterozygote superiority. Under this model we postulate that selection ideally would lead to the establishment of populations of completely heterozygous individuals where each allelic form of a gene would be present once and only once. An ordinary individual under this model would appear as " $a_1a_3$   $b_5b_7$   $c_2c_5$   $d_2d_2$   $e_6e_7$  . . . . ;" such a normal individual falls short of the ideal by possessing certain genes in the homozygous condition (" $d_2d_2$ " in the example). Since, according to this model, the genes retained in a population are retained on the basis of their action in heterozygous condition, we postulate that homozygosis generally leads to a deleterious effect.

There exist genetic techniques by which one can obtain individuals homozygous for entire chromosomes. Under each of the two models outlined above these homozygous individuals are expected to be less viable than normal individuals of a population. Under the model of homozygote superiority, one expects that among the hundreds of genes for which these individuals are homozygous, one or more represent the deleterious genes kept in the population primarily by mutation pressure. Under the other model, these individuals have been made homozygous for many

genes whose existence in the population stems from their behavior in heterozygotes; by definition, these genes are expected to be somewhat deleterious when homozygous.

The two extreme models predict that the relationships between artificially produced homozygotes and the usual individual commonly found in a large population are very different. Under homozygote superiority, these homozygous individuals are very much like the normal individuals with the exception that homozygosity for a few deleterious genes interferes with normal development. Under heterozygote superiority, these homozygous individuals differ widely from the postulated normal individuals by virtue of their homozygosity at many loci which normally are heterozygous for two contrasting alleles.

This contrast in the predicted differences between artificial homozygotes and normal individuals presents an opportunity for distinguishing between the two models experimentally. The experimental approach would consist of determining the effect on the viability of homozygous individuals of new mutations in the heterozygous condition. In the first instance, the vast majority of these new mutations would affect "normal" genes since these are by far the most plentiful; such mutations would simply add to the developmental difficulties of these homozygous individuals. A beneficial mutation would require the coincidence of two rare events: (1) the mutation of a deleterious gene and (2) a change of this gene to a more "normal" allele. Quite the contrary is true in the case of heterozygote superiority. Here a new mutation has an excellent opportunity of affecting a gene which is normally carried in the heterozygous condition. Furthermore, since there are many different states in which the changed gene can exhibit its beneficial effect when heterozygous, it is expected that a sizable proportion of random changes will give rise to one of these "heterotic" states. Therefore, gene mutations beneficial to individuals homozygous for chromosomes taken from a large interbreeding population should not be rare events if heterozygote superiority prevails but should be exceedingly rare under homozygote superiority.

Experimental techniques. Four different second chromosomes were obtained from our experimental population #18 which has had a history of approximately 200 weeks of intensive irradiation followed by 100 weeks with no irradiation. Each of these chromosomes was transferred into the genetic background of our CyL/Pm tester stock (Curly wing, Lobe eye, and Plum eye are three dominant second chromosome genes; the CyL chromosome carries crossover suppressors as well.) Following the initial preparation of the material, appropriate matings were made to produce males homozygous for each of these chromosomes. Half of the males homozygous for any one of the four chromosomes were irradiated with 500r X-rays; the other half were not irradiated. Males of both the X-ray and the non-irradiated controls were mated singly with CyL/Pm females; single Pm/+ sons were obtained from each of these matings. The Pm/+

sons were mated once more with CyL/Pm females. From each of the cultures of the control series three Pm/+ males and eight CyL/+ females were collected; from each culture of the X-ray series three Pm/+ males were obtained. These males and females were mated; the Pm/+ males of the X-ray series to the CyL/+ females of the control and the Pm/+ males of the control to CyL/+ females (not brother-sister matings, however) of the control series.

These matings lead to cultures in which one expects the following classes of flies—(+) indicates an irradiated second chromosome:

Control	1 CyL/Pm	: 1 CyL/+	: 1 Pm/+	: 1 +/+
X-ray	1 CyL/Pm	: 1 CyL/(+)	: 1 Pm/+	: 1 +/(+).

In overcrowded cultures such as those produced by the use of four females, these ratios will be distorted and the distortions serve as measures of relative viability. The CyL/Pm flies can be regarded as having a viability of 1.000 and, by dividing the numbers of flies in the other three classes by the number of CyL/Pm flies (actually, this number plus one), one can obtain estimates of the relative viabilities of these other types of flies. These relative viabilities can be compared between, as well as within, the control or X-ray series. Because of the mating scheme used in setting up these cultures, the Pm/+ flies of neither the X-ray nor the control cultures carry irradiated wild-type chromosomes; hence, the relative viabilities of Pm/+ flies in the two series are expected to be the same.

Experimental results. Tests of 766 control and 764 X-ray cultures have been completed. The results are given below:

Control	CyL/Pm	CyL/+	Pm/+	+/+
	1.000	1.094	1.146	1.008
X-ray	CyL/Pm	CyL/(+)	Pm/+	+/(+)
	1.000	1.115	1.137	1.033
Probability	—	.01-.05	.30-.40	.001-.01

As expected, the relative viabilities of the Pm/+ flies of the two series are the same. However, the relative viabilities of CyL/(+) and +/(+) flies carrying irradiated second chromosomes seemed to be higher than that of the corresponding classes of the control series.

These early results lend strong support to the genetic model of populations based on heterozygote superiority. They must, however, be subjected to extremely close scrutiny in order to determine whether factors other than differential viability have distorted the ratios in a manner duplicating those predicted by the model. A great many subsidiary tests have been made and as yet no explanation of the results other than that based on differential viability has been found.

During discussions of these results with various colleagues it has become apparent that three points need careful elaboration. Granting the assumption that genetic structure of a population is based on heterozygote superiority, it does not follow that an average mutation will be beneficial to an ordinary, heterozygous individual. According to the model, individuals produced by random mating are already heterozygous at most of their loci and, consequently, a random mutation has a very small probability of increasing the frequency of heterozygous loci. Furthermore, with a high percentage of loci already heterozygous, the relative proportions of heterotic and nonheterotic alleles at each locus becomes an important factor. Second, the argument concerning the possible beneficial effect of random mutations outlined above applies to individuals homozygous for chromosomes taken from large populations of cross-fertilizing individuals. In maintaining an inbred line for many generations, one undoubtedly selects, consciously or unconsciously, for constellations of genes which tend to produce normal individuals when homozygous; there is no need to assume that a large proportion of random changes will prove to be beneficial in this type of material. Finally, the apparent increase in the mean viability of wild-type flies in the X-ray series of our experiments, in addition to being unexpected, is not demanded by the argument for heterozygote superiority; this argument predicted that heterotic mutations would occur with a "reasonable" frequency.

Needless to say, the results reported here are extremely exciting and work along these lines—the re-examination of some assumptions implicit in the experimental approach, alteration of experimental techniques, and the extension of the studies to include various doses of radiation—is being continued.

This report covers work done under contract #AT - (30-1) - 557, United States Atomic Energy Commission. The technical assistance of Geneva Branton and Henry Gardner is gratefully acknowledged.

## THE GENETICS OF RESISTANCE TO INSECTICIDES

James C. King and Lauritz Somme

In the annual report of the Biological Laboratory for 1955-1956 it was pointed out that all the evidence obtained from the selection of lines of *D. melanogaster* for resistance to DDT and from the program of intercrossing these lines indicated that the resistant phenotype was produced by a number of different genetic factors. During the past year two resistant lines have been subjected to a genetic analysis to find out just how these factors are apportioned among the chromosomes. The experimental work and the statistical analysis of the resulting data have been completed for one of the lines (SyS-1002). For the other (SyS-102) the experimental work is still in progress.

*D. melanogaster* has four pairs of chromosomes—the X and Y or sex chromosomes, and three pairs of autosomes. One pair of the autosomes consists of two very minute chromosomes which comprise a tiny fraction of the total hereditary material. These were ignored in making the study. If we call chromosomes from flies of the resistant line, resistant, and their analogues from the control stock from which the resistant line was derived, non-resistant, the six chromosomes (three pairs) may form twenty-seven different combinations of the resistant and non-resistant types. Flies of these twenty-seven combinations were produced by crossing both non-resistant and resistant flies to tester stocks which carried dominant markers on the three large chromosomes and then following a scheme of intercrossing the resulting heterozygous offspring through five generations in such a way as to produce the different combinations. These flies when finally obtained carried only wild-type chromosomes from the resistant line and the control; all marked chromosomes had been eliminated by the system of matings.

Approximately twenty thousand flies of each of the twenty-seven combinations were raised and subjected in groups of one thousand to a range of measured exposures to DDT. From the resulting mortality figures, the LD<sub>50</sub> or mean lethal dose was calculated for each of the twenty-seven different chromosome combinations. These figures were used to perform a factorial analysis which enables us to say whether a given chromosome from the resistant line makes a contribution to the resistant phenotype, and just how great this contribution is when the chromosome is present once (heterozygous) or twice (homozygous).

The results of the analysis are very clear-cut. They indicate that each of the three chromosomes investigated contributes an increment of resistance. These increments are not equal, however. The X contributes least, the second chromosome most and the third an intermediate amount. Furthermore, there is no indication of any interaction between chromo-

some pairs. The resistant X, for example, makes its proper contribution no matter what chromosomes make up the other two pairs. Finally, there is no evidence for dominance. The heterozygote in all cases is intermediate between the two homozygotes. One resistant chromosome of a pair contributes half as much as two.

Working with a strain of *D. melanogaster* collected in Japan which is resistant to DDT, Tsukamoto and Ogaki found that the resistance is produced by a single gene located on the second chromosome. Our present experiments have led to a different conclusion, but this does not mean that one of these conclusions must be wrong. The results of the present study do establish beyond doubt that resistance may be produced by a polygenic system in which a number of factors are distributed throughout the chromosome set. It is another illustration of the fact that a given character may be produced in the same species by a single genetic factor or by the cumulative effect of several separate factors.

The fact that we have no evidence of interaction between chromosomes is interesting in another connection. In the annual report for 1955-1956 it was pointed out that when crosses were made between two resistant lines, there was a drop in resistance from  $F_1$  to  $F_2$ . This phenomenon was characteristic of the lines during the early stages of selection. The drop decreased in amount as selection proceeded and after forty generations of selection it disappeared entirely. This drop was evidence of a non-additive interaction between dissimilar genetic factors brought together by the cross. The line which was subjected to the chromosomal analysis (SyS-1002) was one which had shown this interaction when crossed with SyS-1001. The chromosomal analysis is a much more sensitive test for interactions than the comparison of  $LD_{50}$ 's between subsequent generations of a cross between lines. If any appreciable interaction remained in the system, the chromosomal analysis should have uncovered it.

However, the interaction observed in the crosses could have arisen in two ways. It could have been the result of the shuffling of intact chromosomes of the two lines through segregation into new combinations (inter-chromosomal interactions) or it could have arisen as a result of the formation of new combinations within chromosomes produced by crossing over (intra-chromosomal interaction). In the chromosomal analysis crossing over was purposely prevented by the use of chromosomal inversions in the tester stocks and by testing only the  $F_1$  generation of any combination in which any pair of chromosomes was heterozygous for resistance and non-resistance. As a result of the chromosome analysis we can be pretty sure that very little tendency toward interchromosomal interaction remained in the line. The analysis, however, gives us no information on the presence or absence of intra-chromosomal interaction.

Careful study and analysis of the data from the crosses between resistant lines indicates that the decline in resistance from  $F_1$  to  $F_2$  must

have been the result of the presence of different factors for resistance which, when present together, contributed more resistance than the sum of what they contributed when either was present alone. If such factors became separated by either segregation or recombination, a certain increment of resistance would appear to be lost. As a result of continued selection, such factors were apparently bound together so that they rarely became separated. Thus the interaction disappeared. This very interesting phenomenon has been discussed at some length in a paper presented by the senior author of the Xth International Congress of Entomology held at Montreal in August, 1956.

The annual report for 1955-1956 also referred to experiments designed to discover whether successive small doses of DDT in every generation would be more effective in selecting for resistance than a single dose per generation producing the same total mortality. One line (SyS-1101) had been selected by means of small successive doses for eighteen generations and it was not clear whether it had given a greater response than lines SyS-1001 and SyS-1002 which had been selected by means of a single dose per generation. Another line (SyS-1102) was started using small successive doses and was carried through eleven generations. Again the results were negative. Response was no more rapid than it had been in the lines selected by means of a single dose. These results agree with those of somewhat similar experiments carried out on milkweed bugs by Dr. Ramon L. Beard of the Connecticut Agricultural Experiment Station in New Haven.

The work reported here was done under Contract No. DA-49-007-MD-327 with the Medical Research and Development Board, Office of the Surgeon General, Department of the Army. We wish to acknowledge with gratitude the able assistance of Mrs. Patricia D. Myers, Miss Judith Kalish and Mr. Mario Galasso. The chromosome analysis owes a great deal to the competent, intelligent and faithful work of Mrs. Myers and Miss Kalish who helped see it through its most critical phases.

## RELATIONSHIP BETWEEN MUTATION AND BIOCHEMICAL FUNCTION IN BACTERIA.

Ellis Englesberg, Laura Ingraham, and Joanna Hadden

In the past year we have continued our study of the following subjects: (1) The natural requirements for amino acids in *Pasteurella pestis* and the isolation of mutants able to grow independently of these amino acids (meiotrophic mutants). In particular we have concentrated on the so-called requirement for isoleucine and its involvement in the "neutralization" of methionine inhibition; (2) The physiology and genetics of dissimulatory deficient mutants of *Salmonella typhimurium*. A group of three mutants have been studied, which apparently have the same functional deficiency, yet are the result of mutation at different sites in the gene locus. The growth of these mutants is inhibited by glucose, and thus presented to us a unique tool for the study of the so-called "diauxie phenomenon," the phenomenon whereby a microorganism, when presented with two particular utilizable carbon sources, A and B, will selectively use A first, and subsequently when growth on A has been completed, will then adapt to the utilization of B; (3) In collaboration with L. Baron, we have also undertaken a study of the possible linkage in *Salmonella* of the gene controlling the active transport of rhamnose to the site of enzyme activity with those controlling the enzymes involved in rhamnose dissimilation.

The central theme running through all three projects is the inter-relationship between genetic and physiological processes in regulating cellular metabolism.

The So-called Isoleucine Requirement of *Pasteurella pestis* and its Neutralization of the Methionine Inhibition. It has previously been shown that *Pasteurella pestis* requires cysteine, methionine, phenylalanine, valine, and isoleucine for growth. The requirement for methionine has been shown to be the result of a natural break in the biosynthesis of this compound at the conversion of cysteine to cystathionine. It was found that with the isolation of a methionine independent meiotroph ( $me^+$ , previously called  $M^+$ ) both the valine and the isoleucine, as well as the methionine requirement of this organism vanished. Evidence was presented which indicated that although methionine was required, it was also inhibitory (one gamma of 1 methionine per ml severely inhibits growth of the  $me^+$  meiotroph on a cystine, phenylalanine mineral glucose agar medium), and isoleucine was required to "neutralize" the methionine inhibition. Isoleucine was also found to be inhibitory, and valine was required to "neutralize" this inhibition.



It has recently been found that l threonine, dl homoserine, glycine, as well as isoleucine, also relieve the methionine inhibition in an apparently non-competitive fashion. On the basis of these results, it is proposed that the methionine inhibition of *Pasteurella pestis* may be the result of a feedback control mechanism, whereby the presence of excess methionine inhibits the biosynthesis of methionine by inhibiting the synthesis of homoserine. Since homoserine is a common precursor for the synthesis of threonine, isoleucine, as well as methionine (as shown by other workers) the presence of excess methionine, aside from stopping methionine synthesis, inhibits growth as a result of the simultaneous blocking of the synthesis of threonine and isoleucine. The stimulatory effect of glycine may be explained on the basis that this compound may give rise to threonine, as has been postulated by other workers. The possibility that glycine inhibits the penetration of methionine, and therefore neutralizes its inhibitory effect, seems to be ruled out, since glycine does not inhibit the growth of the methionine requiring, methionine resistant mutant in a cysteine-phenylalanine-methionine-supplemented mineral glucose medium. On the same basis, methionine resistance cannot be the result of prevention of methionine from entering the cell.

Dissimilatory Deficient Mutants of *Salmonella typhimurium* and Mutation to Diauxic Resistance. It has been previously shown that mutants in group N, mutants 6-9b, 6-15a, and 6-17c, are able to use various Krebs cycle compounds such as citrate, succinate, fumarate, and malate, but not glucose (or other carbohydrates), glycerol, or pyruvate, as sole sources of carbon and energy, and that growth-factor amounts of glutamate, aspartate, and citrate will bring about excellent growth with glycerol as carbon source. On the basis of these studies it was postulated that mutation in these three cases has led to some deficiency in pyruvate oxidation. Recent comparative manometric studies of mutant 6-9b with the wild type, employing dilute resting cell suspensions, revealed that 6-9b could oxidize pyruvate to the same extent as the wild type but at about  $\frac{1}{3}$  the rate. The addition of ammonium sulfate as a nitrogen source to such a suspension further inhibited pyruvate oxidation by 6-9b after a lag period, but had no effect on the rate of oxidation of pyruvate by the wild type. These results can best be explained by the hypothesis that the deficiency in 6-9b and most likely in the other mutants in this group is in the de novo synthesis of Krebs cycle intermediates, i.e., the synthesis of one or more of these compounds by a pathway other than the Krebs cycle, probably by  $\text{CO}_2$  fixation. (The symbol C- shall be used to describe these mutants.) The applicability of this hypothesis is quite obvious. During growth in a synthetic medium with glucose, for instance, as the sole carbon and energy source and inorganic nitrogen as the sole nitrogen source, the Krebs cycle acts not only as a pathway for energy metabolism, but also provides organic compounds for various synthetic reactions, and thus technically speaking, under these conditions, does not truly operate as a regenerative cycle. For example, oxalacetate and alpha-ketoglutarate

are removed from the cycle, providing the carbon skeleton for the synthesis of aspartic and glutamic acids. Therefore, to keep the cycle going, i.e., to regenerate additional oxalacetate for the condensation reaction with acetyl CoA, outside sources of Krebs cycle intermediates have to be provided. One source of these compounds is the fixation of  $\text{CO}_2$ , for instance, by a  $\text{C}_3 + \text{C}_1$  reaction leading to the synthesis of oxalacetate or malate. In the event of inactivation of one of these necessary reactions, there would be no excess oxalacetate formed, and as a result growth would be inhibited. Therefore, the slow rate of oxidation of pyruvate by  $\text{C}^-$  in the absence of a nitrogen source, as previously mentioned, can be explained on the basis that since amino acid synthesis has been sharply cut, there is still sufficient  $\text{C}_4$  to keep the oxidation of pyruvate going, although limiting the rate of this reaction. With the addition of inorganic nitrogen,  $\text{C}_4$  and  $\text{C}_5$  intermediates are rapidly taken out of the cycle for amino acid synthesis, and this finally depletes the cells of the limiting supply of oxalacetate, and pyruvate oxidation is further depressed.

Although growth-factor amounts of aspartate and glutamate, as well as citrate, stimulate growth of these mutants with glycerol as carbon source, as mentioned above, there is negligible growth when glucose is substituted for glycerol. In fact, the addition of glucose (2.0 gamma/ml) to a medium containing citrate (0.2%) as carbon source severely inhibits growth, and selection occurs for spontaneously occurring "glucose resistant" ( $\text{dg}^r$ ) mutants which are still unable to utilize glucose, glycerol, or pyruvate as sole carbon sources for growth ( $\text{C}^- \text{dg}^r$ ). Similarly fructose and other carbohydrates inhibit growth on citrate, and resistant mutants arise which are specific for the particular carbohydrate contained in the medium on which they were isolated. A comparison of the growth characteristics of the wild type (which we shall call  $\text{C}^+ \text{dg}^s$ ) and mutants  $\text{C}^- \text{dg}^s$ ,  $\text{C}^- \text{dg}^r$ , and  $\text{C}^+ \text{dg}^r$  (the latter was produced by transduction of  $\text{C}^- \text{dg}^r$  with irradiated phage PLT22 previously grown on  $\text{C}^+ \text{dg}^s$ ) has established the following: (1) The wild type exhibits a diauxic type of growth with glucose plus citrate. (2) Mutation to glucose resistance of  $\text{C}^- \text{dg}^s$  is specific for the glucose inhibition. (3) The possession of this glucose resistance marker in an otherwise prototrophic genetic background leads to the abrogation of the glucose diauxic phenomenon, i.e., it leads to diauxic resistance to glucose plus citrate and to other pairs of compounds containing glucose to which the wild type shows diauxic. (4) Compounds which yield rapid growth and which are used constitutively or after a short induction period are generally those compounds which inhibit growth of  $\text{C}^- \text{dg}^s$  on citrate. This correlation is further borne out by the fact that  $\text{C}^+ \text{dg}^r$  has a decreased growth rate when glucose is used as sole carbon source as compared to the wild type. (5) With the wild type, unadapted to use citrate as the sole carbon and energy source, citrate still stimulates growth in the presence of "inhibitory" amounts of glucose.

Since the Krebs cycle has been found to be functioning in all aerobic

and facultative organisms, when properly looked for, it is a safe assumption to make that *Salmonella typhimurium* also possesses a functioning Krebs cycle. Therefore adaptation to growth on citrate or to the oxidation of citrate does not involve the synthesis of enzymes directly involved in the cycle, but rather the synthesis of some carrier (Barrett and Kallio) or active transport system (Davis) or permease (Rickenberger et al.) or another enzyme(s), thus enabling the cell to utilize this compound or a derivative of it, through the Krebs cycle, and as a sole source of carbon and energy for growth. In the diauxic phenomenon between glucose and citrate exhibited by the wild type, therefore, the presence of glucose inhibits the production of what we might call a citrate catabolic transport unit (a term employed to encompass the not so well defined and perhaps only "apparently" different possibilities listed above). Since glucose cannot be used as a source of aspartate and glutamate by the C<sup>-</sup> mutant, this phenomenon results in the inhibition of growth of this mutant. (Davis has shown a similar phenomenon with a mutant of *Aerobacter*.) Since in the wild type, unadapted to use citrate as the sole carbon and energy source, citrate still stimulates growth in the presence of "inhibitory" amounts of glucose, the transport unit is not required for getting citrate within the cell (as has been postulated by Davis with citrate and by Rickenberger et al. with  $\beta$  galactosides), where it may act in this particular fashion, but it is required for the use of citrate as a carbon and energy source. The above evidence indicates further that the glucose block may not be at the cell surface, as postulated by Cohn and Monod.

It is interesting to note that diauxic resistance, dg<sup>r</sup>, has so far been entirely refractory to all attempts at transduction.

Transduction of Rhamnose Utilization from *Salmonella typhimurium* to *Salmonella typhi* by Phage PLT22 and the Nature of Rhamnose Sensitivity and Resistance in *Salmonella typhi*. *Salmonella typhi* is unable to utilize rhamnose as a source of carbon and energy. When rhamnose is added to a nutrient agar medium, which otherwise supports normal growth of this organism, growth is reportedly inhibited, and rhamnose resistant papillae arise, clones from which are still unable to utilize rhamnose (Barkulis). (Recent attempts to isolate spontaneous rhamnose utilizing (R<sup>+</sup>) mutants have been unsuccessful, and it is estimated that the mutation rate to R<sup>+</sup> of *Salmonella typhi* strain 0901S<sup>r</sup>, is less than  $2 \times 10^{-13}$ .)

*Salmonella typhimurium*, strain LT2, on the other hand, is able to utilize rhamnose for growth, and it has been possible to transmit this ability to *Salmonella typhi*, strain 0901S<sup>r</sup>, which is rhamnose negative and rhamnose sensitive (R<sup>-</sup> R<sup>s</sup>), by means of phage PLT22 previously grown on *Salmonella typhimurium* (transduction).

To determine the physiological basis for rhamnose utilization gained by transduction, and its relationship to rhamnose sensitivity and resistance,

an analysis was undertaken for the enzymes involved in the initial steps in rhamnose utilization. Enzyme extracts of the wild type ( $R-R^s$ ) and mutants  $R+R^s$  and  $R-R^r$  were prepared from cells disrupted by treatment with the Raytheon Sonic Oscillator, and assays for rhamnose isomerase and rhamnulokinase were performed according to procedures previously described for characterizing these enzymes in *Pasteurella pestis*. Contrary to expectation, it was found that the wild type ( $R-R^s$ ) and  $R+R^s$  both possessed high rhamnose isomerase and rhamnulokinase activity. These enzymes were found only in extracts prepared from cells grown in the presence of rhamnose, demonstrating that they are inducible enzymes. It was also found that rhamnulose accumulates in the supernatant fluid during the growth of  $R-R^s$  cells. These results indicate that the inability of the prototroph to utilize rhamnose for growth probably stems from a break in rhamnose catabolism subsequent to the formation of rhamnulose phosphate, and is not the result of a "permeability" barrier. An examination of the enzyme preparations of  $R-R^r$ , on the other hand, showed no traces of these two enzymes.

On nutrient agar plates or on a special Endo's agar medium, in the presence of 1.0% rhamnose, the prototroph can best be distinguished from the rhamnose resistant mutants by the papillation of the former, rather than by significant differences in colony size. The so-called inhibition, as reported in the literature, is detectable when the amount of peptone in the medium becomes limiting as a carbon source. A quantitative study of rhamnose sensitivity and resistance has been performed, employing a 0.1%-0.2% casein hydrolyzate mineral rhamnose or rhamnulose medium supplemented with tryptophane and cystine, both amino acids being required for growth of this particular strain of *Salmonella*. These experiments indicated that a definite proportion of the casein hydrolyzate is employed as energy and carbon source by  $R-R^s$  before the inhibition sets in, and furthermore, that rhamnulose itself is non-inhibitory. These results and those of other experiments are consistent with the following hypothesis: There are at least two compounds (A and B) in the casein hydrolyzate which can be used by  $R-R^s$  as a carbon and energy source in the absence of rhamnose. In a casein hydrolyzate rhamnose medium, growth occurs on compound A. After A is completely utilized,  $R-R^s$  adapts to rhamnose conversion, which simultaneously results in preventing the utilization of compound B. Since rhamnose cannot be used as a carbon source by this organism and since rhamnose inhibits the utilization of compound B (the sole utilizable carbon source then present) no further growth occurs. Mutation to  $R^r$  prevents adaptation to rhamnose, and growth continues on B. In many respects, therefore, the rhamnose inhibition resembles the glucose inhibition, or diauxic phenomenon, described in the *Salmonella typhimurium* mutant  $C-dg^s$ , whereby glucose inhibited growth on citrate. One major difference in these two phenomena is that mutation to rhamnose resistance apparently results in the loss in ability to use rhamnose, probably because of a deficiency in ability to

produce a rhamnose transport unit for concentrating rhamnose within the cell, which is required for induction of the rhamnose enzymes, while mutation to glucose resistance allows both glucose and citrate to be used simultaneously.

These studies were aided in part by grants from the Office of Naval Research and The National Microbiological Institute, National Institutes of Health, U. S. Public Health Service. Reproduction in whole or in part is permitted for any purpose of the United States Government.

## GENETICS OF ESCHERICHIA COLI

P. D. Skaar

Two general topics are dealt with in this report: (1) The nature of the conjugation process and (2) the disposition of genes in the section of the genome concerned with tryptophan synthesis.

(1) Conjugation. (a) Material transfer. In bacterial matings, conjugal pairs are formed by cells of diverse mating type (Hfr and F<sup>-</sup> for example). Fertilization consists of the passage of genes between the paired cells prior to their separation. Gene passage can be observed to occur in one direction only (from the Hfr to the F<sup>-</sup> cell).

A considerable body of evidence implicates deoxyribose nucleic acid (DNA) as the important substance of which genes are made. Therefore, in a joint project with Dr. A. Garen (alluded to briefly in a previous report) the following questions were asked: Can DNA transfer between mating cells be measured? If so, is it also unilateral (from Hfr to F<sup>-</sup>) and how much is transferred?

It was found that DNA transfer could be measured by taking advantage of the fact that coliphage T2, during parasitic growth on its bacterial host, efficiently and preferentially converts the phosphorus of bacterial DNA into the DNA phosphorus of new T2 (Hershey). Thus, DNA of T2-resistant Hfr cells was labelled with radioactive phosphorus (P<sup>32</sup>) and then these cells were mixed with unlabelled T2-sensitive F<sup>-</sup> cells. After mating, the F<sup>-</sup> cells were lysed with T2, the T2 purified and the fraction of the P<sup>32</sup> originally in the Hfr now present in the T2 determined. The activity observed was much higher than that found in T2 which had been incubated with the labelled bacteria alone.

That the DNA transfer observed is largely a function of mating is shown by results involving F<sup>-</sup>:F<sup>-</sup> mixtures (where no mating occurs). Labelled T2-resistant F<sup>-</sup> cells were mixed with unlabelled T2-sensitive F<sup>-</sup> cells. After incubation, the sensitive cells were lysed and the resulting T2 assayed as before. The amount of activity observed was only slightly higher than that observed when T2 was incubated in the presence of the labelled component alone.

DNA transfer, then, can be observed in the direction predicted by genetic studies (Hfr to F<sup>-</sup>). Does it also occur in the other direction? This can be tested by a reciprocal of the first experiment. Labelled T2-resistant F<sup>-</sup> cells were mixed with unlabelled T2-sensitive Hfr cells. After mating, the sensitive cells were lysed with T2 and the resulting T2 assayed. The level of activity found in this type of experiment was very close to that found in the F<sup>-</sup>:F<sup>-</sup> experiments and hence significantly

lower than that found when Hfr to F<sup>-</sup> transfer was measured. Thus, material (DNA) transfer is unilateral (from Hfr to F<sup>-</sup>), paralleling gene transfer.

Simultaneously with measures of DNA transfer, minimum estimates were made of the fraction of Hfr cells engaged in fertile matings, by observing the transfer of the gene Lac<sub>1</sub>. As the ratio of Hfr:F<sup>-</sup> cells was altered, both incidence of fertile matings and percent DNA transfer shifted in the same direction.

Estimates of quantity of DNA transfer are difficult to make, due to a number of uncertainties. However, a maximum estimate can be made with some assurance, and this turns out to be of value. To make this estimate, only those mixtures are considered in which the labelled component was in a distinct minority and hence had every chance to participate in a mating. Where Hfr to F<sup>-</sup> transfer was measured, about 0.07% of the activity originally present in the Hfr component appeared in the T2 grown on the F<sup>-</sup> component (in excess of the small amount of activity observed in T2 after incubation with Hfr alone). Where F<sup>-</sup> to F<sup>-</sup> transfer was measured, the comparable figure was about 0.01%. Both of these values are based on a number of experiments showing little variability. The nature of the F<sup>-</sup> to F<sup>-</sup> transfer is not known, but it is clear that if one is interested in that transfer associated specifically with mating, subtraction is called for, giving an Hfr to F<sup>-</sup> transfer value of about 0.06%. Further, a correction should be made for the number of Hfr cells which actually participated in a mating. There is reason to believe that this fraction is close to 100%; but, to maximize the estimate, the assumption is made that only about 1/3 of the Hfr mated, which is somewhat less than the frequency of observed transfer of Lac<sub>1</sub> in these experiments. Applying this correction, one arrives at the figure of 0.18% for the fraction of activity originally present in mated Hfr cells which is detectable among the T2 grown on their F<sup>-</sup> mates.

Now it is clear that T2 is not completely efficient in picking up DNA phosphorus from its host, but its efficiency can be easily estimated. If the DNA of T2-sensitive cells is labelled with P<sup>32</sup> and then these cells lysed with T2 and the phage assayed, it is found that (regardless of mating type) only about 2.6% of the label is recovered. This gives a preliminary estimate of the amount of activity to be expected in the transfer experiments if transfer were complete, subject to two corrections. First, labelling is not entirely specific for DNA (some RNA is labelled for example) and T2 is capable of utilizing this other phosphorus to a limited extent. To make a maximizing correction for this fact, the 2.6% is multiplied by 0.5 giving 1.3%. This means that, if DNA alone is transferred from Hfr to F<sup>-</sup>, and if it completely were to replace the DNA of the receptor cell, not less than 1.3% of the original activity could be recovered in T2. As developed in the preceding paragraph, only about 14% of this amount is actually observed. Since this percentage is so small, a second

possible correction (for dilution) becomes negligible, and can be ignored.

Thus, not more than 14% of the DNA of an Hfr cell is transferred to an F<sup>-</sup> cell with which it mates. These cells are multinucleate; but the maximum number of nuclei expected is 4. If only one nucleus were transferred, one would expect, of course, only 25% transfer of DNA. The remaining discrepancy between this value and the maximum estimate based on observation indicates that entire nuclei are not transferred in bacterial matings.

(b) Integration. In a previous report, a technique was described for obtaining unselected clones derived from single F<sup>-</sup> exconjugants. Hfr T6-sensitive cells are mated with F<sup>-</sup> T6-resistant cells and then exposed to T6 and plated to give isolated colonies. Only F<sup>-</sup> cells survive, but a high proportion of these are fertilized and can be shown to contain one or more genes originally carried by the Hfr parent.

Clones derived from fertilized F<sup>-</sup> cells are surprisingly heterogeneous. Many contain at least two types of recombinants. A few carefully studied clones contained three or four recombinant types. Others appeared to contain as many as eight or more, but these were not dealt with in such a way as to exclude rigorously spurious "recombinants" due to mutation and selection.

If only one of the two or more nuclei of F<sup>-</sup> cells are fertilized, and if reduction division of the zygote nucleus occurs immediately, a maximum of two recombinant types should be expected among the progeny of any fertilized F<sup>-</sup> cell. (Not four, since the transferred genome is incomplete.) The clones with excess recombinant types could mean that reduction division is not immediate and that more than one opportunity for crossing-over exists. Such a situation was inferred from earlier observations on the unstable progeny of K-12 × B crosses (Ann. Report, 1955). On the other hand, they could be due to multiply-fertilized cells. The general excess of F<sup>-</sup> parental types among these clones suggests that this last explanation is not adequate. A more compelling argument, however, would be the unambiguous demonstration of exconjugant clones containing more than eight recombinant types.

(2) The Tryptophan Gene Cluster. As reported last year, all tryptophan mutations in *E. coli*, B/r, appear to be closely linked, as they are in *Salmonella*. The well-known T1 resistance mutation which also results in tryptophan requirement appears to be a large deletion in the same region. The study of tryptophan mutants performed in this laboratory has been paralleled closely by an independent study of Lennox and Yanofsky. It is a pleasure to acknowledge the benefits gained by discussion and free exchange of information with these workers, particularly during the visit of Dr. Yanofsky during the past summer.

The principal landmarks in tryptophan synthesis are: — (A) — anthranilic acid — (B) — indole glycerol phosphate — (C) — indole — (D)



— tryptophan. Mutants blocked at step A are labelled tryA, etc. TryC mutants, those blocked at step C, accumulate indole glycerol phosphate (IGP), a substance incapable of supporting growth of mutants blocked at earlier steps. They also appear to accumulate a small amount of some other substance (probably not anthranilic acid) which allows them to show feeding of other mutants, if sensitive tests are applied. This led to the misclassification of some of the mutants studied. In particular, all three of the mutants originally classified as tryD were found by Yanofsky to accumulate large amounts of IGP. They were, in fact, like other mutants which he had studied in that they lacked two enzymes (those involved in steps C and D). These tryCD mutants are unlike the deletions associated with T1 resistance in that they readily revert to wild type. Reversion, rather than suppression, is inferred from the large size of the colonies which appear on minimal medium; a more satisfying genetic test of this point is under way.

The suggested situation (simultaneous mutation of two closely linked genes) is obviously of great interest with respect to the relation between gene structure and function. Unfortunately, the positioning of tryCD mutations is difficult. TryA, tryB, and tryC mutations appear to be arranged in that order on the linkage map. TryCD and tryD mutations are also to the right of tryB, but are so closely linked to the tryC mutations that no statements on the order in this critical region can be ventured at this time.

Abortive transductions in *E. coli* are detectable microscopically, as elucidated by Ozeki in *Salmonella*. Since the absence of abortive transductions in any confrontation of two similar genes apparently reflects a cis-trans position effect, a powerful tool is provided for the study of the tryptophan cluster, in particular the interesting tryCD types. To date, all that can be said in this regard is that abortive (in addition to true) transductions appear when certain of the tryCD strains are exposed to the phage grown on certain tryC strains. Thus, tryC and tryCD mutations are not necessarily allelic (or do not belong to the same cistron, to use Benzer's terminology). This is understandable in view of recent work by Yanofsky, which indicates that the C step is complex, and that tryC and tryCD mutants generally lack different enzymes. The more interesting assay of interactions between tryCD and tryD mutations is under way.

Further support for the view that a deletion is responsible for the T1 resistant tryptophanless types is provided by the observation that phage grown on these cells is completely ineffective in transducing tryA, B, C, or D to wild type. Such transductions might be expected if the responsible event were an inversion.

In a collaborative study with Yanofsky, about 100 independent T1 resistant (T5 sensitive) mutants of B/r were examined. All were alike in that they required tryptophan for growth and did not accumulate an-

thranilic acid, IGP, nor indole. On the other hand, the T1 resistant tryptophanless mutant used by Novick in chemostat studies apparently carries a deletion only for the tryC, tryD moiety (Yanofsky).

In K-12, T1 resistant mutants are usually not tryptophan requirers, although they are slow growers and may contain a deletion. This mutation is close to the tryptophan region, and three point tests by Yanofsky show that it is close to tryD, probably to the right. It is unnecessary, therefore, to invoke a functional relationship between T1 resistance and tryptophan synthesis. The T1 resistance marker to the right, together with a cystine marker to the left (Lennox and Yanofsky), will be extremely useful in further analysis of the tryptophan region.

These studies were aided by a grant from the National Science Foundation.

## GENETIC STUDIES WITH MAMMALIAN CELLS GROWN IN TISSUE CULTURES

Hermann A. Moser and Sheila Friedman

Recent achievements in the cultivation of mammalian tissues *in vitro* open up a novel approach to the study of genetics in man. Puck and his associates developed plating procedures for single mammalian cells analogous to the quantitative plating of bacteria on semi-solid nutrient media. By this technique single mammalian somatic cells multiply on the bottom of Petri dishes which are covered with liquid nutrient media to form isolated, macroscopic colonies. Earle, Graham and Siminovitch, and others, on the other hand, developed methods for growth in suspension of large mammalian-cell populations. These techniques, and the introduction by Eagle of a basal-growth medium for mammalian cells, permit the precise experimental approach that is now possible with many micro-organisms to be applied also to the study of genetics, radiation effects, biochemistry and physiology in single human cells.

Last year we undertook a project to study spontaneous and induced changes in human cells grown *in vitro*. By applying the new tissue-culture methods for mammalian cells we are now trying to develop techniques for induction and detection of mutations in human somatic cells. We hope that this investigation will finally provide us with quantitative data on the genetic effects of ionizing radiations in man, data which are urgently needed for estimating the accumulation of genetic radiation damage in human individuals and in human populations.

Thus, during the past year, we established a tissue-culture laboratory. We installed various apparatus needed for tissue-culture work, including a large tissue-culture cabinet which can be sterilized by ultra-violet irradiation, and a humidified incubator cabinet to which defined CO<sub>2</sub> air mixtures can be delivered at steady rates. The tissue-culture cabinet was constructed with great skill by Mr. Harry White of the Department of Genetics of the Carnegie Institution of Washington. The CO<sub>2</sub>/air cabinet incubator was necessary for incubating cultures grown in Petri dishes. By using the CO<sub>2</sub>/air incubator we are able to maintain the pH of Petri-dish cultures well within the desired limits (7.2—7.8). Several standard laboratory stocks of cells of human origin were acquired during the past year and successfully maintained. They are the following:

- (1) Strain D-98, human bone marrow, isolated by Berman and Stulberg.
- (2) Strain D-189, malignant human foreskin, isolated by Leighton.
- (3) Strain J-111, acute monocytic leukemia, isolated by Osgood.
- (4) Strain HeLa, human carcinoma of the cervix, isolated by Gey.

Acquisition of Experience in Tissue-Culture Techniques. The cleaning of glassware used for the transfer and cultivation of cells is an important step in the successful operation of a tissue-culture laboratory; but this has often been overemphasized in the past. In our laboratory we have adopted with success a very simple and fast-washing procedure by using Haemo-Sol as the detergent.

We gained experience in the preparation and sterilization of growth media. Eagle's Basal Medium, supplemented with horse serum or serum protein, is used as a basic semi-synthetic medium for stock maintenance (standard laboratory cell lines) and for the study of nutritional variants. Serum protein, a supplement of synthetic tissue-culture media, is being obtained by dialyzing horse serum against distilled water at low temperature for 24 hours. For the cultivation of cells whose nutritional requirements are unknown and therefore may be of a complex nature, we are using now a medium containing enzymatic lactalbumin hydrolyzate—a modification of Melnick's Monkey-Kidney Medium. Owing to the nature of tissue-culture media, their sterilization must be performed by filtration. We have developed a simple technique by which freshly prepared growth medium is filtered through a Selas 02 filter candle directly into a number of small bottles which subsequently may be stored in the freezer.

We have learned to grow and multiply successfully our cell lines, and to prevent bacterial and fungal contaminations of the cultures. For economy, we grow our stock cultures statically in tightly closed milk-dilution or in Blake bottles, using Eagle's Basal Medium supplemented with 10% to 20% horse serum.

Since the major buffer system in the tissue-culture medium is bicarbonate, pH control is more difficult in closed culture devices than in open devices which are kept in an atmosphere containing 5%-10% CO<sub>2</sub>. When Eagle's Basal Medium with the usual bicarbonate concentration (1.68 g/l) is used, freshly prepared trypsinized cultures tend to become unduly alkaline owing to loss of CO<sub>2</sub>. The alkalinity of the culture medium, combined with the effects of the trauma suffered by the cells during trypsinization, often results in inefficient attachment of the cells to the glass surface, cell destruction, and delay in the onset of vigorous population growth. To prevent this we have introduced a simple method of improved pH control in closed-flask cultures. The pH is regulated by adjusting the bicarbonate concentration in the culture medium according to the states of population growth, using Eagle's Basal Medium with low, medium, and high bicarbonate concentrations. When one introduces approximately 100,000 trypsinized cells in 6 ml of growth medium into milk-dilution bottles with tight-fitting rubber stoppers, the scheme (Table I) secures rapid onset of cell sheet formation.

Table 1.

Standard procedure for the growth of human tissue-cultures in tightly closed flasks at 38°C (E\*\*, Eagle's Basal Medium with 0.35 g/l NaHCO<sub>3</sub>; E\*, Eagle's Basal Medium with 0.70 g/l NaHCO<sub>3</sub>; E, Eagle's Basal Medium with 1.68 g/l NaHCO<sub>3</sub>; HoS, horse serum)

Time (days)	Operation	Growth medium used
0	Plating of trypsinized cells (approx. 2500-5000 cells per cm <sup>2</sup> )	E** HoS <sub>20%</sub>
1	Medium change No. 1	E** HoS <sub>20%</sub>
3	Medium change No. 2	E* HoS <sub>20%</sub>
5	Medium change No. 3	E* HoS <sub>20%</sub>
7	Medium change No. 4	E HoS <sub>20%</sub>
9	Medium change No. 5	E HoS <sub>20%</sub>
11	Medium change No. 6	E HoS <sub>20%</sub>

During the past year we have gained a considerable amount of experience in the trypsinization methods used for preparing single-cell suspensions from solid-cell layers grown on glass. Complete cell separation without appreciable loss of viability of the treated cells is a prerequisite for obtaining high plating efficiencies with mammalian cells. In order to find optimal conditions for enzymatic cell separation we compared the efficiency of solutions with identical proteolytic activity of pure trypsin (obtained from Dr. McDonald, Department of Genetics of the Carnegie Institution of Washington), chymotrypsin (also from Dr. McDonald), trypsin 1-300, and pancreatin (3X U.S.P. potency), both obtained from Nutritional Biochemicals Corporation, Cleveland, Ohio, and of amylopsin (Dr. McDonald). Dr. McDonald was kind enough to standardize these enzymes or enzyme mixtures on the basis of their proteolytic activity (Hemoglobin test). In our own experiments we found that all enzyme preparations except chymotrypsin were powerful agents for separation of mammalian cells. Very efficient separation with a minimum of toxicity was obtained with solutions in saline A of either 0.25% pancreatin (3X U.S.P. potency) or 0.005% pure trypsin (McDonald).

We practiced Puck's method for plating of single mammalian cells without the use of a feeder layer. After some early difficulties we succeeded in obtaining macroscopic colonies and high plating efficiencies, using strain D-98 (human bone marrow). While growing single cells on plates we found that Petri-dishes are inconvenient for microscopic observation of clone formation. For this and other reasons we are using in some experiments a special culture dish of our own design for clonal growth of mammalian cells. This new tissue-culture dish proved to be particularly suitable in long-term experiments which require periodic media changes.

Part of our program includes the isolation of cell strains from human male tissue. The cooperation of Huntington Hospital has been secured for a supply of tissue material. Recently we achieved the isolation of connective tissue (human foreskin) and have been able to grow the strain successfully in vitro. The following technique was used for isolation. The organ was finely minced, fragments were treated with a 0.25% trypsin solution at 38°C, and the cells collected, concentrated, and plated out into milk-dilution bottles containing Eagle's Basal Medium. This technique is essentially the same as the one developed by Dulbecco and Vogt for the production of monkey-kidney cell suspensions. Cell extractions from various other male organs (thyroid, tonsils, appendix, prostate, and bone marrow) are in process.

Research. The work reported so far covered the exploratory phase of our project. Our current research activities are aimed (1) at finding conditions under which spontaneous or induced mutations involving certain cell functions (biochemical functions, drug resistance, etc.) become phenotypically expressed in somatic mammalian cells grown in tissue cultures and therefore could be detected (2) at developing the techniques whereby such mutations can be scored quantitatively. The second problem includes the development of selective techniques for phenotypic mutant cells. Recently we have been concentrating on finding a suitable selective technique for the isolation of biochemical mutant cells. Our efforts in this direction seemed justified. The studies of Eagle and his associates at the National Institutes of Health, on the nutritional requirements of mammalian cells, clearly demonstrate that cells derived from various human tissues are auxotrophic with respect to thirteen amino acids and at least seven vitamins. When grown in a medium containing the essential inorganic salts, a carbon source (glucose) the essential amino acids and vitamins, and a small amount of serum protein, these cells multiply vigorously. If one of the essential amino acids or vitamins is omitted from the medium, however, the cells begin to die after a certain time, at rates that depend upon which amino acid or vitamin is lacking. In respect to several of the essential amino acids, the cells do not behave as complete auxotrophs; they are able to synthesize them at very low rates, too low to support cell multiplication in vitro. Thus one would expect the occurrence of mutations which, if phenotypically expressed, would enable the cells to synthesize one of the essential amino acids at a rate high enough to support visible growth (clone formation) in a selective medium. The findings of Puck and of Swim and Parker suggest that mutation from essential nutritional requirement does occur in mammalian cells grown in vitro, and may become phenotypically expressed. For example  $i$ -inositol<sup>-</sup> gives rise to  $i$ -inositol<sup>+</sup>; choline<sup>-</sup> gives rise to choline<sup>+</sup>.

The omission of a single amino acid or a vitamin in Eagle's Basal Medium supplemented with an adequate amount of serum protein furnishes a technique for selecting amino acid prototrophs of mammalian somatic cells. The efficiency of this selective technique depends upon the

degree of residual growth undergone by cells that are exposed to the selective medium. The smaller the amount of residual growth, the better the selective medium. Rapid selective action was observed in our laboratory when bone marrow cells (strain D-98) were exposed to glutamine-deficient Eagle's Basal Medium supplemented with 5% 24-hour-dialyzed horse serum. Table 2 presents the results of an experiment. During an initial phase, wild-type (glut<sup>-</sup>) cells undergo some residual growth in the glutamine-deficient medium, but begin to die off rapidly after the second day, while the controls multiply vigorously in the presence of glutamine. Studies on the response of other selective media on mammalian cell cultures are in progress.

Table 2.

Effect of glutamine-deficient growth medium (Eagle's Basal Medium + 5% dialyzed horse serum) on cultures of human bone marrow cells (strain D-98)

Time after platings (days)	Mean cell counts per culture flask	
	Glutamine-deficient medium	Complete medium (Controls)
0	273 750	273 750
2	523 750	538 750
3	373 750	1 265 000
6	141 250	3 232 500

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

## PSYCHOBIOLOGY SECTION

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

Blocking Effect of Brain Extract. The method used to study the blocking effect of beef brain extract is based on the criteria described by Abramson and Evans for the reaction of the Siamese fighting fish to lysergic acid diethylamide (LSD-25). However, these criteria were modified for the purposes of this investigation and are listed in Table 1.

Table 1.

Inhibition of LSD-25 effect by brain extract. The readings recorded in rows 1a, 2a, and 3a were made 1 hour after brain extract was added to the container, but before LSD-25 was added. The readings in rows 1b, 2b, 3b, and 5b were made 1 hour after LSD-25 was added.

Treatment	Response (No. of fish)			
	Nose up, tail down	Kink in tail	Top of water, tail up	Bottom of container
Experimental containers				
1a Brain extract (2 mg/ml)	0	0	4	0
1b LSD-25 (2 mcgm/ml)	4	4	1	2
2a Brain extract (0.2 mg/ml)	0	0	9	0
2b LSD-25 (2 mcgm/ml)	8*	9	0	2
3a Brain extract (0.02 mg/ml)	0	0	2	4
3b LSD-25 (2 mcgm/ml)	9	10	0	1
Water control				
4a No brain extract	0	0	3	2
4b No LSD-25	0	0	2	4
LSD-25 control				
5a No brain extract	0	0	3	3
5b LSD-25 (2 mcgm/ml)	10	10	0	0

\* One fish dead.

The main criterion utilized was the nose-up, tail-down position of the fish, at an angle of approximately 45 degrees or more. Beef brain extract was prepared in a fashion similar to that described by Florey



and McLennan. In general, the experiments reported here were all run similarly.

Five bottles containing 200 ml of distilled water each were used to observe 50 fish simultaneously, with ten fish in each bottle. Readings were made with two different methods every 15 minutes for a period of four hours or more. The effects of brain extract (2mg/ml, 0.2 mg/ml, and 0.02 mg/ml) on the reaction of the fish to 2 mcgm of LSD-25 per milliliter of solution were compared with a water control (no brain extract) and an LSD-25 control (no brain extract). Table 1 presents, in general, the method of bioassay currently employed.

Until LSD-25 was added, none of the fish showed the typical nose-up, tail-down position except as a normal movement. Fifteen minutes after the LSD-25 was added to the five vessels, all the fish in the LSD-25 control showed the nose-up, tail-down position and continued to do so throughout the experiment. Essentially similar results were obtained in the bottle containing 0.02 mg of crude brain extract per milliliter. In the bottle containing 2 mg of brain extract per milliliter, by contrast, not only was the initial excitatory phase absent, but there was also a lag, with one fish showing the nose-up, tail-down behavior at 45 minutes and only four fish showing this behavior at one hour. Table 1 gives illustrative data obtained at the end of the first hour.

Using unpublished reaction-time curves, it can be readily shown that the fish in the bottle containing 2 mg of brain extract per milliliter acted as if approximately 0.2 mcgm of LSD-25 per milliliter were present. In other words, the brain extract blocked the appearance of the LSD-25 effect, and when this effect finally did make its appearance it resembled that of a much weaker solution of LSD-25 than was actually added.

The question naturally arose: Is the serotonin present in brain extract responsible for the action of the brain extract? Two hours after the fish were exposed to 2 mg of serotonin per milliliter, 2 mcgm of LSD-25 were added. Serotonin does not block the LSD-25 reaction.

Negative results were also found with histamine and  $\gamma$ -aminobutyric acid, as well as with the following amino acids: L-hydroxyproline; L-serine; 3,5-diiodo-L-tyrosine; DL- $\alpha$ -aminobutyric acid; DL-cysteine hydrochloride; L-lysine hydrochloride; DL-methionine; L-tyrosine; DL-valine; DL-tryptophan; L-leucine; DL-phenylalanine; acetyl glycine; L-arginine hydrochloride; DL-threonine; L-histidine hydrochloride; L-glutamic acid; L-valine; acetyl-DL-phenylalanine; glycine; and L-proline. There was possibly slight blocking in L-histidine hydrochloride, for all the fish did not react to LSD-25 immediately.

Lack of material (the result of the difficulty of preparing the crude brain extract in quantity) has prevented our determining where the blocking substance acts or what it is. Conceivably, it could act in several

places (1) outside the fish, forming a loose compound with LSD-25 (2) at the gill membrane or (3) inside the fish itself, as a true pharmacologic inhibitor. The data indicate that some type of equilibrium is set up in which the inhibitory action is dependent on the concentration of the LSD-25 blocking substance present in the liquid.

Future experiments are being designed on mammals and on man to estimate whether the brain extract inhibits the LSD-25 reaction as it does in the fish and whether it will affect the course of clinical psychoses. In view of the small amount of material obtained from beef brain, our present methodology is being scrutinized in an effort to obtain more of the inhibitory substances from beef brain or from other tissues and other animals. Whether the LSD-25 blocking substance is similar to Florey's synaptic inhibitor remains to be determined.

Tolerance to LSD Reaction by Similar Compounds. The experiments with beef-brain blocking substances are only one approach to the possible inhibition of the psychotic process. Another approach is to use compounds already known, like BOL or MLD. We have found that MLD, which is the 1-methyl derivative of LSD-25, is capable of blocking the LSD-25 reaction by development of tolerance when taken several days in advance. This is illustrated in the following table.

Table 2.

Blocking effect of MLD on LSD psychosis. Typical Experiments on Subject B.

a. 3/29/57—50 mcgm LSD orally, no MLD (control).				
	1/2 hr.	1 1/2 hrs.	2 1/2 hrs.	3 1/2 hrs.
# Positive responses	0	13	7	6
Motor behavior	—	Less	—	—
Control	—	—	—	—
Consciousness	—	over alert	—	—
Concentration	—	—	—	—
Mood	—	depressed	—	—
Attitude	—	withdrawn	—	—
Orientation	—	poor	—	—
Memory	—	—	—	—

Remarks by subject: "I have never felt so frightened."

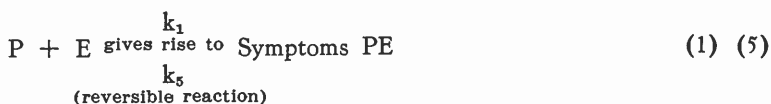
b. 4/12/57—75 mcgm LSD orally, 900 mcgm, total, MLD taken  
6 days prior, in increasing doses to 250 mcgm/day.

	1/2 hr.	1 1/2 hrs.	2 1/2 hrs.	3 1/2 hrs.
# Positive responses	0	0	0	0
Motor behavior	-	-	-	-
Control	-	-	-	-
Consciousness	-	-	-	-
Concentration	-	-	-	-
Mood	-	-	-	-
Attitude	-	-	-	-
Orientation	-	-	-	-
Memory	-	-	-	-

Remarks by observer: "There was no LSD reaction."

A theory of psychosis, published previously, assumes (a) that there is a substance analogous to LSD-25 involved in schizophrenia, and designated here as P; (b) that P has some normal function in the physiology of the emotional processes, but the metabolism of P is disturbed in schizophrenia; (c) that the substance P is regulated by a mechanism like the one proposed here for the development and loss of tolerance to LSD-25, and that a breakdown of this mechanism is involved in P getting out of control.

Thus we would have the mechanism:



—where the normal function of P might be involved, for instance, in the

accommodation of the organism to stress situations.  $E_t$  would be the bal-  
last against emotional explosion while the reaction  $k_5$  would be the "safety  
valve" to bring about the necessary emotional response. Without such a  
safety valve  $E_t$  could act to eliminate P before the organism could make  
the necessary response. The  $k$ 's in equations (1) to (5) are reaction ve-  
locity constants. Our experiments with MLD may lead to a more accurate  
study of this theory and possibly to a technique of approaching the  
chemical control of the psychotic process.

The Effect of LSD in Schizophrenia. LSD (Lysergic Acid Diethylamide)  
produces a schizophrenia-like state in man, but LSD is not supposed to  
affect the schizophrenic the way it affects normal people.

The work in the Biological Laboratory on lower organisms like fish  
and snails has been correlated with a project set up at the State Hospital  
in Central Islip to ascertain if more delicate methods of communicating  
with schizophrenics could be developed. Two methods have been used  
(1) a questionnaire method, and (2) the group-interview method. The  
group-interview method was structured by having a normal "stablemate"  
with whom a schizophrenic may identify. Although the questionnaire was  
found useful in studying the action of LSD in normal individuals, it does  
not as readily detect the LSD reaction in schizophrenics. A more compli-  
cated type of standard content analysis of the conversations may lead to  
improved methods of communication with schizophrenics. Table 3 is an  
example of the type of content-analysis employed.

Table 3  
Ratio of Expressive to Instrumental Activity for Patient  
Under Placebo and LSD-25

Condition	Date	Number of Expressive Acts Number of Instrumental Acts	Ratio
Placebo	2/9/56	$\frac{19}{129}$	.15
	3/22/56	$\frac{24}{183}$	.13
LSD	3/1/56	$\frac{44}{192}$	.23
	3/15/56	$\frac{19}{86}$	.22

Expressive acts include the showing of solidarity and antago-  
nism, the showing of tension and of tension release, the expres-  
sion of agreement and disagreement

Instrumental acts include the giving and asking for orientation, the giving and asking for opinion, the giving and asking for suggestion

a) The ratio of Expressive to Instrumental Acts is almost twice as high for the patient during the LSD sessions as during the Placebo sessions.

b) This finding is to some extent, but by no means entirely, accounted for by the greater number of laughter acts under the LSD condition.

c) The change in the balance between the two kinds of behaviors occurring for this patient under the two conditions should be contrasted to the stability in the ratio of expressive to instrumental activity found in our group experiment.

d) This difference in the reaction of normals to LSD is interesting since, contrary to previous claims, the schizophrenic patient shows more change in behavior (in this aspect) than do the normals.

We believe that our present method is a more sensitive technique of detecting the LSD response in hospitalized patients.

Aerosols. The use of penicillin aerosols for therapy of the lungs was developed at this Laboratory during World War II. It therefore seems desirable, in view of the possible importance of psychochemicals in therapy, to investigate LSD-25 in a similar fashion. We have therefore succeeded in standardizing the administration of therapeutic aerosols to study this to a limited extent.

## BIBLIOGRAPHY

- Abramson, H. A. Lysergic acid diethylamide (LSD-25) XXII. Effect on transference. *J. Psychol.* 42: 59-98 (1956)
- L. H. Geronimus and L. J. Ingraham. Lysergic acid diethylamide (LSD-25) XXIII. Comparative effects of LSD-25 and related ergot drugs on brain tissue respiration and on human behavior. *J. Psychol.* 42: 157-168 (1956)
- B. Sklarofsky, M. O. Baron, and H. H. Gettner. Blocking effect of brain extract on lysergic acid diethylamide reaction in Siamese fighting fish. *Science.* 125: 397-398 (1957)
- B. Sklarofsky, M. O. Baron, and H. H. Gettner. Lysergic acid diethylamide antagonists. *A.M.A. Archives Neurol. and Psychiat.* 77: 439-445 (1957)
- Abramson, H. A. *The Patient Speaks.* Vantage Press, New York, N.Y. 239 pp. (1957)
- Psychic factors in allergy and their treatment. *N.Y.S.J. Med.* 56: 3361-3364 (1956)
- Psychogenic eczema in a child. *Ann. Allergy* 14: 375-381 (1956)
- Englesberg, Ellis, and L. Ingraham. The use of meiotrophic mutants in the elucidation of the amino acid requirements of *Pasteurella pestis*. *Bacteriol. Proc.* 51 (1957)
- Englesberg, Ellis. Mutation to rhamnose utilization in *Pasteurella pestis*. *J. Bacteriol.* (In press)
- The physiological basis for rhamnose utilization by a mutant of *Pasteurella pestis*. I. Experiments with resting cells; the isolation of lactic aldehyde. *J. Bacteriol.* (In press)
- The physiological basis for rhamnose utilization by a mutant of *Pasteurella pestis*. II. A single mutational event leading to the production of two enzymes. *Arch. Biochem. and Biophys.* (In press)
- and L. Ingraham. Meiotrophic mutants of *Pasteurella pestis* and their use in the elucidation of nutritional requirements. *Proc. Nat. Acad. Sci. U. S.* (In press)
- King, James C. Some light on population dynamics provided by lines of *D. Melanogaster* selected for resistance to DDT. *Proceedings Xth International Congress of Entomology.* (In press)
- Moser, H. Contributions to the theory of the continuous bacterial growth apparatus. I. Kinetics of growth of homogeneous populations. *Proc. Nat. Acad. Sci. U. S.* 43: 222-226 (1957)
- Kinetics of bacterial growth in the chemostat. Abstracts National Biophysics Conference, Columbus, Ohio 50-51 (1957)

- Skaar, P. D. and Alan Garen.  
The orientation and extent of  
gene transfer in *Escherichia*  
*coli*. Proc. Nat. Acad. Sci. U. S.  
42: 619-624 (1956)
- Skaar, P. D., Alan Richter and  
Joshua Lederberg. Correlated  
selection for motility and sex-  
incompatibility in *Escherichia*  
*coli* K-12. Proc. Nat. Acad. Sci.  
U. S. 43: 329-333 (1957)
- von Borstel, R. C., and H. Moser.  
Differential ultra-violet irradiation  
of the *Habrobracon* egg  
nucleus and cytoplasm. Progress  
in Radiobiology, edited by  
J. S. Mitchell, B. E. Holmes,  
and C. L. Smith. Oliver and  
Boyd, Ltd., Edinburgh. 211-215  
(1956)
- Wallace, Bruce. Chromosomal  
polymorphism and coadaptation.  
Fifth Poultry Breeders'  
Round Table. (1956)
- The role of sub-vital mu-  
tations in the genetics of popu-  
lations. Fifth Poultry Breeders'  
Round Table (1956)

## REPORTS OF SUMMER INVESTIGATORS

Berman, Leonard, New York University College of Medicine, New York, N.Y.—My objectives this summer were twofold: (1) To discover a mutant of the Klebsiella phage (KP) which would produce no enzyme and will not plate on the mucoid strains of Klebsiella Pneumonia. This is essential in order to study the genetic apparatus of enzyme production. To this end I did a series of platings of phage variant #1 which produces little or no halo on H<sub>1</sub>, the capsuleless mutant of KP. Any atypical plaques were picked and tested on the mucoid strain of bacteria. No enzymeless mutants were found. (2) To find a way in which high titers of enzyme can be grown en mass in broth flasks. This is necessary in order to be able to get sufficient enzyme to conduct a thorough study of its physical and chemical properties. To this end I ran a series of lysates with varying multiplicities of infection and various combinations of bacterial and phage mutants. I achieved phage titers as high as  $2.5 \times 10^{10}$ /ml and enzyme titers as high as 1:128 on spot test. As the multiplicity of infection rose above 1, the enzyme titer leveled off. Also as might be expected there was no enzyme produced in lysates involving H<sub>1</sub>, and less produced by phage variant #1 than by phage variant #3.

Bernheimer, Alan W., New York University College of Medicine, New York, N.Y.—Two unrelated problems were investigated: (1) In collaboration with Miss Betty Freeman, a study was made of the action of streptolysin 0, a toxic protein formed by hemolytic streptococci, on the isolated beating heart of the mouse. A modified Langendorff technique was used involving cannulation of the aorta and perfusion of the heart by way of the coronary arteries with Ringer solution at constant temperature. The frequency and amplitude of contraction were recorded by means of a lever and kymograph. Small amounts of streptolysin 0 caused a temporary decrease in coronary flow and a gradual or sudden decrease in force of contraction usually accompanied by bradycardia and in some instances by arrhythmia. The effects were qualitatively similar to those that have been observed in the isolated perfused guinea-pig heart but the mouse heart appeared to be appreciably less sensitive, on a weight basis, than the hearts of larger animals. No difference in sensitivity to streptolysin 0 was found between hearts of normal mice and hearts of mice that has been injected the day before with a sublethal dose of streptolysin 0. (2) In collaboration with Miss Lois Schwartz, preliminary observations were made on the response of tadpoles of *Rana pipiens* to the intraperitoneal injection of materials known to be antigenic in mammals. The materials injected were bovine serum albumin, whole heparinized carp blood, and a bacterial toxin, streptolysin 0. In no instance were specific antibodies detectable either in the blood plasma or in the extravas-



cular fluid of the amphibian larvae. It is possible that a more prolonged course of injections given under conditions in which metamorphosis is inhibited would lead to different results, and it would be desirable to repeat the experiments with this modification. In the light of recent immunological observations, an attempt was made to see whether exposure of tadpoles to an appropriate antigen leads to inhibition of antibody formation in the adult. Carp blood was injected into frogs that had received carp blood also as tadpoles. The blood plasma of the frogs was found to contain antibodies against carp erythrocytes (hemagglutinins) suggesting that inhibition of antibody formation did not occur. Incidental to this study it was observed that blood and extravascular fluid of both frogs and tadpoles contain a relatively potent inhibitor of streptolysin O. The inhibitor is present in unimmunized animals; it varies in titer from individual to individual; and its nature and significance remain to be elucidated.

Caspari, Ernst, and Blomstrand, Ingbritt, Wesleyan University, Middletown, Conn.—Pigment Formation in the Testis of *Ephestia*. A pigment derived from tryptophan via kynurenine appears in the testis sheath of *Ephestia* during the last larval instar. The alleles *Rt* and *rt* determine the quality of the pigment and the time of its appearance. In order to investigate the action of these genes on the time of pigment formation 4-10  $\mu$ g kynurenine was injected into last instar larvae of known age and of the genetic constitutions *aa Rt Rt* and *aa rt rt*. The injections resulted in formation of pigment in the testis but not in the eye as is the case after injections in the pupal stage. The larval testis undergoes a period during which it reacts on kynurenine injection with pigment formation. This period ends in the prepupa, about two days before pupation. The beginning of this period has not yet been definitely established.

In the course of these experiments it was observed that during the development of the testis another pigment appears in the outermost layer of the testis. It appears in the middle of the last larval instar and disappears in the pupal stage about 9 days after pupation. It is yellow, dissolves easily in water and in alcohol, but not in fat solvents, and has a strong fluorescence. It becomes colorless when exposed to light and behaves in paper chromatograms identically with synthetic riboflavin. This pigment is present in almost all strains examined including *aa*; but the gene *wa* in homozygous condition inhibits the formation of the yellow pigment as well as of the kynurenine-derived pigments.

Doermann, A. H., Chase, Martha C., and Kataja, Eva I., University of Rochester, Rochester, N.Y.—During the summer of 1955, a method had been developed for obtaining strains of bacteriophage T4 which carried, in their genomes, two or more *rII* markers. This material was put to use in two ways during the summer of 1956. An extensive series of two-, three-, and four-factor crosses was completed which was designed

to test linearity and additivity over very short intervals of the genetic map of the virus. These experiments showed that linearity and additivity characterize recombination even within the confines of the individual functional genes. The experiments yielded, in addition, a surprising result: a very high positive correlation of recombination events in adjacent regions is observed when very short intervals of genetic structure of these viruses are scrutinized.

The second use to which the double-rII mutants were put involves an associated problem which must be analysed before the complexities of the recombination mechanism in phage can be understood in terms of a precise, quantitative model. Edgar's (Cold Spring Harbor Symposium on Quantitative Biology 21:109, 1956) work indicates strongly that the positive correlation already mentioned arises as a consequence of recombinant formation from residual heterozygotes (Hershey and Chase, Cold Spring Harbor Symposium on Quantitative Biology 16:471, 1951). The double-rII's were used in preliminary experiments to measure the genetic length of the heterozygous region. Double-rII phages were crossed to wild-type, and the resultant heterozygotes tested to see whether their overlap regions included one or both *r* loci. Three double-rII's, differing in the extent of the recombination interval between the component *r* loci, were used in these experiments. The results indicate a mean overlap length of five to eight map units. Additional experiments are required for greater precision in determining the mean length and even for a rough estimate of the distribution of lengths around the mean value.

Edgar, R. S., University of Rochester, Rochester, N.Y.—Work initiated during the winter was continued in an attempt to further characterize the mechanism of recombinant formation from non-recombinant heterozygotes in T4. In support of earlier observations (see discussion following paper by Streisinger in Cold Spring Harbor Symposium Quant. Biol. XXI) it was found that the mean burst size of  $r^+$  recombinants arising from  $r_1r_3/r_2$  heterozygotes was about 20. This is to be contrasted to mean burst size of about 1 for  $r^+$  recombinants arising from a parental mixed infection. This observation permits the conclusion that the intracellular growth of heterozygotes is not comparable to a mixed infection by the two parental types. Together with previous information it suggests that the heterozygotes may give rise to large (therefore presumably early-formed) clones of recombinants. This is not found in bacteria mixedly infected with the parental types. Crosses involving independently isolated *h* mutants, active against K-12/4, revealed at least 3 *h* loci in the genome of T4. One on the second-linkage group and two on the third-linkage group.

Fraser, D. K., and Luria, S. E., University of Illinois, Urbana, Ill.—Lysogenization with phage P22 of *Salmonella typhimurium*. A technique for the isolation of single cells by micromanipulation was applied to the

problem of the establishment of lysogeny in *Salmonella typhimurium* by phage P22. Newly infected cells are isolated in droplets of broth under mineral oil and the progeny cells are separated as they divide. This method permits separation of all cells of a clone as far as the 5th or 6th generation. The droplets are observed for lysis or multiplication of the cells and the contents of each drop can be removed and tested for phage or for lysogeny.

When a cell is infected with a mixture of two temperate mutants differing by two plaque-type markers, the clone formed contains: (1) subclones of sensitive cells; (2) subclones lysogenic for one parental or one recombinant type; and (3) occasional subclones that continue to carry both parental types (or a parental and a recombinant type) but segregate out cells stably lysogenic for a single prophage type. No cells lyse during the development of such a clone. Sensitive subclones may still be segregated for several generations after infection, and recombinant phage can appear in subclones that are still segregating sensitive cells.

Clones were also studied from cells infected with mixtures of  $c_1$  and  $c_2$  mutants of P22. Each of these mutants lyses almost 100% of the infected cells, but mixed infection leads to lysogeny for  $c_1$  (Levine). The phages used differed also in two plaque-type markers. Infection was generally done with 10  $c_2$  and 1 or 2  $c_1$  phages per cell, in order to follow the markers of the minority parent and to obtain evidence for their multiplication before persistent lysogeny or lysis. In these clones some progeny cells survived, others lysed. The following types of subclones were observed: (1) Subclones consisting of sensitive cells; (2) subclones stably lysogenic for prophage  $c_1$ , either parental or recombinant for other markers; (3) subclones with  $c_1$  and  $c_2$  carried through as many as nine or ten generations, all cells eventually lysing and producing phage mixtures; (4) subclones with  $c_2$  carried alone for one cell division, after which both cells lysed; (5) subclones with  $c_2$  carried through three or four generations through a single line of descent, with repeated segregation of sensitives; (6) occasional subclones with  $c_1$  and  $c_2$  carried together (without segregation of sensitives) in persistent "mixed lysogenics." These segregate cells that lyse and produce  $c_2$  (alone or as majority type), and cells stably lysogenic for  $c_1$  (parental or recombinant for other markers).

The results show that extensive multiplication and recombination may take place before the permanent establishment of lysogeny, as defined by the formation of clones without sensitive or lytic segregants.

Granick, S., The Rockefeller Institute for Medical Research, New York, N.Y.—Preliminary studies indicated that *Euglena* might be a useful organism for studies on porphyrin and chlorophyll synthesis. A medium was found which permitted rapid and dense growth of *Euglena* with a minimum of paramylum formation. The medium consists of 1 part of

Hutner's synthetic medium (Proc. Soc. Exp. Biol. & Med. 69, 279 [1948]) + 1 part of 2% tryptone (Difco).

Part of the summer was used in the preparation of papers for publication.

Igalsohn, Fred F., New York University College of Medicine, New York, N.Y.—This summer I attempted a study of the kinetics of phage penetration. The phages employed in this work were T6r+ and T5. The T6r+ required tryptophane for adsorption to its host. In working with this phage, the bacteria were first starved by the Benzer method and almost complete adsorption obtained before addition of nutrient. After addition of nutrient, the Waring blender was used at suitable intervals to stop penetration by stripping the infecting phage off the bacteria. Temperature was varied in different tests to try to slow down penetration. Using T5, the bacteria were not starved but experiments were made to find whether or not calcium could be used to start penetration. The Waring blender was also used to determine whether inactivation of free T5 occurred in buffer containing 10 micrograms per milliliter of gelatin. It was found that 18.5% inactivation occurred in one-minute blending, and 75% inactivation in two-minute blending.

Kalle, G. P., University of Illinois, Urbana, Ill.—Thymineless Death and Synchronized Division in *Salmonella Typhimurium*. Thymine deficiency was induced in *S. typhimurium* by growing it in a synthetic medium containing glucose, sulfathiazole and a mixture of known end products of folic acid metabolism, including amino acids, purines, and thymine. Bacteria adapted to such a medium depend on thymine for growth. If thymine is removed, they show a typical "thymineless death" as reported by Cohen and Barner for a thymineless mutant of *E. coli* 15 (Proc. Natl. Acad. Sci., 40:885, 1954; and J. Bact. 68:81, 1954) and for *E. coli* B in sulfanilamide medium (J. Bact. 71:588, 1956). For the first half hour after removal of thymine, there is little or no decrease in viable count. Later, the viable count decreases at a logarithmic rate and after the next two hours less than 1% of the cells survive. When bacteria starved of thymine for 30 minutes receive an excess of thymine, there is an initial lag after which the viable count increases exponentially. If instead of thymine an excess of pABA is added, the cells divide sooner and faster than with thymine. If, however, both pABA and thymine are added in excess, the cells, after an initial lag of about 40 minutes, start to multiply synchronously and continue to do so for at least five successive generations. The doubling time of synchronously multiplying cells is 20 minutes for *S. typhimurium*, that is, several minutes shorter than for bacteria growing exponentially in the same medium. Infection with bacteriophage P22 at various stages of the synchronized bacterial growth cycle gives an approximately constant frequency of lysogenization, without any sign of cyclical fluctuations.

Maramorosch, Karl, The Rockefeller Institute for Medical Research, N.Y., N.Y.—Cross-Protection Studies of Two Types of Corn Stunt Virus. The virus disease of corn known as corn stunt was first described in 1945. Recently it was found that two distinct types of corn stunt occur in nature. A study was made to establish whether the two diseases, named Rio Grande and Mesa Central, were caused by strains of the same virus or by unrelated viruses. Both types of virus were transmitted in a greenhouse by two known leafhopper vectors of corn stunt. If a corn leafhopper acquired either type of virus by feeding for one day on a diseased plant, the insect became infective after 2 or 3 weeks and remained infective for life. A large number of insects were fed for 2 weeks on plants with Mesa Central disease, and for the following 2 weeks on plants with Rio Grande disease. These insects transmitted first Mesa Central and afterwards Rio Grande virus. When the order of feeding on diseased plants was reversed, the insects seemed protected against Mesa Central, as only Rio Grande virus was transmitted. In other tests plants were simultaneously infected with both viruses. Signs of Mesa Central were often observed in the beginning, later giving way to the more severe Rio Grande disease. However, doubly infected plants which initially came down with Rio Grande disease never showed signs of Mesa Central. The protection of plants and insects by Rio Grande against Mesa Central virus indicated that these viruses constitute related strains. Rio Grande virus probably moves or multiplies faster and thus suppresses the transmission and symptom expression of Mesa Central.

Niu, M. C., The Rockefeller Institute for Medical Research, New York, N.Y.—It was planned to write an article on embryonic induction and also, if possible, to explore the possibility of studying the incorporation of radioactive amino acids into the giant nerve fibers of the squid. In view of the difficulty of obtaining specimens and keeping them alive in the laboratory, it was not possible to carry out the study of protein synthesis in the giant nerve fibers.

Novick, Aaron, Committee on Biophysics and Department of Microbiology, University of Chicago, Chicago, Ill.—An attempt was made to develop techniques for the determination of the quantity of the enzyme  $\beta$ -galactosidase in a single *E. coli* bacterium. Two methods were tried and gave fair success. In the first, single bacteria in micro droplets under oil were observed with the phase-contrast microscope. As a substrate the non-inducing sugar  $\beta$ -phenyl-galactoside was used. Bacteria with  $\beta$ -galactosidase can utilize this sugar, while bacteria without this enzyme cannot. As a result, bacteria with  $\beta$ -galactosidase grow on  $\beta$ -phenyl-galactoside; but since no new enzyme is synthesized, there will be a decrease in the enzyme per cell as a result of growth. Hence a cell with the enzyme should give descendants whose clone size is determined by the amount of  $\beta$ -galactosidase in the original cell. In the second method bacteria were

plated on medium containing  $\beta$ -phenyl-galactoside as the only substrate and in the presence of an inducer concentration that would support the synthesis of enzyme in induced cells but would not initiate synthesis in un-induced bacteria. Under these conditions the induced cell should form a visible colony while the un-induced should not.

Roman, Herschel, University of Washington, Seattle, Wash.—The routine testing of mutants was continued for the purpose of finding differences among the alleles of each of seven loci affecting the synthesis of adenine in yeast. (The techniques and basic results were described in this summer's Symposium on Quantitative Biology.) Information on the point of physiological block of each of the seven mutant types was sought through the application of the Bratton-Marshall test for diazotizable amines released into the growth medium. The test gave a positive result only for the two mutant types which produce a red cell-limited pigment as a consequence of the block in adenine synthesis, and the amine released by each of the two types gave a color reaction suggestive of 5-amino imidazole ribotide. Some encouraging results were obtained with azaserine which indicate that this antibiotic effectively arrests cell division in yeast and may be useful in studies of mutation.

Wassermann, F. E., New York University College of Medicine, New York, N.Y.—Efforts were made during the summer to determine conditions necessary for effective simultaneous infection of bacterial hosts with phage T5 and some serologically related phages. The aim was to design experiments to investigate the genetic control of serological specificity. In connection with this, parental phages T5 and PB as well as some hybrid phages were further tested for genetic markers suitable for this work.

Watson, J. D., and Risebrough, R. W., The Biological Laboratories, Harvard University, Cambridge, Mass.—Preliminary experiments were done with the small phage—Phi-X174 (F-10). Attempts were made to obtain high titer stocks and to purify this virus for electron microscope examination. A number of host range mutants were isolated and preliminary crosses made to see if the different mutants would give genetic recombination when crossed among themselves.

Yanofsky, C., Department of Microbiology, Western Reserve University School of Medicine, Cleveland, Ohio—Examination of the Tryptophan Synthetase Produced by a Temperature-Sensitive Mutant of *Neurospora*. Only one of the mutants of *Neurospora* blocked in the conversion of indole to tryptophan is capable of growth in the absence of a tryptophan supplement. This strain,  $td_{24}$ , will not grow on unsupplemented minimal medium at 30°C or below but is capable of limited growth at elevated temperatures. Suppressed stocks of this strain exhibit the same type of temperature-sensitivity but grow much better. Experiments were

performed to determine the amount of tryptophan synthetase (the enzyme which converts indole to tryptophan) produced by these strains, and whether an inhibitor of tryptophan synthetase is formed at low temperatures. The results of these analyses revealed that there are trace amounts of tryptophan synthetase in extracts of  $td_{24}$  and  $td_{24}$ -suppressed stocks, but much less than would be expected from the growth rates of these strains. It was also found that the tryptophan synthetase content of these strains was fairly constant regardless of the growth temperature and regardless of whether or not tryptophan was included in the culture medium as a growth supplement. Attempts to detect a tryptophan synthetase inhibitor in the various extracts were unsuccessful as were attempts to increase the tryptophan synthetase activity by various fractionation and enzymatic treatments. Thus we are unable to account for the unusual properties of these strains.

# COURSE ON GENETICS OF FILAMENTOUS FUNGI

June 18-July 7, 1956

Instructors: G. Pontecorvo and E. Käfer, Dept. of Genetics, The University, Glasgow, Scotland

The applicants for this course, offered for the first time in 1956, exceeded the capacity enrollment. This is a measure of the interest in the subject and in particular in the techniques developed at the University of Glasgow for genetic analysis, both through the sexual cycle and through mitotic recombination.

The course consisted of selected exercises with the homothallic *Aspergillus nidulans* and with the asexual *Aspergillus niger*. These exercises included the isolation of nutritional mutants, crossing, synthesis of heterokaryons, synthesis of diploids, analysis of mitotic segregants, and chromosome mapping.

M. S. Fox, The Rockefeller Institute for Medical Research—Pneumococcus transformations.

W. M. Stark, Lilly Research Laboratories—Problems of variation in industrial microbiology. —

E. D. Garber, University of Chicago—A genetic approach to pathogenicity.

W. Szybalski, Rutgers University—A beginning with *Streptomyces* genetics.

R. W. Barratt, Dartmouth College—Allelism at the glutamic dehydrogenase locus in *Neurospora*.

R. H. Pritchard, The University, Glasgow—Negative interference and all that.

E. Käfer, The University, Glasgow—Mitotic haploidization.

C. Yanofsky, Western Reserve University—Genetic control of tryptophan synthetase formation in *Neurospora*.

H. Roman, University of Washington—Genetic Studies in Yeast. Saskatoon, Saskatchewan, Canada

The course was attended by the following students, ten of whom hold doctor's degrees; nine being graduate students. Three of the participants came from industrial research laboratories.



Donald F. Bacon, M.S., Yale University Medical School, New Haven, Conn.

Raymond W. Barratt, Ph.D., Dartmouth College, Hanover, N.H.

Jack Nicol Coetzee, M.D., University of Pretoria, Pretoria, South Africa

John L. Converse, M.S., MB Division, Fort Detrick, Md.

Robert S. Edgar, Biological Labs., University of Rochester, Rochester, N.Y.

Dorothy I. Fennell, B.A., Quartermaster Research and Development Center, U. S. Army, Natick, Mass.

Maurice S. Fox, Ph.D., The Rockefeller Institute for Medical Research, New York, N.Y.

Edward D. Garber, Ph.D., University of Chicago, Chicago, Ill.

William F. Grant, Ph.D., McDonald College of McGill University, Quebec, Canada

Hans Martin, Ph.D., University of Texas, Austin, Tex.

William S. Medart, St. Louis, Mo.

Haruo Ozeki, Carnegie Institution, Cold Spring Harbor, N.Y.

John B. Routien, Ph.D., Chas. Pfizer and Co., Inc., Brooklyn, N.Y.

W. Max Stark, Ph.D., Lilly Research Labs., Indianapolis, Ind.

Constance Thomas, B.S., University of Wisconsin, Madison, Wisc.

Robert Davies Tinline, Ph.D., Science Service, Dept. of Agriculture, Saskatoon, Saskatchewan, Canada

William H. Trejo, B.S., Squibb Inst. for Medical Research, New Brunswick, N.J.

Takashi Yura, Yale University, New Haven, Conn.

Auditor:

Waclaw Szybalski, Ph.D., Rutgers University, New Brunswick, N.J.

## COURSE ON BACTERIOPHAGES

July 13-31, 1956

Instructors: A. H. Doermann, University of Rochester; S. E. Luria, University of Illinois.

Assistant: Robert S. Edgar, University of Rochester.

The intensive three weeks' course on bacteriophages was given for the twelfth time in the summer of 1956. As in the previous summer, the course followed a stepped-up schedule which permitted covering some of the recent developments in the field. A guest instructor, Dr. Norman Melechen of the Carnegie Institution of Washington, supervised an experiment which utilizes the radio-isotopes, phosphorus-32 and sulfur-35. In addition to the intensive laboratory sessions, eight seminars were presented by investigators active in phage research. The speakers and their topics were:

- M. H. Adams, New York University—The enzymes of viruses.
- J. D. Mandell, Carnegie Institution—Inactivation of T4r by anti-serum in distilled water.
- S. E. Luria, University of Illinois—Lysogeny, lysogenization, and all that.
- G. Streisinger, Carnegie Institution—The inheritance of sweetness in T-even bacteriophages.
- J. D. Watson, Harvard University—Structure of small viruses.
- A. H. Doermann and R. S. Edgar, University of Rochester—Genetic recombination in bacteriophage T4.
- N. Melechen, Carnegie Institution—Nucleic acid economy in phage synthesis.
- M. Levine, University of Illinois—Lysogenization with Salmonella bacteriophage.

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

- Donald F. Bacon, M.S., Yale University Medical School, New Haven, Conn.
- Gene M. Brown, Ph.D., Massachusetts Institute of Technology, Cambridge, Mass.

John M. Buchanan, Ph.D., Massachusetts Institute of Technology,  
Cambridge, Mass.

Pei Wen Chen, D.V.M., University of Rhode Island, Kingston, R.I.

Robert Chanock, M.D., The Children's Hospital Research Founda-  
tion, Cincinnati, Ohio.

Jack Nicol Coetzee, M.D., University of Pretoria, South Africa

Joel G. Flaks, Ph.D., Massachusetts Institute of Technology, Cam-  
bridge, Mass.

Tetsuo Iino, University of Wisconsin, Madison, Wisc.

Etta Käfer, Ph.D., The University, Glasgow, Scotland

Eva Kataja, University of Rochester, Rochester, N.Y.

S. M. Kindler, Ph.D., New York University College of Medicine,  
New York, N.Y.

John Leahy, Ph.D., University of California, Berkeley, Calif.

W. David McBride, D.D.S., National Institutes of Health, Bethesda,  
Md.

Newton E. Morton, Ph.D., University of Wisconsin, Madison, Wisc.

R. H. Pritchard, Ph.D., The University, Glasgow, Scotland

Herschel Roman, Ph.D., University of Washington, Seattle, Wash.

Rex S. Spendlove, Ph.D., University of Connecticut, Storrs, Conn.

Thomas B. Stim, M.A., Fort Detrick, Frederick, Md.

Auditor:

Lucy W. Clausen, Columbia University, New York, N.Y.

## COURSE ON BACTERIAL GENETICS

August 2-22, 1956

Instructors: M. Demerec, E. M. Witkin, V. Bryson, P. E. Hartman, and P. D. Skaar, in collaboration with E. L. Lahr and H. Moser.

The course on selected methods in bacterial genetics research, first given in 1950, was offered for the seventh 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:

Donald F. Bacon, M.S., Yale University Medical School, New Haven, Conn.

Earl Beck, Ph.D., Fort Detrick, Frederick, Md.

John M. Buchanan, Ph.D., Massachusetts Institute of Technology, Cambridge, Mass.

Jack Nicol Coetzee, M.D., Institute for Pathology, University of Pretoria, Pretoria, South Africa

Jean M. Cummings, Ph.D., Western Reserve University, Cleveland, Ohio

Memory P. F. Elvin, B.A., University of Pennsylvania, Philadelphia, Pa.

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

Sheldon Goldberg, Ph.D., Institute of Microbiology, Rutgers University, New Brunswick, N.J.

Donald M. Green, Grad. Stud., University of Rochester, Rochester, N.Y.

Arthur P. Harrison, Ph.D., Vanderbilt University, Nashville, Tenn.

Mildred T. Hyatt, M.S., U. S. Quartermaster Research and Development Center, Natick, Mass.

Fred F. Igalsohn, M.A., New York University College of Medicine, New York, N.Y.

John Leahy, Ph.D., University of California, Berkeley, Calif.

Harvard Reiter, Grad. Stud., New York University College of Medicine, New York, N.Y.

Robert A. Roosa, Grad. Stud., University of Pennsylvania School of Medicine, Philadelphia, Pa.

Sidney Silverman, Ph.D., Fort Detrick, Frederick, Md.

Roger Weinberg, Ph.D., Fort Detrick, Frederick, Md.

Sidney Yaverbaum, Ph.D., Fort Detrick, Frederick, Md.

Auditor:

S. A. Mayyasi, Fort Detrick, Frederick, Md.

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

- W. Braun, Institute of Microbiology, Rutgers University—Factors influencing bacterial population changes.
- V. Bryson, Institute of Microbiology, Rutgers University—Bacterial resistance to antibiotics.
- R. C. Clowes, Carnegie Institution—Genetic and biochemical analysis of cystine mutants in *Salmonella*.
- M. Demerec, Carnegie Institution—Transduction in *Salmonella*; the mechanism and some of the results.
- N. H. Giles, Yale University—Genetic control of adenylsuccinase in *Neurospora*.
- P. E. Hartman, Carnegie Institution—Genetic and biochemical analysis of histidine mutants in *Salmonella*.
- S. E. Luria, University of Illinois—Recombination between *Escherichia coli* and *Shigella*.
- P. D. Skaar, Biological Laboratory—Sexual recombination in *Escherichia coli*; mechanism and results.
- E. M. Witkin, College of Medicine, State University of New York—Time factor in induction of mutations by ultraviolet in *Escherichia coli*.
- C. Yanofsky, Western Reserve University—Biochemical and genetic analysis of tryptophan mutants in *Escherichia coli*.
- T. Yura and H. Ozeki, Yale University and Carnegie Institution—Genetic and biochemical analysis of purine mutants in *Salmonella*.

## NATURE STUDY COURSE

July 2—August 3, 1956

August 6—24, 1956

- Instructors: John A. Gustafson, Department of Science, State University Teachers College, Cortland, N.Y.  
Marvin Rosenberg, Biology Instructor, Northport Central High School, Northport, N.Y.  
Jill A. Lamoureux, Port Washington, N.Y.
- Assistants: Shayna Rosenberg, Northport, N.Y.  
Donna Granick, New York, N.Y.

During the summer of 1956 the usual Nature Study Course for children ran for five weeks; from Monday, July 2 to Friday, August 3. Ninety-three children, ranging in age from six to thirteen years, were enrolled in these classes. They were separated into six groups according to age and experience in nature study. Each instructor had two classes. (In August, in response to a request from parents, Marvin Rosenberg conducted a new and special class in which there were 18 registrants).

All classes this year were conducted during the morning hours, which necessitated rearrangement of the hours and a shortening of the class sessions from the traditional two hours, to one and one half hours each. Each class met three times a week instead of twice as in previous years; some classes meeting on Monday, Wednesday and Friday mornings and others meeting on Tuesday, Thursday and Friday mornings. As could be expected, this schedule taxed the facilities and the staff somewhat on Friday mornings. However, the advantage of having all classes meet in the morning offset this disadvantage.

Students were divided into Beginner, Junior, Intermediate, and Senior groups, according to age. Within each of these groups they were further separated on the basis of whether they were first-year students, or repeaters. All first-year students were given a general Nature Study course, while repeaters were given more advanced work in one or more special fields. These special fields were selected on the basis of student interest. They included bird study, marine invertebrate study, insect study, plant study, and earth science. This specialization solved the recurring problem of former years of what to give repeaters which would be challenging to them, and also would develop their interest further.

Wawepex Laboratory was again the headquarters for the courses. Improvements to the building during the preceding winter made it well suited for nature study courses. Each teacher had a room, and there was ample space for the storage and display of "trophies" brought in from

the field. As much time as possible was spent in the field, but time was allocated by each instructor also for the examination of specimens and the necessary indoor preparation that goes with field work.

At the end of the course each class took an extended all-day trip by automobile to parts of Long Island where unusual natural phenomena could be seen. These trips were the highlights of the course, and were very worth-while. Each child brought a picnic lunch, and several of the parents volunteered to drive on each trip. Trips were taken to Jones Beach, Mill Neck, Bayville Beach, and Tackapausha Preserve.

Three Tuesday evenings were devoted to the showing of films in the auditorium of the Lecture Hall. These programs were open to the public, and many children came with parents and friends. Some of the films were shown to younger children on Wednesday mornings.

On Friday, August 3, an open house was held at the Laboratory at which time the work of the students was on display for all to see. Refreshments were served on the lawn.

The following students were enrolled in the course:

Adams, Grace	*Englesberg, Barbara Joan
Adams, Lucy	Galehouse, Shelly
Andrews, John	Gartland, Thomas
Arena, Joseph	Gottlieb, Gail
Arthur, John MacDonald	Griffiths, Mark
Atkins, Gregory Todd	Guille, James
Atkins, Roger Stuart	Harding, Douglas
Augustin, Ronald	**Harris, Jean
**Baer, Melinda C.	Hart, Kathryn
Barnes, Bear	Hicks, John W.
Barnes, Phoebe	Hilbert, Lee
Barnes, Robin	Hoguet, George
Bernheimer, Alan	**Hollander, Jane
Bernstein, Elizabeth	Isaak, Laurie
Bernstein, Theodore A.	Jazombek, Bonnie
Bowne, Mary Elisabeth	Johnson, Benjamin Bates
Buckley, James Lawrence	Kafka, Robert
Camp, Ralph N.	Kane, Kevin
Cooke, George C. III	Karpen, Daniel
Countey, Christopher	**Knight, Jesse W.
Crawford, Jean	Kornblueh, Nancy
Davis, Deborah	Korwan, William S.
*Dropkin, Gregory	Lally, Philip Jeffrey
Drosin, Larry	Lally, Stephen
Elder, Sarah	Luria, Daniel David
Emmet, Katharine T.	**Mack, Stephen

MacMurray, Bruce	Seaman, H. Bogart, Jr.
McGuire, Kevin	Simons, Lavinia A.
Meyer, Allen	**Slater, Robert N.
*Mindell, William	Smith, Douglas
Mulligan, William	*Solomon, Janet
*Muma, Dee	Thayer, William Craig
Nardiello, William	Thiele, Peter S.
Nathan, Laura	Thomson, Paul
Nields, Laura	Thomson, Sydney
**Niu, McYing	Turner, James M.
Norins, Wendy	Wagner, Glenn Arthur
Olson, Gregory	**Wallace, Peter
Page, Donald	Warren, Constance B.
Page, Kenneth	Warren, Virginia G.
Page, Mark N.	**Weissman, Jo.
Pierce, Elizabeth Gay	Werkley, Christopher
Pivnick, Carol	Wheeler, Bruce R.
Powers, Diana C.	White, Katherine Ann
Powers, Francis C., Jr.	*Witken, Joseph
Read, C. S., Jr.	Witzenburg, James
**Roman, Ann	Wyckoff, Curtis
**Schaeffer, Colin Sim	Young, Thomas B. F.
Schaeffer, David E.	Young, William F.
**Schaeffer, Debora	

\*Attended August course.

\*\*Attended both July and August courses.



## WORKSHOP IN NATURE STUDY

June 25-July 6, 1956

Instructors: John A. Gustafson, Department of Science, State University Teachers College, Cortland, N.Y.  
Marvin Rosenberg, Biology Instructor, Northport Central High School, Northport, N.Y.

During 1956 a new course, the Workshop in Nature Study, was offered for the first time. Designed primarily for teachers in the elementary grades, it was offered for credit from the State Education Department under the auspices of the Association. Two in-service credits were awarded to those successfully completing the course of study.

The workshop was conducted from June 25 to July 6, Monday through Friday each week, with sessions from 9:00 a.m. to 12 noon and from 2:00 p.m. to 4:00 p.m. The morning sessions were devoted to field trips to various types of natural habitats, and the afternoon sessions to lectures, discussions, and project work. There were thirteen teachers enrolled, two men and eleven women. Some of them commuted as many as forty miles each way to attend.

The course was designed to give teachers background information and experience in nature study to help them in their teaching careers. It was not a "methods" course, although teaching techniques useful in the elementary classroom were occasionally brought out. Each student chose a project at the beginning, and reports of these projects were given on the morning of the last day. Each student received a certificate from the Association as proof of successful completion of the requirements.

An all-day automobile trip was taken to Tackapausha Preserve, Jones Beach, and the Bayard-Cutting Arboretum. This trip nicely culminated the field work, and was acclaimed as most rewarding.

The workshop seemed to fill a need, and it is planned to conduct it again in 1957. All of the students this year were enthusiastic about the course, and their good word will undoubtedly interest others. Because of their enthusiasm and interest, they were a superior group to teach, and the course as a result was most delightful.

Those in attendance were:

Ackerman, Edna  
Adams, Hazel  
Crawford, Maxine  
Mangi, Martha E.  
Messe, Rebecca  
Milliken, Henry O. Jr.  
Picone, Marye E.

Rapacz, Eleanor  
Reiter, Eleanor  
Rugg, Antoinette J.  
Schoppe, Dorothea  
Thomas, Beatrice M.  
Tomlinson, Alfred

## 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 (in press)

\*Out of print.

## LABORATORY PERSONNEL

- Abramson, Harold A.—Research Psychiatrist  
†Abramson, Harold A., Jr.—Technical Assistant  
Baron, Myrna—Research Assistant  
\*Beaubian, Wendell—Dining Hall Manager  
Bell, Marshall L.—Maintenance Man  
Bennett, James, Jr.—Maintenance Man  
Borsching, Arlene—Typist  
Branton, Geneva—Technical Assistant  
\*Burtch, Ethel—Typist  
\*Conway, Richard—Janitor  
Demerec, M.—Director  
\*Doermann, August H.—Instructor, Bacterial Viruses Course  
Englesberg, Ellis—Bacteriologist  
Franzese, Eleanor—Business Manager  
Fremont-Smith, Nicholas—Research Assistant  
Friedman, Sheila—Research Assistant  
\*Galasso, Mario—Research Assistant  
Gardner, Henry—Technical Assistant  
Gillies, Gloria—Research Assistant  
\*Gustafson, John—Nature Study Course Instructor  
Hadden, Joanna—Research Assistant  
†Hershey, Harriet D.—Research Assistant  
\*Hewitt, M. Cary—Technical Assistant  
†Holmes, Jeanette—Research Assistant  
Hyde, Olive—Administrative Assistant  
Ingraham, Laura—Research Assistant  
Isenberg, Alice—Technical Assistant  
†Jarvik, Murray E.—Psychologist  
Kalish, Judith—Research Assistant  
Kennard, John F.—Gardener  
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

- \*Mayr, Susanne—Technical Assistant
- McMullen, Ellen—Research Assistant
- Meissner, Richard—Superintendent of Buildings and Grounds
- †Milton, Ulysses—Maintenance Man
- Moser, Hermann—Geneticist
- †Myers, Patricia D.—Research Assistant
- Neviackas, Gwendolyn—Stenographer
- \*Olsen, Mary Joan—Technical Assistant
- Reddy, William F.—Maintenance Man
- \*Rolfe, William—Research Assistant
- \*Rosenberg, Marvin—Nature Study Course Instructor
- †Schilling, Harold C.—Carpenter
- Skaar, Palmer D.—Geneticist
- Sklarofsky, Bernard—Psychobiologist
- Sokoloff, Alexander—Guest Investigator
- Sokoloff, Olga—Research Assistant
- \*Somme, Lauritz—Research Assistant
- †Thurston, Robert K.—Superintendent of Buildings and Grounds
- Wallace, Bruce—Geneticist; Assistant Director
- †Wallace, Miriam—Research Assistant
- Warren, Katherine Brehme—Executive Editor of Symposia, and of “Mutants of *D. melanogaster*.”
- \*Wolsk, David—Research Assistant
- \*Zotz, Barbara—Technical Assistant

\*Summer or temporary.

†Resigned during the year.

## SUMMER RESEARCH INVESTIGATORS

- \*Adams, Mark H.—New York University College of Medicine, New York, N.Y.  
Berman, Leonard—New York University College of Medicine, New York, N.Y.  
Bernheimer, Alan W.—New York University College of Medicine, New York, N.Y.  
Blomstrand, Ingbritt—Wesleyan University, Middletown, Conn.  
Caspari, Ernst—Wesleyan University, Middletown, Conn.  
Doermann, A. H.—University of Rochester, Rochester, N.Y.  
Fraser, Dorothy K.—University of Illinois, Urbana, Ill.  
Goldfarb, A. R.—The Chicago Medical School, Chicago, Ill.  
Granick, Sam.—The Rockefeller Institute for Medical Research, New York, N.Y.  
Hotchkiss, Rollin—The Rockefeller Institute for Medical Research, New York, N.Y.  
Igalsohn, Fred. F.—New York University College of Medicine, New York, N.Y.  
Kalle, G. P.—University of Illinois, Urbana, Ill.  
Luria, S. E.—University of Illinois, Urbana, Ill.  
Maramorosch, Karl—The Rockefeller Institute for Medical Research, New York, N.Y.  
Niu, M. C.—The Rockefeller Institute for Medical Research, New York, N.Y.  
Novick, Aaron—University of Chicago, Chicago, Ill.  
Risebrough, R. W.—Harvard University, Cambridge, Mass.  
Roman, Herschel—University of Washington, Seattle, Wash.  
Wasserman, F. E.—New York University College of Medicine, New York, N.Y.  
Watson, James D.—Harvard University, Cambridge, Mass.  
Yanofsky, Charles—Western Reserve University, Cleveland, Ohio

\*Deceased

## REPORT OF THE SECRETARY

A meeting of the Executive Committee was held at the home of President Ames on July 12, 1956, with Dr. Demerec and five members present. The annual financial statement was discussed; dates were chosen for the Open House (September 23) and the Board of Directors' meeting (November 25). Mr. Hoyt Ammidon was nominated for membership on the Board of Directors to fill the vacancy in the class of 1957 caused by the resignation of Mr. Roosevelt.

The 33rd Annual Meeting of the Association was held in the Lecture Hall at Cold Spring Harbor on July 24, 1956, with President Ames presiding and 22 members present. The chief acts of the Association during the previous year were reviewed by the Secretary, the report being duly accepted. The following members were named by the Nominating Committee and elected or re-elected to the Board of Directors to serve until 1960: Dr. Mark Adams, Dr. Crispin Cooke, Mrs. G. S. Franklin, Dr. E. C. MacDowell, Mr. William B. Nichols, Mrs. A. M. White, Jr., and Dr. B. H. Willier. Mr. Hoyt Ammidon was elected to fill the vacancy in the class of 1957. The report of the Treasurer, indicating that the year had ended with a small cash balance, was approved. Attention was called to the magnitude of the operation, which in the previous year had exceeded \$220,000. The report of the Laboratory Director, Dr. Demerec (see Annual Report—1955-56) was accepted. President Ames described a new pamphlet which was being prepared, interpreting the aims and activities of the Biological Laboratory.

The 73rd meeting of the Board of Directors of the Association was held on November 25, 1956 in the Lecture Hall at Cold Spring Harbor, with nine members present and Mr. Ames presiding. Regret was expressed at the death of Mr. Marshall Field, charter member and for 23 years an officer of the Board, and of Dr. Mark Adams, a member of the Board since 1952. The Assistant Secretary was instructed to forward letters to the families of these members, expressing the deep sympathy of the Board and acknowledging its own loss of these valued members. A fellowship fund was then established by the Board in memory of Dr. Adams. Mrs. Franklin reported on the proposal of a Women's Committee to raise \$1,000 from a small, selected group for the refurbishing of Blackford Hall. Dr. Demerec reported briefly on the activities of the preceding summer (see Annual Report 1955-'56). The usefulness of the new motel was noted. Dr. Vernon Bryson was elected a member of the Board of Directors to fill the vacancy in the Class of 1960 caused by the death of Dr. Mark H. Adams.

The 74th meeting of the Board of Directors was held on April 28, 1957 in the Lecture Hall at Cold Spring Harbor, with 15 members present and President Ames presiding.

Minutes of the previous meeting were reviewed, approved and ordered placed on file. Discussion of the proposed budget, the personnel and current problems ensued. Work of a special committee on the refurbishing of Blackford Hall was noted; and mention was made of the public meeting being planned for June 7th in connection with the Planned Parenthood Group, at which a panel of Symposium speakers will discuss population problems. After full discussion of the budget, it was formally approved. The date of October 27, 1957 was proposed for the next meeting of the Board of Directors.

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, 1957 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, 1957 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 except with respect to depreciation on equipment. Such depreciation has been charged against net worth whereas for the preceding year ended April 30, 1956 it had been charged against that year's operations. The effect of this change has been a reduction in the excess of expense over income for the year ended April 30, 1957 by \$11,588.06.

MAIN AND COMPANY  
Certified Public Accountants

New York, N.Y.  
June 24, 1957



## BALANCE SHEET

April 30, 1957

### ASSETS

General and Endowment Fund			
Cash			\$ 30,973.22
Investments (market value \$71,881.54)			69,992.00
Accounts receivable:			
On grants and contracts for special research		\$28,368.72	
Other	\$7,728.34		
Less: Reserve for uncollectible accounts	1,093.74	6,634.60	35,003.32
Inventory of books			16,086.44
Deferred expenses			7,310.50
Land, buildings and equipment			292,891.61
			<u>\$452,257.09</u>
Special Funds			
Cash in Bank			\$ 1,438.62
Investments (market value \$15,454.58)			15,708.00
			<u>17,146.62</u>
<b>Total</b>			<b>\$469,403.71</b>

## LIABILITIES AND NET WORTH

### General and Endowment Fund

#### Liabilities:

Accounts payable	\$ 15,447.52	
Accrued payroll and taxes	1,881.06	
Grants and contracts for special research	75,988.59	

#### Total liabilities

\$ 93,317.17

Reserve for Scientific Research

20,000.00

Endowment Fund:

Dr. William J. Matheson Bequest

20,000.00

Net worth

318,939.92 \$452,257.09

### Special Funds

Blackford Memorial Fund:

Principal \$ 5,000.00

Charles Benedict Davenport

Memorial Fund:

Principal \$4,934.75

Unexpended income 1,204.95 6,139.70

Charles Benedict Davenport,

Junior, Fund:

Principal 1,037.12

Temple Prime Scholarship Fund:

Principal \$2,500.00

Unexpended income 105.50 2,605.50

Dorothy Frances Rice Fund:

Principal \$2,304.84

Unexpended income 59.46 2,364.30 17,146.62

#### Total

\$469,403.71

## STATEMENT OF NET WORTH

For the Year Ended April 30, 1957

Balance, May 1, 1956		\$354,916.46
Less:		
Additional provision for obsolescence of volumes of yearly Symposia of Quantitative Biology published prior to May 1, 1954	\$17,036.35	
Excess of expense over income for year ended April 30, 1957	7,352.13	
Depreciation for year on equipment	11,588.06	35,976.54
Balance, April 30, 1957		\$318,939.82

# STATEMENT OF GENERAL FUND INVESTMENTS

April 30, 1957

No. of Shares or Par Value		Book Value	Market or Redemp- tion Value
11	American Can Company	\$ 491.75	\$ 488.13
12	American Telephone and Telegraph Company	1,794.07	2,133.00
\$2,000	Dow Chemical Company Convertible Debenture Bonds, 3% due 7/1/82	2,062.50	2,602.50
22	General Motors Corporation	995.50	937.75
50	General Motors Corporation, \$5.00 Preferred Stock	5,012.50	5,575.00
\$5,000	General Motors Acceptance Corporation Debentures, 3½% due 3/15/72	4,937.50	4,718.75
\$5,000	General Telephone Corporation, 15-year Convertible Debentures, 4% due 5/1/71	5,287.50	5,175.00
21	Merck and Company, Inc. \$4.00 Second Cumulative Convertible Preferred Stock	2,443.88	2,383.50
4	Minerals and Chemicals Corporation of America	137.65	117.50
300	Northwest Production Corporation	1,956.15	1,950.00
124	Phillips Petroleum Company	4,420.49	6,029.50
\$5,000	Phillips Petroleum Company, Convertible Subordinated Debentures, 4¼% due 2/15/87	5,412.50	5,618.75
4	Pittsburgh Plate Glass Company	330.50	310.00
\$5,000	Twelve Federal Intermediate Credit Banks Consolidated Collateral Trust Deben- tures, 3.20% due 5/1/57	4,996.88	5,000.00
\$5,000	Twelve Federal Land Banks Consolidated Federal Farm Loan Bonds, 2¾% due 5/1/58	4,937.50	4,948.44
\$5,000	Twelve Federal Land Banks Consolidated Federal Farm Loan Bonds, 2¼% due 5/1/59	4,834.38	4,840.63

9	Square D Corporation	254.25	264.38
\$5,000	United States Treasury Bonds, 2¼% due 12/15/62-59	4,906.25	4,690.63
\$5,000	United States Treasury Bonds, 2½%, due 6/15/72-67	5,018.75	4,484.38
\$10,000	United States Treasury Bonds, 2½%, due 11/15/61	9,687.50	9,525.00
\$100*	United States Savings Bond, Series F, due 9/1/60	74.00	88.70
	<b>Totals</b>	<u>\$69,992.00</u>	<u>\$71,881.54</u>
* Maturity Value			

### STATEMENT OF SPECIAL FUND INVESTMENTS

April 30, 1957

Maturity Value		Book Value	Market or Redemption Value
\$ 500*	United States Treasury Bond, 2½%, due 3/15/70-65	\$ 500.00	\$ 449.38
100	United States Savings Bond, Series J, due 9/1/68	72.00	72.40
1,400	United States Savings Bond, Series F, due 9/1/60	1,036.00	1,241.80
14,000	United States Savings Bonds, Series K, 2.76%, due 9/1/66	14,000.00	13,594.00
100	United States Savings Bond, 2½% Series G, due 9/1/60	100.00	97.00
	<b>Totals</b>	<u>\$15,708.00</u>	<u>\$15,454.58</u>
* Par Value			

## LAND, BUILDINGS AND EQUIPMENT

April 30, 1957

Land:			
Purchased with funds raised through public subscription	\$52,198.22		
Land purchase from Estate of Mary E. Jones	15,674.99		
Henry W. de Forest land	12,000.00		
Airlsie 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:			
Airlsie building	\$ 5,000.00		
Blackford Hall*	19,000.00		
Cole Cottage	2,105.00		
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	120,656.19	
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		23,176.12	34,764.20
<b>Total</b>			<b>\$292,891.61</b>

\* Built on land leased from Wawepex Society.

## STATEMENT OF INCOME AND EXPENSE

Income: For the Year Ended April 30, 1957

Grants, contracts and research fees		\$181,797.83
Contributions:		
Dues	\$ 9,851.75	
Wawepex Society	2,100.00	
John D. Jones Scholarship	700.00	
Walter B. James Fund	180.00	12,831.75
Book sales—Symposium of Quantitative Biology		18,042.23
Dining hall		17,050.45
Rooms and apartments		17,295.10
Summer activities		10,306.36
Registration fees for annual symposium		270.00
Interest and dividends on investments		1,569.81
<b>Total income</b>		<b>\$259,163.53</b>
Expense:		
Expenditures directly chargeable against grants and contracts for special research:		
Salaries	\$100,514.59	
Supplies	33,083.38	
Expense of participants in annual symposium and related costs	22,500.00	
Other expenses	7,151.34	\$163,249.31
Cost of books sold and publication expense—		
Symposium of Quantitative Biology		8,775.59
Dining hall		21,114.34
Rooms and apartments		10,669.37
Research expense		2,586.43
Summer Activities		5,283.12
Distribution of John D. Jones Scholarship		715.00
Buildings and grounds maintenance:		
Salaries	\$ 18,350.92	
Materials and supplies	7,947.61	
Heat, light and water	4,061.50	30,360.03
General and administrative:		
Salaries	\$ 8,946.69	
Insurance	1,921.81	
Printing and stationery	1,733.73	
Telephone, telegraph and postage	942.46	
Equipment	261.35	
Other	4,650.19	18,456.23
Provision for loss on accounts receivable		314.24
Provision for obsolescence of inventory of books		4,992.00
<b>Total expense</b>		<b>\$266,515.66</b>
Excess of expense over income		<b>\$ 7,352.13</b>

STATEMENT OF UNEXPENDED GRANTS AND CONTRACTS FOR SPECIAL RESEARCH  
April 30, 1957

	Authorized Amount	Amount Expended to April 30, 1957 (Including fees to Association)	Unexpended Balance April 30, 1957
Josiah Macy, Jr. Foundation			
Three year grants expiring June 30, 1958	\$ 46,200.00	\$ 10,716.57	\$ 35,483.43
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	11,491.98	8,308.02
National Science Foundation			
Eighteen month grant expiring July 31, 1958	12,500.00	1,334.57	11,165.43
Special summer research grant from National Science Science Foundation			
Grant for three years expiring June 30, 1958	15,000.00	12,480.14	2,519.86
Grants for annual symposia:			
Carnegie Corporation	15,000.00		15,000.00
National Science Foundation	6,500.00		6,500.00
The Population Council, Inc.	5,000.00		5,000.00
United States Public Health Service	5,175.00		5,175.00
United States Atomic Energy Commission:			
One year contract expiring June 14, 1957	19,850.00	15,986.44	3,863.56
One year contract expiring February 28, 1958	38,610.00	6,028.17	32,581.83
United States Department of the Army, Office of the Surgeon General			
One year contract expiring May 31, 1957	33,087.00	25,814.71	7,272.29
United States Department of the Navy, Office of Naval Research			
Three year contract expiring March 31, 1958	30,360.00	19,703.81	10,656.19
United States Public Health Service			
One year grant expiring December 31, 1957	17,012.00	12,238.96	4,773.04
	<b>\$266,184.00</b>	<b>\$116,500.10</b>	<b>\$149,683.90</b>

# ORGANIZATION OF THE ASSOCIATION

## OFFICERS

President Amyas Ames	Vice-President Mrs. Walter H. Page	Secretary E. C. MacDowell
Vice-President & Treasurer Grinnell Morris	Assistant Secretary B. P. Kaufmann	
Laboratory Director: M. Demerec		Assistant Director: Bruce Wallace

## BOARD OF DIRECTORS

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 .....	Huntington, 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. Alexander M. White, Jr. ....	Oyster Bay, 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.
Grinnell Morris .....	Oyster Bay, N.Y.
Arthur W. Page .....	Huntington, N.Y.
Franz Schneider .....	Oyster Bay, N.Y.

To serve until 1958

Amyas Ames .....	Cold Spring Harbor, N.Y.
George W. Corner .....	Carnegie Institution
Th. Dobzhansky .....	Columbia University
Mrs. Maitland A. Edey .....	Brookville, N.Y.
Rollin D. Hotchkiss .....	Rockefeller Institute
Ernst Mayr .....	Harvard University
Mrs. Walter H. Page .....	Cold Spring Harbor, N.Y.

Members Emeriti

R. C. Leffingwell .....	Oyster Bay, N.Y.
Ross G. Harrison .....	Yale University



## EXECUTIVE COMMITTEE

Amyas Ames

Mrs. G. S. Franklin

William B. Nichols

E. C. MacDowell

Arthur W. Page

Grinnell Morris

Mrs. Walter H. Page

## WOMEN'S COMMITTEE

Chairman—Mrs. Alexander M. White, Jr.

Vice-Chairman—Mrs. George S. Franklin

Secretary—Mrs. Edward S. Blagden

Treasurer—Mrs. Walter H. Page

## FINANCE COMMITTEE

Grinnell Morris

William B. Nichols

Amyas Ames

## BUILDINGS AND GROUNDS

Mrs. George S. Franklin, Chairman

Mrs. Percy Jennings

B. P. Kaufmann

William B. Nichols

## SCIENTIFIC ADVISORY COMMITTEE

George W. Corner, Chairman

L. C. Dunn

E. C. MacDowell

Edwin J. Grace

Alfred E. Mirsky

Alexander Hollaender

## FORMER PRESIDENTS, LABORATORY DIRECTORS, AND BOARD MEMBERS

### Presidents

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

### Laboratory Directors

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

### Directors

Abbott, Lyman 1896-1901	de Forest, H. W. 1912-17; 1924-25
Adams, Mark H. 1951-56	de Forest, R. W. 1902-17
Atkins, C. D. 1915-23	Denbigh, J. H. 1923
Ayer, J. C. 1930-33	Detwiler, S. R. 1928-42
Ayres, H. M. 1892-1900	Doubleday, F. N. 1908-11
Backus, T. J. 1890-1901	Draper, George 1924-43
Blackford, Eugene 1890-1904	Field, Marshall 1924-47
Blackford, Mrs. Eugene 1906-17	Fisher, G. C. 1924
Bleeker, C. M. 1926-45	Fisk, H. D. 1924
Bleeker, T. B. 1946-51	Flinsch, Rudolph 1909-17
Blum, E. C. 1923	Francis, Mrs. L. W. 1923
Boody, D. A. 1890-1917	Frick, Childs 1924-29
Brackett, G. C. 1904-08	Gager, C. S. 1915-17
Brower, G. V. 1899-1917	Hall, C. H. 1890-95
Brown, Addison 1890-1913	Harris, R. G. 1930-36
Brown, J. S. 1908-17	Harrison, R. G. 1926-51
Bumpus, H. C. 1903-12; 1927-30	Haskins, Caryl P. 1946-55
Butler, N.M. 1903-17	Healy, A. A. 1896-1921
Chambers, Robert 1932-54	Heckscher, August 1902-17
Cochran, D. H. 1890-1902	Hendrix, Joseph 1890-97
Cole, K. S. 1940-43	Hicks, Henry 1924-53
Cole, W. H. 1934-52	Hoagland, C. N. 1890-98
Coombs, W. J. 1890-1910	Hooper, F. W. 1890-1914
Crittenden, W. H. 1922-23	Hoyt, Colgate 1902-17
Crozier, W. J. 1928-44	Hulst, G. D. 1894-1900
Davenport, C. B. 1903-44	Huntington, L. D. 1894-1900
Davenport, W. B. 1916-17	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  
 Mayer, A. G. 1903-17  
 Merle-Smith, Mrs. Van S. 1931-50  
 Mickleborough, John 1890-1917  
 Mills, D. H. 1946-52  
 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  
 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. 1927-56  
 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  
 Stimson, H. L. 1925-36  
 Smith, H. C. 1913-17  
 Stewart, J. H. J. 1893-1917; 1924-26  
 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. 1923-43  
 Webb, Alexander 1890-1902  
 Weld, F. M. 1914-17  
 Wetmore, C. W. 1902-07  
 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	John & Mary Markle Foundation
Mrs. Ethel Clyde	Mrs. Van Santvoord Merle-Smith
Mrs. Henry W. de Forest	Arthur W. Page
Mrs. Leonard Elmhirst	Rockefeller Foundation
Marshall Field	John M. Schiff
Russell C. Leffingwell	Wawepex Society

## PATRONS

Contributions of at least \$500

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

## SUSTAINING MEMBERS

<p>W. H. Alston            Amyas Ames            Charles E. Ames            Mrs. Charles E. Ames            Hoyt Ammidon            Mrs. Henry H. Anderson            Mrs. Donald Arthur, Jr.            Mrs. Paul Atkins            Mrs. Daniel Bacon            C. E. Barkalow            Mrs. C. E. Barkalow            Edmund Bartlett            Mrs. Edmund Bartlett            Lloyd V. Berkner            Frederick Bernheim            Alan Bernheimer            Loren C. Berry            Sydney Bevin            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            Louis H. Buck            Mrs. Louis H. Buck            Dean Burk            W. Burns            Mrs. Towbridge Callaway            Mrs. H. Schuyler Cammann            John L. Carey            E. W. Caspari            McKean Cattell</p>	<p>Frank L. Clough            Mrs. Henry E. Coe            William Rodgers Coe, Jr.            Mrs. William Rodgers Coe, Jr.            John K. Colgate            Mrs. John K. Colgate            Robert B. Colgate            Crispin Cooke            George W. Corner            Duncan Cox            Mrs. Duncan Cox            John L. Cox, 2nd            Clinton H. Crane            Mrs. Clinton H. Crane            Miner D. Crary, Jr.            Mrs. Miner D. Crary, Jr.            Arthur M. Crocker            Paul Cushman            Mrs. Paul Cushman            F. Trubee Davison            Mrs. F. Trubee Davison            Henry P. Davison, Jr.            Mrs. Henry P. Davison            Raymond de Clairville            Mrs. Henry W. deForest            Robert F. de Graff            Max Delbruck            M. Demerec            Mrs. M. Demerec            Mrs. William Denby            Mrs. Richard Derby            Th. Dobzhansky            J. P. Downer            Russell E. Duvernoy            Walter K. Earle            Ferdinand Eberstadt            Mrs. Ferdinand Eberstadt            Mrs. Maitland A. Edey            Joseph R. Eggert, Jr.            Mrs. Joseph R. Eggert, Jr.            Henry Ehlers, Jr.            Mrs. Henry Ehlers, Jr.            George L. Fair</p>
---	---

Julian D. Fairchild  
Ugo Fano  
M. H. Farnham  
Ernst Fischer  
George H. Fonde  
Mrs. George H. Fonde  
Nevil Ford  
Mrs. Nevil Ford  
Mrs. George S. Franklin  
George S. Franklin, Jr.  
Childs Frick  
A. H. Funnell  
Chauncey B. Garver  
Mrs. William C. Gay  
John Gerdes  
Mrs. John Gerdes  
Mrs. E. S. Gilman  
Mrs. Robert N. Gilmore  
H. Bentley Glass  
C. Fitzhugh Gordon  
Joseph Gots  
H. A. Gottfried  
Edwin J. Grace  
Charles V. Graham  
Walter S. Gubelmann  
Arthur Gwynne  
Mrs. Arthur Gwynne  
Mrs. Joanna J. Hadden  
Morris Hadley  
Mrs. Winston Hagen  
Bruce Wood Hall  
Mrs. Joseph W. Hambuechen  
Paul L. Hammond  
Mrs. Paul L. Hammond  
Mrs. Montgomery Hare  
Henry U. Harris  
Augustin Hart, Jr.  
Mrs. Augustin S. Hart  
Caryl P. Haskins  
Mrs. Caryl P. Haskins  
Charles F. Havemeyer  
Horace Havemeyer, Jr.  
R. W. Hawkins  
R. Graham Heiner  
Mrs. R. Graham Heiner  
J. E. Hellier

Charles L. Hewitt  
Mrs. Robert L. Hoguet, Jr.  
Alexander Hollaender  
Davenport Hooker  
Clarence A. Horn  
Mrs. George S. Hornblower  
William B. Hornblower  
Rollin D. Hotchkiss  
J. Taylor Howell  
Mrs. J. Taylor Howell  
Oliver Iselin  
Mrs. Myron Jackson  
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  
E. Elizabeth Jones  
Robert Kafka  
Mrs. Robert Kafka  
Martin D. Kamen  
Morris I. Karpen  
Mrs. Morris I. Karpen  
B. P. Kaufmann  
Francis C. Kiel, Jr.  
Mrs. Francis C. Keil, Jr.  
James C. King  
William A. Korwan  
Ernest L. Lahr  
Mrs. Ernest L. Lahr  
Orin T. Leach  
Mrs. Randall J. Le Boeuf, Jr.  
Mrs. P. H. T. Le Boutillier  
Mrs. Burton J. Lee, Sr.  
Russell C. Leffingwell  
Mrs. Russell C. Leffingwell  
Paul E. Letz  
Mrs. Paul E. Letz  
David A. Lindsay  
Mrs. David A. Lindsay  
George N. Lindsay, Jr.  
Mrs. George N. Lindsay, Jr.  
Mrs. Vladimir S. Littauer

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  
Mrs. John M. Martin  
William H. Mathers  
Ernest Mayr  
Mrs. Harvey C. McClintock  
Ross McFarland  
Mrs. Ross McFarland  
John Meirs  
Mrs. Van S. Merle-Smith  
Leo M. Meyer  
William H. Miller  
Alfred E. Mirsky  
Mrs. Douglas M. Moffat  
Walter V. Moffitt  
Grinnell Morris  
Mrs. Grinnell Morris  
Mrs. Ray Morris  
Hermann Moser  
Stuart Mudd  
John R. Muma  
Mrs. John R. Muma  
Charles J. Mundo, Jr.  
Mrs. Alfred E. Munier  
Robert Cushman Murphy  
Vincent A. Nardiello, Jr.  
James V. Neel  
Winthrop Neilson  
Mrs. Winthrop Nielson  
H. H. Neumann  
Mrs. George Nichols  
John Treadwell Nichols  
William Nichols  
John W. Nields  
Mrs. John W. Nields  
Anthony J. Nittoli  
Hawley M. Norins  
Juliet Nourse  
Mrs. Charles P. Noyes

Mrs. D. Chester Noyes  
Robert G. Olmsted  
George D. O'Neill  
Mrs. George D. O'Neill  
Arthur W. Page  
John H. Page  
Mrs. Walter H. Page  
Isabel Peters  
William C. Pierce  
Esther Pivnick  
Francis T. P. Plimpton  
Mrs. Arthur W. Pope  
Keith R. Porter  
Mrs. Edward Everett Post  
Frank C. Powell  
Francis C. Powers  
Mrs. Francis C. Powers  
Mrs. Charles Pratt  
Mrs. Frederick R. Pratt  
H. Irving Pratt  
Mrs. H. Irving Pratt  
Richardson Pratt  
Theodore H. Price  
G. Hale Pulsifer  
Mrs. G. Hale Pulsifer  
E. Racker  
Roland L. Redmond  
Mrs. Roland L. Redmond  
Mrs. Lansing P. Reed  
Mrs. Gordon Rentschler  
Oscar W. Richards  
Bernard J. Ridder  
Mrs. Bernard J. Ridder  
R. Oliver Rippere  
Harry C. Robb  
John K. Roosevelt  
Walter N. Rothschild, Jr.  
Mrs. Walter N. Rothschild, Jr.  
J. A. Rousmaniere  
Theodore S. Roxlau  
Mrs. Stanley M. Rumbough  
Russwood Drugs, Inc.  
John Rutherford  
Theodore F. Savage  
Mrs. Theodore F. Savage  
John M. Schiff

Richard Schlaugies  
Francis O. Schmitt  
Franz Schneider  
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  
Theodore E. Stebbins  
Curt Stern  
Mrs. M. Chase Stone  
Mrs. Richard Storrs  
Edward K. Straus  
Waclaw Szybalski  
Mrs. T. C. Takami  
Eugene S. Taliferro  
Mrs. Eugene S. Taliaferro  
Stanley Tarrant  
E. P. Taylor, Jr.  
Mrs. Henry C. Taylor  
Evan W. Thomas, II  
Mrs. Evan W. Thomas, II  
Norman Thomas  
Irving A. Tittler  
Ernest T. Turner  
William J. Turner  
D. S. Tuthill  
Mrs. Edmund S. Twining, Jr.  
Philip Wadsworth

Mrs. Philip Wadsworth  
Roy A. Waggener  
Jean Irwin Wagner  
Mrs. Jean Irwin Wagner  
Bruce Wallace  
William J. Wardall  
Ethelbert Warfield  
Bradford A. Warner  
Mrs. Bradford A. Warner  
Charles O. Warren  
Katherine Brehme Warren  
Gordon J. Watt  
Wawepex Society  
Mrs. Percy S. Weeks  
David Weld  
Mrs. David Weld  
Mrs. Francis M. Weld  
Thomas G. Wheelock  
Alexander M. White  
Mrs. Alexander M. White  
John C. White, Jr.  
Mrs. John C. White, Jr.  
Douglas Williams  
B. H. Willier  
William W. Willock, Jr.  
Henry S. Wingate  
J. A. Wiseman  
David I. Wolsk  
Guy W. Wood  
W. Wilton Wood, Inc.  
Mrs. Willis D. Wood  
Sewall Wright  
Mrs. Herbert H. Zeese



## 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 - L A W S**

### **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, 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. He and the Secretary shall jointly execute on behalf of the Association all contracts, leases and other corporate instruments.

Sec. 2. The Vice Presidents shall perform the duties of President in order of their seniority in case of the absence or inability of the President.

Sec. 3. 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. 4. 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. 5. 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 or 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.

July—1957.

---

## A BEQUEST FOR THE BIOLOGICAL LABORATORY

The Biological Laboratory of the Long Island Biological Association, whose work and organization are 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 375 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