Annual Report

OF

THE BIOLOGICAL LABORATORY

1958-1959

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-Ninth Year

1958-1959

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

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REVIEW OF THE YEAR

A Message to the Members

The past year has been an active one for the Laboratory with many accomplishments and a further focusing of the problems ahead of us. The 1958 summer courses for research workers and the summer nature study course for children were well filled and in fact oversubscribed. Our year-round research program was greatly strengthened with the appointment of Dr. Arthur Chovnick who joined us in January, 1959. Dr. Chovnick has also been made Assistant Director of the Laboratory and has assumed many of the administrative functions of the Director. Our XXIV Annual Symposium held in June, 1959, on the topic "Genetics and Twentieth Century Darwinism" was a success with two hundred participants including guests from fifteen foreign countries.

During the past year a Committee of Directors of the Association headed by Dr. Crispin Cooke conducted a membership drive which almost doubled our individual supporters. For the 1957-58 year our membership was 385, and it is now about 700. As shown in our financial report, membership contributions this year totalled $13,777 compared to $8,850 a year earlier. The significance of increased support from our neighbors is increaseingly evident in our efforts to obtain research or other grants outside the community.

As mentioned in detail in the Director's report, we have received a sizable four-year grant from the National Science Foundation to rehabilitate our facilities, both for housing and research. This will fill a long felt and important need for the Laboratory.

Some progress has been made toward providing an adequate research building for our year-round staff, and development of a "research stabilization fund". This year will be critical and considerable effort to this end must be made in order to assure continuation of our outstanding research program.

In anticipation of the retirement next year of our Laboratory Director, Dr. Demerec, a summary of the current status and future plans for the Laboratory has been prepared. To aid the Board of Directors in determing a wise and constructive policy to insure a vigorous, outstanding, and broadening program in the future, we have been most fortunate to persuade a group of distinguished scientists to advise us. The committee which will meet at the Loboratory shortly consists of Dr. Edward L. Tatum, Chairman; Drs. S. E. Luria, Ernst Caspari, J. D. Watson, Edgar Zwilling, and W. D. McElroy. We are most grateful and deeply indebted to these gentlemen for their interest and help.

The coming months can be of great significance to our Laboratory and we bespeak your increased support as it approaches its seventieth year of service to mankind.

Nevil Ford
Chairman

Walter H. Page
President

REPORT OF THE DIRECTOR

Our efforts to work out long-range plans for the functioning of the Biological Laboratory have continued during the past year. There is general agreement that year-round research constitutes the key activity of the Laboratory, for it supplies the essential background for all our other activities, including the Symposia, summer courses, and summer research. If we are to maintain this research program at a high level, we must provide staff members with good working facilities and give them some assurance of continuity with the Laboratory. At present we cannot meet either of these requirements: research facilities are not adequate, nor do we have a financial reserve to assure the salaries of key staff members. Efforts are therefore being concentrated on two objects, the construction of a modern laboratory building and the accumulation of a "research assurance" fund. In the meantime, arrangements have been made with the administration of the Carnegie Institution of Washington to extend for a three-year period our use of laboratory facilities in one of the buildings of the Department of Genetics where much of our research is now located. In line with these long-term plans, a campaign was conducted during the past year to interest more members of the community in the work of the Laboratory. This drive was very successful and local membership in the Association has more than doubled.

Recently we obtained a four-year grant, in the amount of $135,000, for improvement of our facilities, particularly those related to summer activities. Part of the first year's allotment has been used to install equipment for cafeteria service in the dining room, and to build another motel-like residence building which provides 8 additional rooms and 6 baths for the accommodation of summer guests.

The Laboratory has been included this year in the educational program of the National Science Foundation, and will conduct two new summer projects, a Summer Conference for college teachers of genetics, and an Undergraduate Research Participation Program. The genetics conference is designed to give three weeks of training in recent developments in genetics to twenty college teachers; and the undergraduate program will make it possible for ten college students to assist various members of the staff in research during the summer months. Our Nature Study program benefits this year from the establishment of ten scholarships for students in Suffolk County schools by the Huntington Federal Savings and Loan Association.

STAFF

Major changes have occurred during the year among both research and office staff. Dr. Bruce Wallace, assistant director, resigned to join the faculty of Cornell University, and Dr. J. C. King moved his research program to Columbia University. Dr. Arthur Chovnick came to us from the University of Connecticut in January, 1959, to take the position of

assistant director. Other additions to the research staff were: Dr. Paul Margolin and Dr. Abraham Schalet, who joined the group working in bacterial genetics, and Dr. Horace M. Mazzone, who became associated with the electron microscopy group.

Miss Olive Hyde, administrative assistant, left us in the spring of 1959 for family reasons. Her place was taken by Miss Clara Wooldridge, who will also have charge of editing the Symposium volume. Mrs. Henry O. Nilsson was appointed assistant to the business manager.

RESEARCH

Concurrently with changes in the scientific staff, the pattern of research at the Laboratory has undergone modifications. Dr. Chovnick brought with him from Connecticut an extensive program of studies of genic structure, employing Drosphila as experimental material, as a replacement for Dr. Wallace's work on population genetics. Our research schedule has been enlarged by studies with the electron microscope being conducted under the direction of Dr. B. P. Kaufmann.

The accomplishments of the various research units, reported more fully in a later section of this Report, are summarized briefly below.

After a short period spent in establishing and equipping his laboratory, Arthur Chovnick now has investigations under way on several problems. For some time he has been studying the structural and functional organization of a small region of genetic material concerned with the production of red eye pigment in the fruit fly, Drosophila melanogaster. Three active sites have now been uncovered within this functional unit. A major feature of the analysis is that the region under investigation represents the smallest unit thus far resolved in a macroorganism. Revealed in the course of this study was an unusual phenomenon, which has been called "gene conversion." Experiments designed to answer a number of questions about the phenomenon have indicated quite clearly that it is related to genetic recombination, but differs in certain ways from the classical process of "crossing over." Quite possibly this occurrence is a regular event, overlooked in past investigations, which is discernible only in analyses of very small regions of genetic material.

James C. King, during a second year of a study of development aspects of population genetics, has concentrated on rates of development. In a population consisting of three discrete strains of Drosphila, measurements were taken at several embryonic stages. It was found that the different strains had different growth rates at the various development stages but that hatching time was the same for all. Phenotypes in a crossbreeding population which has reached equilibrium are determined by complicated biochemical systems. Biochemical reactions hold the integrated genetic system within limits, so that any given population has its proper pattern of development. Since October, 1958, Dr. King's project has been continued at Columbia University.

Paul Margolin has begun a study of the genetic locus that controls production of the amino acid, leucine, in the bacterium Salmonella typhimurium. By means of the technique known as transduction, he has determined the linear order, within this locus, of thirty-two independent sites at which spontaneous mutations may occur, and has studied their complementation patterns. Working with the various leucine-requiring mutants (auxotrophs), he has compared their capacities to mutate back to normal leucine production (prototrophy) under the influence of treatment with 2-aminopurine or ultraviolet light. Auxotrophs resulting from mutation at different sites proved to have different, characteristic, rates of induced back-mutation.

A. Schalet, also working with Salmonella typhimurium, has studied induced mutability at ten different sites of a locus governing the production of another amino acid, tryptophane. The chemicals used to induce mutation were ethyl sulfate, ethyl methanesulfonate, bromoethyl methanesulfonate—all alkylating agents—and manganous chloride. Although all four chemicals induced mutations at some of the sites, manganous chloride was in general the most potent mutagen. Each chemical elicited a specific pattern of mutational response; but on the whole the patterns produced by the three alkylating agents resembled one another more closely than they resembled the patterns associated with manganous chloride and with ultraviolet radiation (the latter studied by another investigator). This finding is consistent with the idea that alkylating properties play a major role in bringing about mutagenic effects.

Hermann Moser has continued his studies of spontaneous and induced changes in human cells grown in vitro. Information obtained by investigating the molecular nutritional requirements of single epithelial somatic cells made it possible to establish a chemically defined minimal medium in which to grow heteroploid human cells. Such a medium is useful for the rapid selection of mutants which are deficient in certain biochemical cell functions. Dr. Moser also has begun selection experiments designed to detect inheritable radiation resistance in somatic cell populations grown in the laboratory.

In collaboration with Dr. Irwin Fand, of Creedmoor State Hospital and Adelphi College, Dr. Moser has undertaken some experiments, by the clonal cell culture method, to demonstrate the cytotoxic effects reported by Fedorov to be associated with acute mental illness. The preliminary results show that cytotoxicity in patients with acute paranoid schizophrenia ranges from 40 to 80 per cent higher than in normal control subjects.

The work of installing and adjusting the new electron microscope, directed by B. P. Kaufmann, has been under way for many months. The instrument, acquired under the terms of a grant from the National Institutes of Health, is now in good operating condition for the program to study fine structure of cellular components. Photographs can be

taken at a direct magnification of 200,000 X, which can be increased by enlargement to a potential magnification of nearly 1,000,000 X or a useful working magnification of about 800,000 X. Studies of chromosomes during somatic and meiotic mitoses have been begun. Also, in cooperation with Dr. Moser, a combination biochemical, cytochemical, and electron microscope analysis is being made of the differences between progenitor and mutant forms in mammalian cells grown in tissue cultures. Dr. Horace Mazzone joined the electron microscopy program in January, 1959.

H. A. Abramson and his collaborators have continued their studies of the psychobiological effects of LSD-25 (lysergic acid diethylamide), and have also investigated such new derivatives as LME (methyl ethyl LSD), LMP (methyl propyl LSD), LMI (methyl isopropyl LSD), and LEP (ethyl propyl LSD). Experiments have been made with Siamese fighting fish and with volunteer, non-psychotic human subjects, to test the effects of substances which block the aberrant behavior reaction usually elicited by LSD. Variables involved in the blocking response are being studied. It has also been found that treatment with some derivatives of LSD leads to an acquired tolerance for the chemical. Observations of effects of LSD treatment on communication among schizophrenic patients are being continued at Central Islip State Hospital.

Alexander Sokoloff has been studying the phenotypic variation produced by changes in climate and food supply in Drosophila populations originating in different localities. His findings so far seem to show that lower temperatures result in larger individuals. When strong competition for food arises, however, flies develop a minimal survival size, and the effect of temperature on size is nullified. While taking measurements of mounted wings of D. pseudoobscura, Dr. Sokoloff found a number of specimens with folded ventral bristles; and this character also appeared among laboratory strains of D. melanogaster. Further studies will be made of the genetics of this character.

Katherine Brehme-Warren has continued her preparation of a second edition of the reference book "The Mutants of Drosophila melanogaster." First published in 1944 under the authorship of Dr. Calvin B. Bridges and Dr. Brehme-Warren, the book has been out of print for several years. The extensive work of preparing a revised edition is being sponsored by the National Science Foundation, and the volume will be published by the Carnegie Institution of Washington.

Brief reports about the research of summer investigators will be found in a later section of the report.

SYMPOSIUM

The twenty-fourth Cold Spring Harbor Symposium on Quantitative Biology, held during the eight days from June 3rd to 10th, 1959, was

attended by about 200 scientists, including 30 from other countries. The topic of this year's meetnig was "Genetics and 20th Century Darwinism," and its purpose was to bring together outstanding investigators in the fields of genetics, ecology, anthropology, and paleontology who are interested in problems of organic evolution. By organizing the meeting, the Biological Laboratory joined a number of scientific organizations throughout the world in recognizing the 100th anniversary of the publication of Charles Darwin's book, "Origin of Species."

The program dealt with the theory of the gene, the raw materials of evolution, natural selection and race formation, ecological systems and social organization, fossil records as they relate to rates of evolution, and trends in evolution. The introductory lecture, "Where are we?" was delivered by Professor Ernst Mayr of Harvard University, and the concluding lecture, "The synthetic approach to organic evolution," was given by Professor G. L. Stebbins of the University of California. A committee composed of Professors Th. Dobzhansky (chairman), C. S. Coon, and Bruce Wallace was in charge of the organization of the program.

The Symposium was supported by funds received for the purpose from the Carnegie Corporation of New York, the National Science Foundation, The Rockefeller Foundation, the U. S. Atomic Energy Commission, and the U. S. Public Health Service.

TEACHING

The children's Nature Study Courses during the summer of 1958 were expanded in scope and interest, and attracted a large number of students. Mr. Marvin J. Rosenberg, of the Department of Science, Northport Central High School, was again in charge of the program, with Mrs. Jill A. Lamoureux of Port Washington and Mr. Otto Heck of Levittown as collaborators. Miss Donna Granick, who had assisted with the course for the past five years, joined the staff of regular instructors this year. Assistants were Misses Gail Garaghty, Gay Adams, and Cynthia Hotchkiss, and Mr. Struan Robertson. As an innovation this year, students were able to choose from a variety of specialized subject-matter classes in addition to the general nature study offered previously. Conservation, entomology, seashore life, and vertebrate zoology were among the most popular of the special subjects taught. Enrollment in the main classes, held during July, was 147; and 20 students took part in a supplementary session in General Nature Study during August.

Fourteen elementary school teachers were enrolled in the two-week Workshop in Nature Study, offered in 1958 for the third season. 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 participating teachers upon their completion of the course.

The course in techniques and problems of research with bacterial viruses was offered to research workers and graduate students for the fourteenth consecutive time in the summer of 1958. Dr. F .W. Stahl, of the University of Missouri, and Professor S. E. Luria, of the University of Illinois, were in charge of the course, assisted by C. M. Steinberg of the California Institute of Technology. A number of prominent scientists in this field of research contributed to the course as special lecturers. Eighteen students and two auditors were enrolled.

Following the bacterial viruses course, the ninth session of the course in selected methods of bacterial genetics research continued for three weeks during July. This course, too, had a capacity enrollment of eighteen students plus one auditor. It was conducted by Drs. M. Demerec, Evelyn M. Witkin, V. Bryson, and P. E. Hartman; and a schedule of lectures by other noted workers in the field was arranged to complement the teaching program.

A new advanced course, offered for the first time in the summer of 1958, dealt with methods of growing and analyzing human cells in laboratory tissue cultures. It was taught by Dr. H. Moser of the Biological Laboratory and Dr. E. H. Y. Chu of Yale University, assisted by Leila Diamond, Sloan-Kettering Institute for Cancer Research, Jean Thompson, Huntington, New York, and Dr. K. Tomizawa, the Biological Laboratory. Ten seminar lectures, presented by investigators active in tissue culture or related fields, supplemented the laboratory sessions; and a demonstration of tissue culture glassware was arranged. Fourteen students and six auditors took part in this course.

LECTURES

As part of the 1958 program of courses, invited speakers presented a series of seminar lectures, open to all members of the Laboratory as well as visitors from neighboring scientific institutions. During the Nature Study courses, movie films shown on two Tuesday evenings to students, parents, and friends were well attended.

Professor Mogens Westergaard, of the University of Copenhagen, one of the Laboratory's distinguished foreign visitors during the summer of 1958, gave an illustrated lecture on Tuesday, July 15, for members of the Long Island Biological Association and their friends. Dr. Westergaard, a member of the Board of Governors of the Artic Institute of North America, spoke about Greenland, the country and its flowers, and showed slides of his own color photographs of Artic flora.

The Lecture Hall was used by several local groups and organizations during the year covered by this report. On September 10, 1958, Mr. Oakes A. Plimpton showed a collection of color pictures taken by him during a Natural History Museum expedition to Baja, California, and a trip to Mexico. The annual meeting of The Nature Conservancy, Long Island Chapter, was held in the auditorium on Friday evening, November

21. On January 29, 1959, the Huntington League of Women Voters arranged a meeting for the public, addressed by an adviser to the Department of State, on the subject of foreign relations. The American Association of University Women, Huntington Township Branch, used the auditorium for an educational meeting on March 18; and the Board of Directors of The Nature Conservancy met in the lounge on May 25.

On June 10 and 11, immediately after the close of the 1959 Symposium meetings, the Lecture Hall and surrounding lawns were the scene of an experimental documentary filming, televised under the direction of the American Institute of Biological Sciences, to be used for biology teaching at the college level. The film, on the subject of speciation, featured informal discussion among several prominent biologists and paleontologists, most of whom had been participants in the Symposium program.

SPECIAL EVENTS

The annual open house demonstration and tea was held on Sunday afternoon, September 21, 1958, and was attended, in spite of very unfavorable weather, by a large number of Association members and their friends. Scientific exhibits displayed in Blackford Hall illustrated current projects at the Laboratory and at the neighboring Department of Genetics; and staff members were on hand to demonstrate the exhibits and discuss their research with the visitors. At 4:30 p.m. Dr. Lloyd V. Berkner, President of Associated Universities, Inc., gave an informal address in the Lecture Hall on "The Meaning of Research." Tea was then served in the dining room under the supervision of Mrs. J. Taylor Howell and other members of the Women's Committee.

SCHOLARSHIPS

In the summer of 1958 the following students and investigators received scholarships from the Laboratory, to help toward their tuition fees and living expenses.

John D. Jones Scholarships: Dr. S. Banic, Instiute of Microbiololgy, Medical Faculty, Ljubljana, Yugoslavia; LuBelle Boice, University of Illinois, Urbana, Ill.; Morton Davidson, New York University, New York; Dr. Thomas D. Grace, The Rockefeller Insttiute for Medical Research, New York, N.Y.; Dr. Karl Maramorosch, The Rockefeller Institute for Medical Research; and Bernard Weisblum, State University of New York, Brooklyn, N.Y.

U. S. Public Health Service Research Trainee Stipends: Richard Almond, Harvard University, Cambridge, Mass.; Dr. Elias Balbinder, Carnegie Institution of Washington, Cold Spring Harbor; Robert C. Baumiller, Fusz Memorial Bellarmine House of Studies, St. Louis, Mo.; Dr. David G. Catcheside, The University, Birmingham, England; Dr. Carlo Cocito, Institute of Microbiology, Rutgers University, New Brunswick, N.J.; Morton Davidson, New York University College of Medicine,

New York, N.Y.; Dr. Fritz Kaudewitz, Max Planck Institute for Virus Research, Tubingen, Germany; Dr. Urs Leupold, Institute of General Botany, Zurich, Switzerland; Marian Martinello, New York University College of Medicine, New York, N.Y.; Milton Schlesinger, University of Michigan, Ann Arbor, Mich.; Drasko Serman, Johns Hopkins University, Baltimore, Md.; Dr. G. S. Watson, Australian National University, Canberra, Australia; and Jeanette Winter, New York University College of Medicine, New York, N.Y.

BUILDINGS AND GROUNDS

With funds from the National Science Foundation grant mentioned earlier, we began the work for general improvement of our facilities. A new "wing" for the Arthur W. Page Building was constructed according to plans prepared by Mr. E. Everett Post, architect. This addition has eight rooms and six bathrooms. Part of the first floor of Hooper House was remodeled to make an apartment consisting of two rooms, kitchen, and bathroom. Extensive remodeling was also carried out in the north apartment of the Henry W. de Forest Building. The Walter B. James Laboratory was adapted for use by Dr. Chovnick and his group. Equipment for cafeteria style service was purchased and set up in the Blackford Hall dining room; and two additional air-conditioning units were installed in the Davenport Laboratory room where the summer courses are conducted.

FINANCES

The expenses of the Symposia, of full-time research, of part of the Laboratory's educational program, and of special improvements to its facilities, are being met by grants received from the organizations mentioned in the following section.

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

ACKNOWLEDGMENTS

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

For research of Dr. Bruce Wallace, terminated in August, 1958; the U. S. Atomic Energy Commission.

For research of Dr. J. C. King, transferred in September, 1958, to Columbia University: the National Science Foundation.

For research of Dr. A. Chovnick and his group: National Science Foundation and U. S. Public Health Service.

For research of Dr. B. P. Kaufmann and Dr. Horace M. Mazzone: U. S. Public Health Service.

For research of Dr. H. Moser: U. S. Atomic Energy Commission.

For research of Dr. H. A. Abramson and his group: Josiah Macy, Jr. Foundation.

For research by Dr. P. Margolin and Dr. A. Schalet: U. S. Public Health Service.

For research of Dr. A. Sokoloff: National Science Foundation.

For preparation of a second edition of "The Mutants of Drosophila Melanogaster," by Dr. Katherine Brehme-Warren: National Science Foundation.

For the Cold Spring Harbor Symposia: Carnegie Corporation of New York, National Science Foundation, The Rockefeller Foundation, U. S. Atomic Energy Commission, and U. S. Public Health Service.

For a Summer Conference for College Teachers of Genetics: National Science Foundation.

For an Undergraduate Research Participation Program: National Science Foundation.

For the summer courses for research workers and graduates: U. S. Public Health Service.

For rehabilitation, renovation, and enlargement of facilities: National Science Foundation.

I wish to express our sincere thanks to the following members of the Association, who entertained our guests at dinner parties during the 1959 Symposium: Mr. and Mrs. William Rogers Coe, Jr., Dr. and Mrs. Crispin Cooke, Mr. and Mrs. Paul Cushman, Mr. and Mrs. Robert de Graff, Mr. and Mrs. Ferdinand Eberstadt, Mr. and Mrs. Maitland A. Edey, Mr. and Mrs. Joseph R. Eggert, Jr., Mr. and Mrs. Richard S. Emmet, Mrs. George S. Franklin, Mr. and Mrs. Nevil Ford, Mr. and Mrs. David Ingraham, Mrs. Burton J. Lee, Mr. and Mrs. Robert Lindsay, Mr. and Mrs. William B. Nichols, Mr. and Mrs. Walter H. Page, Mr. and Mrs. Franz Schneider, and Mr. and Mrs. Cornelius W. Wickersham, Jr. We are especially grateful to Mrs. David Ingraham for her efficient organization of the dinner party schedule.

It gives me pleasure to acknowledge the assistance of Mrs. J. Taylor Howell and other members of the Women's Committee who provided refreshments for the Open House Demonstration and Tea last September.

We owe a special debt of gratitude to the Membership Committee, and in particular to Mrs. David Ingraham and Mrs. William S. Smoot, for their active and effective participation in the campaign for new members.

Our many thanks go once again to the Wawepex Society for its continued generous support of the Laboratory, and to the friends who contributed this year, either as old or as new members of the Association.

REPORTS OF LABORATORY STAFF

STUDY OF A COMPLEX LOCUS IN DROSPHILA

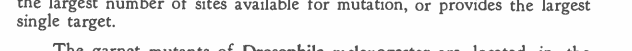
Arthur Chovnick, David Weisbrot and Jane MacMillan

The now common phenomenon of pseudoallelism has served to focus attention upon the operational nature of our notions concerning the structural and functional organization of the genetic material. Investigations of the spatial relationships of functionally related alterations of the genetic material in Drosophila consistently reveal a small number of distinct genetic loci. Moreover, clustered about each of the spatially distinct loci, there are mutants which have not exhibited recombination inter se; and these constitute members of a multiple allelic system in the classic sense. These observations suggest a genetic fine structure not sensibly altered from the classic view of discrete genetic units separated by non-genic materials, with recombination restricted to the non-genic, interstitial, regions. By way of contrast, similar systems in microorganisms exhibit a high degree of spatial resolution. Such systems lend favor to the notion of a segment of genetic material to be effectively a continuum, capable of alteration at many separable sites.

In order to contribute to the resolution of the apparent discrepancy between microbial and macroorganism studies, investigation was initiated into the structural organization of the garnet locus of **Drosophila melanogaster.** The logic behind the selection of the garnet locus stems from an earlier report by Muller that the garnet locus responds to X-irradiation with the highest frequency of visible mutation for any locus in this organism. This observation suggested that perhaps the garnet locus has the largest number of sites available for mutation, or provides the largest single target.

The garnet mutants of **Drosophila melanogaster** are located in the vicinity of 44.4 on the X-chromosome. These mutants alter the pigmentation of the eyes and larval Malphigian tubules. Initial experiments designed to investigate the possibility of pseudoallelism at this focus were performed as follows:

Females were produced which were heterozygous for all combinations of the mutants g, g^2 and g^{50}. In addition, such females were heterozygous for marker genes adjacent to garnet. The markers used in this study and their locations are:



dy—dusky; s—sable; g—garnet; pl—pleated; sd—scalloped; f—forked.

Assay of segregating of the heterozygous females was made by crossing to males with X-chromosomes carrying a garnet mutant, and one of the closely linked markers. In Table 1, there are recorded data collected in three groups of experiments involving an assay of 405,183 segregating

X-chromosomes from heterozygous females. Most of the assayed chromosomes carried a garnet mutant, and may, or may not, have carried one or more of the closely linked markers. Twelve exceptional (non-garnet) individuals were recovered, and successfully bred to verify their X-chromosomal constitutions. The non-garnet chromosomes thus recovered fell into two classes. Eight possessed marker genes which indicated that they were associated with recombination in the garnet region (non-starred, Table 1). The remaining four non-garnet chromosomes (starred, Table 1) possessed markers indicating no recombination in the garnet region.

An interpretation of these data in terms of recombination within a complex locus ordinarily would demand that all of the exceptional chromosomes be associated with marker recombination. Several cases of aberrant segregations termed "gene conversions" have been found in microbial studies. The results of these studies, and the present study, resemble markedly the behavior of the mutable reddish-alpha locus of **Drosophila virilis** studied by Demerec. Reddish-alpha was seen to "mutate" to wild-type only in heterozygotes, and such "mutation" was correlated with recombination in the region of the locus. However, not all mutations of reddish-alpha were associated with marker recombination.

Table 1

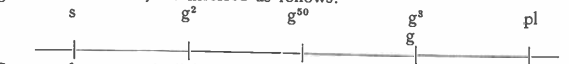
Assay of Segregating X-Chromosomes From Free-X Females of The Indicated Genotypes

Group	Female Genotype	Non-Garnet Chromosomes Recovered					Total Chromosomes Assayed
		s + pl	s++	+++	dy + pl	dy++	
1. A	s g +/+ g^2 pl	2	-	-	-	-	68,051
B	dy g +/+ g^2 pl	-	-	-	1	-	65,251
C	dy g + sd/+ g^2 pl +	-	-	1*	1	-	33,200
2.	s g^{50e} +/+ g^2 pl	2	1*	-	-	-	73,499
3.	dy g pl/+ g^{50e} +	-	-	2*	-	2	165,182
Totals							405,183

It will be seen from the data to be presented, that for the garnet locus, an interpretation in terms of our modern understanding of genetic fine structure, is inescapable. Moreover, a similar interpretation of reddish-alpha would serve to clarify this most interesting system.

Restricting attention for the moment to those non-garnet chromosomes associated with marker recombination (non-starred, Table 1) the

regular behavior of marker movement is consistent with that found in pseudoallelic recombination. The recombinations of Groups 1 and 2 (Table 1) place g^{50} and g to the right of g^2. The recombinations of Group 3 place g to the right of g^{50}. Thus a pseudoallelic order of the garnet mutants may be inferred as follows:



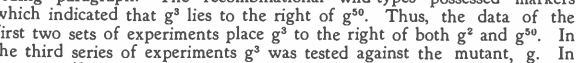
Support for a pseudoallelic interpretation of the data of Table 1 comes from experiments with other garnet mutants.

Evidence From Studies With The Mutant, g^3

Introduction of a fourth mutant, g^3, to the study, may serve as a test for the pseudoallelic interpretation of the preliminary data of Table 1. If we are indeed dealing with pseudoalleles, then the pattern of recombinations with g^3 should be consistent, and localize g^3 in the garnet segment. Experiments were carried out assaying meiotic products of all heterozygous combinations between g^3 and the mutants studied earlier. In addition to studying free-X females, attached-X heterozygotes were assayed The significance of incorporating attached-X heterozygotes into the study will be discussed in a later section.

The results obtained with free-X and attached-X females were quite consistent and served to complement each other. Non-garnet chromosomes were recovered from g^2/g^3 heterozygotes. These fell into two categories, those associated with recombination for the adjacent markers, and those which have been classified as aberrant segregants. Restricting attention to the recombinational wild-types, the data indicate that g^3 falls to the right of g^2.

Studies investigating the location of g^3 with respect to the mutant g^{50} yielded wild-types, in the same two categories as described in the preceding paragraph. The recombinational wild-types possessed markers which indicated that g^3 lies to the right of g^{50}. Thus, the data of the first two sets of experiments place g^3 to the right of both g^2 and g^{50}. In the third series of experiments g^3 was tested against the mutant, g. In 197,875 offspring assayed from both free-X and attached-X females, no wild-type individuals were recovered from this heterozygous combination. The complete failure to obtain non-garnet chromosomes from g/g^3 heterozygotes in both free-X and attacher-X chromosomes consistently places g^3 to the right of g^2 and g^{50}, localized to the region of g. The linkage relations of the four garnet mutants thus far tested many be summarized as follows:



One feature of interest in connection with the g^3 experiments, is the fact that in this series we find the first heterozygote which fails to yield

recombinational wild-types. It should be noted, that failure to recover recombinational wild-types is paralleled with a similar failure to recover aberrant segregants.

Evidence From Studies With The Mutant, g*

A fifth mutant, g*, serves as a novel sources of evidence for the pseudoallelic interpretation described above. Attempts to mark the g* bearing chromosome with the closely linked markers, s(sable) and pl (pleated) failed repeatedly. A linkage experiment was carried out, and it was seen that crossing over in the regions v-dy-g-f is suppressed radically in the presence of g*. In summary, the linkage data indicated that g*, a radiation induced mutant, is associated with a rearrangement whose breaks are to the right of dusky, and to the left of forked. Examination of salivary gland chromosomes of g* heterozygotes revealed a small inversion in the garnet region.

In earlier sections, it was inferred that the origin of non-garnet chromosomes from mutant heterozygotes is associated with crossing over within a complex locus. If this interpretation is correct, then heterozygotes of g* and all other garnet mutants should fail to yield non-garnet segregants due to the suppression of crossing over in the garnet region afford by the inversion associated with g*. Experiments were run assaying segregating X-chromosomes of heterzygotes of g* and mutants marking each end of the garnet segment (g^2 and g^3). From some 98,000 segregating X-chromosomes recovered from the heterozygotes g^*/g^2, no wild-types were recovered. Similarly from g^*/g^3 heterozygotes, approximately 79,000 chromosomes were recovered, and none were wild-type. It should be emphasized again, that the failure to recover non-garnet chromosomes associated with recombination for adjacent markers is correlated with the failure to recover aberrant segregants. In a negative fashion, these experiments support the pseudoallelic interpretation as described above.

* Denotes a small inversion in garnet region

Experiments With The Mutant g^{s1}

In Table 2, are summarized data obtained from experiments with a sixth mutant, the spontaneous mutant g^{s1}. From heterozygous females, g^{50}/g^{s1}, five non-garnet chromosomes were recovered in approximately 133,000 chromosomes assayed. Four individuals survived to reproduce, two yielding chromosomes exhibiting marker recombination, and two were aberrant segregants (starred, Table 2). The marker recombination in the non-garnet chromosomes associated with marker recombination, reveal that g^{s1} lies to the right of g^{50}, presumably in the cluster with g and g^3. A more interesting experiment involved a repetition of this experiment with one slight, but most significant variation. In this second experiment, g^{s_1} was tested against g^{50}, but the g^{s1} bearing chromosome also contained two major inversions straddling the garnet region suppress-

Table 2

Assay of Segregating X-Chromosomes From Free-X Females of The Indicated Genotypes

Female Genotype	Non-Garnet Chromosomes Recovered			Total Chromosomes Assayed
	s**	***	Died without progeny	
$\frac{s\ g^{50e}\ +}{+\ g^{s1}\ sd}$	2*	2	1	132,930
$\frac{s\ g^{50e}}{In(1)\ d\ 1\ kg,\ v\ g^{s1}\ In(1)\ B^{M1}}$	0	0	0	122,262
Totals				255,200

ing crossing over most radically. In this second experiment (Table 2) it should be noted that no wild-type chromosomes of any type were recovered in 122,262 chromosomes assayed. In addition to localizing g^{s1} in the garnet segment, these experiments also support the notion of the complex locus nature of garnet. Here again we see that suppression of crossing over leads to suppression of both recombinational wild-types and aberrant segregants.

The Double Mutant Chromosome

The data presented above lead to the conclusion that we are dealing with recombination between pseudoalleles at the garnet locus. It should be noted that throughout this investigation, such recombination is studied from the non-garnet chromosomes arising from mutant heterozygotes. According to classic notions concerning recombinational phenomena, we should expect that the recombinations involved:

(1) A reciprocal exchange of parts between the synapsed chromosomes.
(2) Such exchange should take place at exactly the same point on each chromosome.

Thus, by assay of meiotic products of a pseudoallele heterozygote, we should recover the double mutant chromosome in addition to the wild-type crossover product. From the free-X studies, it was impossible to detect any individuals that might have carried such a crossover product. It was inferred that such a chromosome was produced, and that the individuals carrying this chromosome could not be distinguished from individuals carrying a single mutant chromosome.

In order to examine this question further, we turned to study recombination in attached-X heterozygotes. The assay procedures used are the now, well established, procedures described by Lewis and others. The major feature provided by the attached-X females is that one recovers two of the four strands involved in a meiotic event. Thus, it is possible

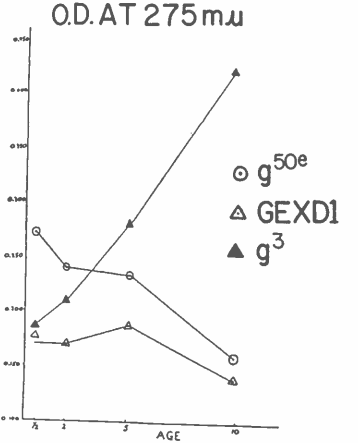
to recover the double mutant strand (if it is produced) in the same individual possessing the non-garnet chromosome.

The results obtained to date from a study of several systems of attached-X heterozygotes are entirely consistent with the inferences drawn from the free-X studies. From one crossover category, it should be possible to recover a double mutant-bearing chromosome. When such presumptive double mutants are isolated, they are found to be indistinguishable (by eye) from one or the other single mutant. In order to establish the genetic constitution of presumptive double mutants, two operational procedures are possible. These are described in the following paragraphs.

Physiological Identification Of The Double Mutant

Another aspect of our investigation is concerned with the functional relationships of the garnet pseudoalleles. From these studies, it was seen that the garnet mutants produce quantitative changes in two groups of eye pigments, believed to be related in their biosynthesis. The two groups of pigments are the yellow, fluorescent pteridines and the red pigment complex. Spectrophotometric studies of pigment extracts indicate that the former has a maximum optical density in the ultra-violet at 275 μ, while the latter reaches a peak at 485 μ.

From study of the attached-X heterozygote $sg^{50}+/+g^{3}pl$, there were recovered both presumptive double mutant chromosomes, and presumptive single mutant chromosomes. These chromosomes, along with



known g^{50} and g^3 bearing chromosomes were subjected to a series of studies designed to identify chromosomal types. The results of these experiments are summarized in Figure 1.

A series of replicate cultures were grown at 25°C. Adult males emerging within defined time limitations were set in cultures to age for varying time periods before pigment extraction. Extracts of eye pigment from males of the three genotypes g^{50}, g^3 and GEX-D1 (presumptive double mutant) were assayed for both red and yellow components. The experiment was run several times with the same results. GEX-D1 is significantly different from both g^{50} and g^3 when the time course of development of yellow pigment is examined. In Figure 1, it can be seen that g^3 and GEX-D1 start out at twelve hours with approximately the same pigment concentration. However, the pigment concentration in g^3 increases with time to approach a value near that of the wild-type. On the other hand, GEX-D1 declines to a very low value by ten days. The g^{50} strain starts out with a pigment concentration very much greater than g^3 and GEX-D1, and declines until it reaches approximately the same value as GEX-D1 at ten days.

With respect to the red pigment complex, g^{50} and GEX-D1 cannot be separated, both maintaining very low levels of this pigment over a ten day period. However, both differ from g^3 in that the concentration of red pigment in g^3 individuals increases in time.

Control spectrophotometric experiments with garnet bearing chromosomes isolated from the attached-X heterozygote, and believed to be single mutant bearing were tested. The results confirmed the distribution of marker genes identifying the strand as carrying single mutants.

Recombination Studies To Identify The Double Mutant Chromosome

A second demonstration of the double mutant nature of GEX-D1 is possible by means of recombination experiments. The most direct approach would be to produce heterozygous females, g^{50}, $g^3/+$ +, and to assay the meiotic products of such females for crossovers carrying either g^{50} or g^3 alone. This approach cannot succeed since it would be impossible to distinguish the single mutants from the double mutant at all times. Another approach would be to produce three categories of heterozygous females ($+g^{50}g^3/++g^3$; $+g^{50}g^3/g^{50}+$; $+g^{50}g^3/g^2++$), and to assay their meiotic products for non-garnet recombination products. If GED-D1 is a double mutant, then non-garnet chromosomes should be recovered only from the heterozygous combination $+g^{50}g^3/g^2++$. This experiment was carried out, and the results obtained are summarized in Table 3. The data of Table 3, along with the physiological data described in the previous section provide conclusive evidence of the production of

Table 3

Recombination Studies To Verify The Double Mutant GEX-D1, Isolated From Attached-X Females Heterozygous for g^3 and g^{50e}.

Genotype	+++	Total
$\frac{GEX\text{-}D1}{+ + g^3}$	0	71,679
$\frac{GEX\text{-}D1}{+ g^{50e} +}$	0	79,250
$\frac{GEX\text{-}D1}{g^2 + +}$	2	39,867
Total	2	190,996

multiple mutants as reciprocal products of crossing over within a complex locus.

Aberrant Segregants Or "Gene Conversion" At The Garnet Locus

Turning next to the aberrant segregants observed throughout the course of this investigation, two questions concerning these aberrant types were asked:

a. Do they represent changes in the garnet segment of the X-chromosome, or might they involve mutation at some other locus in the form of a suppressor or garnet?

b. Muller (1946) and Schalet (1957) studying radiation induced and spontaneous visible mutation respectively, have recovered garnet mutations. Among the garnets thus recovered, there have been several subliminal or wild-type isoalleles. These mutants are wild-type when homozygous, but exhibit a garnet phenotype when heterozygous with certain garnet mutants. Should the aberrant segregants be due to changes with-

in the garnet segment, might they be partial or subliminal mutations similar to the wild-type isoalleles?

Three types of experiments were performed which were designed to answer these questions. This material is published elsewhere, and a summary of the conclusions drawn follows:

The origin of the aberrant garnets does not involve suppressor mutation, but is concerned with an alteration within the garnet segment of the X-chromosome. The aberrant segregants produce a phenotype indistinguishable from that produced by the wild-type, and thus are not partial mutants.

The next question of interest is further concerned with the nature of the process giving rise to the aberrant segregants. Are they the result of a random process such as spontaneous reverse mutation or is their origin in some way associated with the specific genotypes from which they segregated? All of the aberrant segregants might be explained as complete reverse mutations of one or another of the garnet mutants. Estimate of the frequency of occurrence of such events, according to this hypothesis, in the genotypes in which they occurred is $q=1.3 \times 10^{-5}$. Should spontaneous reverse mutation be the mode of origin of the aberrant segregants, then such events would be expected to occur in all genotypes, including homozygotes with a similar low frequency.

On the right hand side of Table 4 there are listed six genotypes from which no aberrant segregants were recovered. The maximum mutation frequency permitted by these data for a null event, using a 5% Poisson confidence limit is $q=4 \times 10^{-6}$. On the basis of these data, it is possible to reject the hypothesis of spontaneous reverse mutation.

Table 4

Summary of Recombination and "Conversion" Data

Female Genotype	Recombinations	"Conversions"	n	Female Genotype	Recombinations	"Conversions"	n
g^2/g^*	4	1	166,502	g^{50e}/g^{50e}	0	0	177,286
$+g^{50e}+/+ g^2 pl$	2	1	73,499	g^3/g^3	0	0	108,141
$+ g^{50e}+/+ g^3 pl$	6	1	78,200	$dy\ g\ pl/+ g^3 +$	0	0	197,875
$+g^{50e}+/dy\ g\ pl$	2	2	165,182	$+g^3 pl/dy\ g^w +$	0	0	78,685
$+g^3+/+ g^2 pl$	6	4	208,872	$+g^2 pl/dy\ g^w +$	0	0	97,841
$+ g^{50e}+/+ g^{el}\ ad$	2	2	132,938	$\frac{l^{J1}\ ac^{J1}\ In\ 49\ v^{el}g^{H1}}{+\ g^{50e}}$	0	0	122,262
Totals	22	11	825,193		0	0	782,090

* several marker combinations

Returning to Table 4, it should be noted that here are listed all of the genotypes that have been tested to date. On the left side of the

Table are listed those genotypes from which "conversions" emerge. In all genotypes from which recombinational wild-types occurred, "conversions" also were obtained. On the right hand side of the Table are listed those genotypes from which no recombinants were obtained. No "conversions" were obtained in the homozygotes, nor were they recovered from our heterozygotes that did not yield recombinants. The g/g^3 heterozygotes yielded no recombinants, permitting the localization of g^3 and g to the same general region of the garnet segment. There was no recombinants from g^3/g^*, and g^2/g^* genotypes, and this is associated with an inversion suppressing recombination in the garnet region. The fourth heterozygous system of interest is the $g^{50}e/g^{s1}$ combination. Such heterozygotes yield both recombinations and conversion (Table 4, left side). By inserting major inversions on the g^{s1} bearing chromosome in regions which lead to suppression of crossing over in the garnet region it is seen (Table 4, right side) that both recombination and conversion are suppressed.

From these observations, it becomes clear that conversion may involve an exchange between homologous chromosomes. The next question that is to be asked of this system concerns the nature of the exchange. Does this exchange, like crossing over, produce a reciprocal strand that is different from the original strand before conversion? It is possible to ask this question in experiments utilizing attached-X heterozygous females. The operational approach to answering this question, follows the approach used in identifying double mutants (see earlier discussion). Should it turn out that the reciprocal strand is different than it was before conversion, then it would be clear that we are dealing with a reciprocal exchange process that does not involve movement of adjacent markers (possibly double crossing over). On the other hand, should we find that no reciprocal exchange occurs in conversion then we know that we are dealing with a new type of event. The experiments designed to answer this question are now in progress, and the direction of future investigation depends upon the outcome of these experiments.

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THE RADIATION PROJECT

Bruce Wallace and C. V. Madden

Research on irradiated Drosophila populations and on viability mutations was terminated during the past year. No experimental work was done beyond that reported in the last Annual Report. The final six months under Contract No. AT-(30-1)-557, U. S. Atomic Energy Commission, were spent in making calculations, preparing manuscripts, and in an occasional visit to the Cornell University campus where facilities were being prepared in the Department of Plant Breeding for a continuation of this type of study.

POPULATION STUDIES ON EMBRYONIC DEVELOPMENT RATES

James C. King

In the Annual Report for 1957-58 a summary was given of the first year's work on a research project designed to investigate the developmental aspects of population genetics. Since that report was written, the work has been concentrated on the study of developmental rates mentioned there on page 23.

By means of the techniques described in the earlier report, measurements have been made of the mean time taken for arriving at a given developmental stage, and of the dispersal of individuals around this time. Three strains of **Drosophila meanogaster** and five stages of the developing embryo were measured. The strains constitute one large population (Sy) and two closely inbred lines (SySP and I_1). The five stages are I extending germ band, II extended germ band, III contracting germ band, IV contracted germ band, and V hatching.

The results of these measurements indicate that the three strains develop at different rates, but the differences cannot be described in simple terms such as faster or slower. Instead, relative rates between any pair of strains are constantly shifting from stage I to stage V. To use the metaphor of a race with respect to another, one strain gets a fast start, later slows down, then puts on a spurt and catches up but finally lags in the home stretch and reaches the finish line (hatching) second. If we compare Sy with SySP, the latter is ahead at stage I; at stage II, Sy has overtaken it; at stage III, SySP is again ahead; at stage IV, the two are running a dead heat; but by stage V, Sy has pulled out front and wins with an hour to spare. If we compare Sy with I_1, for the first four stages, Sy is ahead, although not equally so at every stage, but at hatching time there is no difference between the two strains.

These results are of very great interest because they suggest hypotheses for explaining the relationships between populations, their genetic systems and the biochemical and embryonic processes through which the information contained in the genetic material forms the characters observed in successive generations of adults.

Every cross-breeding population which has existed long enough as a distinct entity in a given environment to approach equilibrium, has a characteristic distribution of phenotypes. It also has its own integrated genetic system, which means that the embryos produced by the random union of eggs and sperms grow into adults having the usual characteristics of the population. Since each individual's genetic material actualizes his adult characters through the process of development, it would be surprising if every population did not have its proper pattern of development. This is precisely what our results indicate with respect to developmental rates.

Within every living organism an extremely complicated system of biochemical reactions is constantly going on. These reactions are interrelated through interactions or feedbacks which have the effect of maintaining the organism as a functioning unit. They hold its physiological states within limits which enable it to continue living and which are characteristic of the population to which it belongs. During development, the biochemistry of the organism effects not merely the cyclical changes which keep it alive, but, in addition, produces a series of directional changes which result in growth, differentiation and the construction of its morphology. Feedbacks keep these directional changes within limits which finally result in the normal adult.

In the Drosophila embryo, a set of interacting biochemical systems determines the structural pattern of the larva and the potentialities of the anlagen, or buds, which will eventuate in the characters of the adult. Thus, differences in the rates of different, interacting biochemical systems must be responsible for the differences in morphology, pigmentation, and physiology which will characterize the adult flies. Differences in rates of embryonic development must mirror differences in the rates of the chemical reactions directing development. When we observe different patterns of developmental rates in two populations, we are seeing one kind of evidence of the different processes at work building the two different distributions of characters, one for each population.

Embryonic rates of development are very sensitive to small changes in environment. Consequently, when embryos of two populations are compared, every effort must be made to keep the environment as constant as possible. Even so, errors of measurement are large. These can be reduced by improvements in technique and by repeated observations. As we build up more accurate and more extensive comparisons of the development patterns of different populations, these should help us to understand how the genes in different populations produce the kinds of individuals they do. As our understanding grows, we should be able to give more complete descriptions of the process and to design more effective experiments for testing our hypotheses and further increasing our knowledge.

This work was supported by the National Science Foundation under grant number NSF-G3844. On October 1, 1958 the project was transferred from The Biological Laboratory to the Department of Zoology at Columbia University where it is being continued under grant number NSF-G6098.

GENETIC STUDIES WITH MAMMALIAN CELLS GROWN IN TISSUE CULTURES

Hermann Moser and Carol V. Madden

One of the fundamental topics of biology is the study of somatic cell variation and its causes in man and other mammals. As Curt Stern recently pointed out, somatic cell variation is the essence of development. The fundamental question is whether cell variation in the multicellular organism is reversible and only superimposed on basic stable properties, or the result of irreversible changes in the genetic material of the cells at fixed times and in fixed locations. Whatever the final answer to this intriguing problem, genetic changes and mechanisms undoubtedly occur or operate in somatic cells, and they certainly play a major part in certain cases of atypic cyto-differentiation. Somatic mutations, if followed by phenotypic expression and favorable somatic selection, may lead to atypic tissue formation. Similarly, development abnoralities may be initiated by mitotic recombination or atypic mitotic segregation in heterozygous cells. The occurrence of unbalanced karyotypes by ploidization may perhaps yield cells with superior growth characteristics and thus give rise to various forms of tumorous growth. Yet still undetected mechanisms of transfer of genetic material from cell to cell (by infectious particules or by direct contact) analogous to those operating in microorganisms may perhaps play a role in somatic cell variation.

Although somatic cells can be studied now under fairly controlled conditions in vitro, genetic analysis is still hindered by a number of technical difficulties. The major difficulties are the following:

1) Stabilization of karyotype and growth characteristics in primary (euploid) cell cultures is still difficult to achieve in vitro even temporarily, i. e., over a period of several months.

2) Growth media for single cells are still only partially defined and possibly sub-optimal.

3) Conditions most suitable for phenotypic expression of somatic mutations in vitro (haploidy) are not available yet.

Therefore part of our program deals with methodology (karyotype stabilization, nutritional studies) while the other part of our research activities avails itself of already established methods for the study of heteroploid cells (lethal effect of radiations, development of radiation resistance).

Karyotype Stabilization in Euploid Somatic Cell Cultures. The maintenance of primary cell cultures—obtained by trypsinization of biopsy materials—with stable chromosomal configuration (karyotype) and stable growth characteristics is of prime importance for systematic genetic analysis of somatic cells in vitro. Experiments designed to obtain stable euploid cell cultures from human somatic tissues—such as those recently reported by T. Puck—are in progress in our laboratory. A short outline

of the problems involved, and of our approaches will be presented at this time.

Cytotypes and Karyotypes in Primary Cultures.

a) Cytotypes. In actively growing in vitro cultures of somatic cells, two distinct cytotypes are observed; cells with fibrolastic morphology (elongated spindle-shaped cells with needle-like extensions) or cells with epithelioid morphology (polygonal-shaped cells). Epithelioid cells form tightly packed colonies; fibroblasts—which are migratory to some extent —form loosely packed colonies with large surface area and rough, not well defined edges. Primary cell cultures are usually mixtures of both cell types, in contrast to established cell cultures which are uniform in respect to cytotype.

b) Karyotypes. Cytogenetic analysis of primary cell cultures obtained from man and other mammals clearly demonstrates the fact that they are composed almost exclusively of euploid cells. Only a small percentage of cells are polyploid. Thus primary cultures of various materials exhibit genetic uniformity (in terms of chromosome number) in sharp contrast to established cell cultures which are invariably heteroploid.

Adaptation and Selection in Primary Cultures. When cells are extracted from the organism and cultivated in vitro, "profound adaptive processes go on during this period in which the cell makes the transition from a possibly non-multiplying condition in the body to a state of very high reproductive rate in vitro" (Puck, Cieciura and Fisher, 1957). Undoubtedly selection processes play a role in many instances once growth is initiated in primary explants. Since primary cell cultures generally are heterogeneous, i. e., cultures composed of cell types or genotypes with different selective values, selective processes (overgrowth of epithelioid cells or fibroblast-like cells?) take place in primary cell cultures because of differential reproduction rates, survival rates, migratory capacities or durations of initial lag. Early clone isolations in primary cultures minimize the effects of selection and preserve the initial cell types and karyotypes.

Karyotype Transformation in Primary Cultures. When grown under conventional conditions of tissue culture, primary (euploid) cell cultures, after several passages (culture transplants), undergo a drastic transformation (heteroploid cell transformation). During this process alterations of the chromosomes, both in number and morphology, occur spontaneously concurrent usually with a temporary refractory state (non-multiplying phase) of cell reproduction. After the refractory state, aneuploid cell types begin to replace the euploid karyotypes in the cell population and finally a new aneuploid karyotype dominates in the cell culture which has now attained an unlimited capacity to grow in vitro (established cell culture). The causes of karyotype transformation in vitro are not known; however, the observations indicate that selection favors aneuploid karyotypes in the conventional tissue culture environments.

Malignant Cell Transformations in Primary Cultures. Closely related to karyotype transformation in vitro is the conversion to malignancy of normal primary (euploid) cell cultures, i.e., the conversion to a condition where the cell culture produces a tumor upon inoculation into hosts (cancer patients). Evidence of a parallelism between karyotype transformation and malignant cell transformation has been obtained by Moore, et al., (1956) and Southern, et al., (1957).

Experimental Approaches to the Genetic Analysis of Euploid (primary) Somatic Cells (Karyotype Stabilization). Our approach to creating experimental conditions which inhibit heteroploid cell transformation in primary cell cultures, i.e., permit the indefinite maintenance of euploid cell cultures in a state of active growth is similar to, and in part guided by the approaches of Puck, et al. It is based on the belief in the inadequacy of conventional tissue culture media and conditions for maintaining normal cell cultures indefinitely. Puck's experiments demonstrate that the refractory state of growth of diploid cell cultures can be avoided or delayed for long periods if cell nutrition is optimum, and cytotoxicity (of serum factors) kept low. Experiments are under way in our laboratory to test three different growth media (with minimal toxicity levels), containing foetal serum as the only macromolecular supplement, for their capacity to maintain euploid cell cultures in the state of active growth. Anaerobiosis is minimized, control of pH and temperature strict, and nutrition kept optimal.

Studies on the Molecular Requirements of Single Human Somatic Cells For Growth in Vitro. Rapid selection of rare biochemical mutants in large somatic cell populations demands the availability of selective growth media, for biochemical mutants are variations of the minimal growth medium, defined for non-mutant cells of a given cell strain.

It it is to be used for selection of nutritional variants, the minimal medium must meet the following qualifications:

1) It must support the sustained growth of single cells as well as the growth of mass cultures.

2) It must be chemically defined and be of such a composition that the omission of a single one of the components supresses the growth of every single standard-type cell.

The Basal Growth Medium of Eagle et al. does not meet all the requirements listed above. However, studies on the nutritional requirements of single cells of clonal cell line D-98C6b (Detroit-98 clone) carried out during this year have led to a semi-synthetic minimal growth medium for established cell lines. Similar studies are in progress to determine the minimal requirements for growth of euploid human somatic cells.

Analysis of the "Dilution Effect" in Basal Growth Medium (TCM-1). We have reported last year that Eagle's Basal Medium (EBM) supplemented with extensively dialyzed serum (Basal Growth Medium, TCM-1) supports massive growth of dense cell populations but loses its

potency as a function of decreasing cell density of the inoculum (dilution effect). During the past year we began a quantitative cellular analysis of the "dilution effect". This analysis is by now near completion but will be paralleled by physiochemical studies of the dilution phenomenon.

Supplementation of BGM. Clonal growth of widely separated somatic cells in Basal Growth Medium can be initiated and maintained by supplementation of this medium. The activity or efficiency of a given supplement in promoting growth of single cells, i.e., of suppressing the dilution effect, in BGM (TCM-1) is defined (in per cent) as $A=(Ee/Ec)$. 100 where Ec is the plating efficiency obtained in standard growth medium (TCM-2) and Ee the plating efficiency obtained in supplemented TCM-1. Since Ee and Ec cannot exceed 100%, A is less than 100% if $Ec=100\%$, A is less than 200% if $Ec=50\%$, A is less than 100% if $Ec=10\%$, etc. In strain D-98C6b the plating efficiency in standard growth medium is usually around 60% (control value). In this case A is less than 167%. It is important to note that the efficiency of the supplemented TCM-1 in promoting growth of single cells is equal or higher than that of the standard growth medium (TCM-2) whenver A is greater than 100%. A supplement is considered inactive if A is less than 3%. The nutritional studies reported here were carried out with a modified D-98C6b strain, D-98C6bM, which exhibits in standard growth medium a plating efficiency of only 10—20%; thus A values exceeding 167% were observed. In the experiments reported here usually from 200-400 cells were plated in 100 mm diameter Petri dishes; they were washed with phosphate buffered saline immediately after their attachment to the glass, i.e., before their treatment with experimental growth media.

The Effect of Molecular Serum Fractions on the Growth of Single Cells in BGM. The molecular portion of serum can be easily separated from the macromolecular portion (serum protein complex) by dialysis, ultrafiltration, or by protein precipitation techniques.

a) Supplementation of Basal Growth Medium with Serum Ultrafiltrate. Addition of fresh adult horse serum ultrafiltrate (AHoSUFf) to the Basal Growth Medium (TCM-1) at a concentration of 10% promotes the growth of single D-98C6bM cells. But the activity of the UF varies a great deal from batch to batch and often low plating efficiencies, i.e., activities, are observed in UF supplemented BGM (see Table 1). These experiments demonstrate that the dilution effect is not the result of denaturation of serum proteins but the result of the removal of certain molecules, required for the growth of single cells, from serum, during dialysis.

b) Supplementation of Basal Growth Medium with Serum Ultrafiltrate Fractions. Boiling of AHoSUF for 10 minutes results in the formation of a precipitate. When the supernatant UF fraction (AHoSUFBf) is aded to the BGM it exhibits an activity in promoting growth of single cells of D-98C6bM averaging 131% of the activity of standard growth medium (TCM-2) (see Table 1). In contrast to supplementation with

fresh ultrafiltrate low activities are not obtained with this UF fraction, indicating the removal of cytotoxic materials from UF during heat treatment.

Autoclaving of activated ultrafiltrate (AHoSUFbf) for 40 minutes at 15 lb. pressure yielded a supernatant UF fraction with an activity of 123%. In another case, autoclaving of fresh UF for 20 minutes yielded activity of only 27%, (See Table 1). We therefore conclude that the molecular (dialyzable) compounds of adult horse serum necessary for the growth of single cells are heat stable molecules.

Table 1

Effects of Supplementation of Basal Growth Medium (TCM-1) with Serum Ultrafiltrate and Fractions of Serum Ultrafiltrate

Supplement	Description	Concentration	Activity
$AHoSUF_f$	Fresh adult horse serum ultrafiltrate	10%	260%
"	"	10%	29%
$AHoSUF_{BF}$	AHoSUF, boiled, precipitate removed	10%	197%
"	"	10%	123%
"	"	10%	87%
"	"	10%	43%
"	"	10%	278%
"	"	10%	61%
$AHoSUF_{AF}$	AHoSUF, autoclaved, precipitate removed	10%	27%
$AHoSUF_{BFAAF}$	$AHoSUF_{BF}$, twice autoclaved, filtered	10%	123%
AHoSUF-C3	AHoSUF treated with strong acidic cation exchange resin	10%	0%
AHoSUF-A4	AHoSUF treated with strong basic anion exchange resin	10%	4.4%

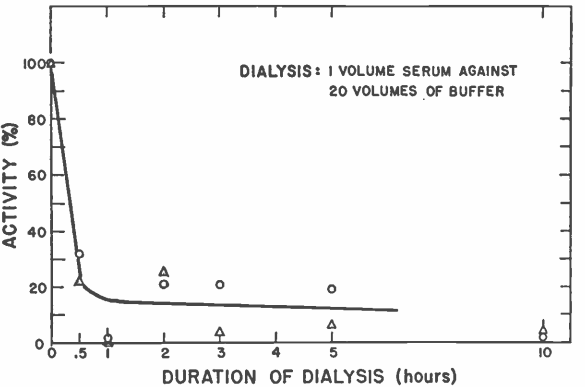
Treatment of UF with ion exchange resins yielded further information on the nature of the active molecular compounds of serum. In these experiments pooled serum ultrafiltrate was mixed with an excess of strong acidic cation exchange resin (Duolite C3) and a strong basic anion ex-

changer (Duolite A4) and stirred at room temperature for 12 hours. The treated ultrafiltrates were filtered and incorporated into BGM at a concentration of 10%. The tests showed that almost all the activity of UF is taken up by either ion exchange resin, indicating possible amphoteric nature of the active molecular species (see Table 1).

The Effect of Serum Dialysis on the Growth of Single Cells in Basal Growth Medium. Dialysis removes from seum the molecular species necessary for the growth of single cells but not necessary for the growth of dense cell cultures. Dialysis experiments conducted under controlled conditions reveal some of the physicochemical properties of the molecular species involved.

During the past year we carried out several dialysis experiments. Figure 1 illustrates the change in potency of diayzed serum (supplemented at a concentration of 10% to Eagle's Basal Medium) of promoting growth of single cells as a function of time of dialysis. In these experi-

Figure 1



ments a volume of 30 ml of adult horse serum was dialyzed against 600 ml buffer 6.8 gr NaCl/L) at 7°C under agitation. Under these conditions the activity of serum decreases by approximately 70-80% during the first 30 minutes of dialysis. This result indicates that small or medium sized molecules are composing the active molecular fraction of serum.

The Effect of Growing Mass Cultures on the Growth of Single Cells in Basal Growth Medium (feeder-layer experiments). The study of the effects of mass cultures on the growth of single cells in Basal Growth Medium provided further information on the mechanism of the dilution

effects. Mass cultures of D-98C6bM were grown in Basal Growth Medium on a glass disc which fits the base of a Petri dish and contains a circular hole. This disc culture is introduced into a Petri dish which has been seeded previously with a small number (200-400) of single cells. When the mass culture reaches a high density and cells tend to detach from the glass, it is replaced by a fresh, actively growing mass culture, approximately at intervals of five days. The mass cultures exert a profound feeder effect on the single cells in the center of the plate. After 12 to 14 days of incubation well developed macroscopic colonies appear in the center of the dish. Obviously dense monolayer cultures synthesize and release into the supernatant Basal Growth Medium the molecular compounds which must be supplied to single cells in order to initiate and maintain their sustained multiplication. These factors may be identical with those in the molecular portion of serum.

The Effect of Defined Molecular Species on Growth of Single Cells in Basal Growth Medium (Supplementation Experiments). In order to obtain an operational minimal growth medium for somatic cells we had to define the molecular compounds which must be added to the Basal Growth Medium in order to initiate and sustain growth of single cells. Two approaches are possible to achieve this goal:

1) Fractionation of the molecular portion of serum (serum ultrafiltrates or serum dialyzates) or of the supernatants of mass cultures grown in BGM by techniques such as, for example, ion exchange chromatography. Purification of the active compounds and identification of the active molecules.

2) Supplementation of the Basal Growth Medium with defined molecular species suspetced of having activity. (Trial tests).

During the past year we have conducted a large number of supplementation experiments. These experiments proved to be time consuming but yielded the information necessary for the establishment of a semi-synthetic minimal growth medium for D-98C6bM cells.

a) Supplementation of Basal Growth Medium with Lactalbumin Hydrolysate. Lactalbumin hydrolysate is an inexpensive source of essential and non-essential amino acids and amino acid conjugates (peptides). Supplementation at a concentration of 2.5% did not produce any activity in BGM (see Table 2).

b) Supplementation of Basal Growth Medium with sodium pyruvate. Sodium pyruvate is an important metabolite involved in anaerobic and aerobic conversion of glucose to lactate. Supplementation of sodium pyruvate showed definte activity (20-87%) in initiating and sustaining Growth Medium at a concentration of 1 mM/L. The effect of pyruvate, Gorwth Medium at a concentration of lmM/L. The effect of pyruvate, however, was reduced in the presence of ATP (1 mg/L) or Vitamin B_{12}

(1 mg/100ml) (see Table 2).

c) Supplementation with Synthetic Mixture NCTC-107, sodium-pyruvate and ATP. Synthetic mixture NCTC-107 is a complex xynthetic mixture which contains, in addition to the essential growth factors (EBM) all the non-essential amino acids, non-essential vitamins, a non-essential amide (glucosamine), reducing agents (glutathione, ascorbic acid and cysteine), coenzymes (diphosphopyridine nucleotide, triphosphopyridine nucleotide, coenzyme A, thiamin pyrophosphate, flavin adenine dinucleotide, uridine triphosphate), deoxyribose derivatives of nucleic acid and pyrimidines (deoxyadenine, deoxycytidine, deoxyguanosine, thymidine and 5-methyl cytosine), 3 unsaturated fatty acids, cholesterol, glucuronolactone, sodium glucuronate and sodium acetate. Supplementation of this

Table 2
Effects of Supplementation of Basal Growth Medium (TCM-1) with Chemically Defined Compounds

Supplement (s)	Concentration (s) (in TCM-1)	Activity
Lactalbumin hydrolysate	2.5%	0%
Sodium pyruvate	0.1 mM/L	0%
Sodium pyruvate	1.0 mM/L	20%
Sodium pyruvate	1.0 mM/L	87%
Sodium pyruvate	5.0 mM/L	1%
Sodium pyruvate	10.0 mM/L	0%
ATP (1 mg/L) + Sodium pyruvate	0.1 mM/L	2%
ATP (1 mg/L) + Sodium pyruvate	1.0 mM/L	10.2%
ATP (1 mg/L) + Sodium pyruvate	10.0 mM/L	1%
ATP (1 mg/L) + NCTC-107 (50%) + Sodium pyruvate	1.0 mM/L	0%
Vitamin B_{12} (1 mg/100 ml) + Sodium pyruvate	1.0 mM/L	3.4%

mixture at a concentration of 50% in combination with sodium pyruvate (1 mM/L) and ATP (1 mg/L) to the BGM yielded no activity in single D-98C6bM cells (see Table 2). This indicates that coenzymes, nucleic acid derivates, cholesterol and reducing agents do not promote the growth of single D-98C6bM cells as reported by Sato and Puck in Hela-S3. The effect of pyruvate on the other hand is suppressed in the presence of the complex mixture, perhaps due to toxic effects of coenzymes, ATP or reducing agents.

d) Supplementation with components of the tricarbolic acid cycle. Adition to the Basal Growth Medium of pyruvate (1 mM/L), citric acid (30 mg/L), succinic acid (2 mg/L), lactic acid (100 mg/L) fumaric acid (2 mg/L) and alpha-ketoglutaric acid (2 mg/L) increased the activity of BGM in promoting colony formation by single D-98C6bM cells to 50% (see Table 3). Further supplementation with ATP (1 mg/L), however, reduced the activity in accordance with earlier experiments, to 11% (see Table 3).

e) Supplementation with pyruvate, oxalacetic acid and non-essential amino acids. Addition to the Basal Growth Medium of sodium pyruvate (1 mM/L), oxalacetic acid (13 mg/100 ml) in combination with the seven non-essential amino acids (alanine, aspartic acid, asparagine, glycine, glutamic acid, proline, serine) at a concentration of 0.2 mM/L yielded a

Table 3

Supplementation of Basal Growth Medium (TCM-1) with Molecular Components of the Tricarbolic Acid Cycle

Supplements	Concentrations	Activity
sodium pyruvate +	1 mM/L	
citric acid +	30 mg/L	
succinic acid +	2 mg/L	54.1%
lactic acid +	100 mg/L	
fumaric acid +	2 mg/L	
alpha-ketoglutaric acid	2 mg/L	
sodium pyruvate +	1 mM/L	
citric acid +	30 mg/L	
succinic acid +	2 mg/L	
lactic acid +	100 mg/L	11.2%
fumaric acid +	2 mg/L	
alpha-ketoglutaric acid +	2 mg/L	
ATP	1 mg/L	

plating efficiency of approximately 60-65%, i.e., a plating efficiency that is usually obtained with standard growth medium TCM-2 in strain D-98C6b (see Table 4) and fully developed colonies. Supplementation with the seven non-essential amino acids along, however, only a much reduced plating efficiency and many not fully developed colonies (see Table 4). It has recently been shown by Lockhart and Eagle (1959) that "non-essential" amino acids, notably serine, are necessary and sufficient nutritional supplements for growth of single Hela, conjunctiva and KB cells. Neuman and McCoy on the other hand demonstrated that single Walker Carcinoma 256 cells require—unlikely heavily inoculated colonies—either pyruvate, oxalacetate, or alpha-ketoglutarate for growth.

Table 4

Supplementation of Basal Growth Medium (TCM-1) with Non-essential Amino Acids, Pyruvate and Oxalacetic Acid

Supplements	Concentrations	Plating Efficiency	Activity
alanine +	17 mg/L		
aspartic acid +	25 mg/L		
asparagine +	10 mg/L		
glycine +	15 mg/L	6%	24%
glutamic acid +	29 mg/L		
proline +	23 mg/L		
serine	21 mg/L		
alanine +	17 mg/L		
aspartic acid +	25 mg/L		

asparagine +	10 mg/L		
glycine +	15 mg/L		
glutamic acid +	29 mg/L	61%	243%
proline +	23 mg/L		
serine +	21 mg/L		
sodium pyruvate +	110 mg/L		
oxalacetic acid	130 mg/L		
TCM-2 (control)	— — —	25%	100%

Our experiments indicate that the D-98C6bM cell has a greater demand for quasi-essential growth factors, i.e., growth factors that need not be supplied to heavily inoculated monolayer cultures in Basal Growth Medium, than for example, Hela or Walker Carcinoma 256 cells, since they require both non-essential amino acids plus pyruvate for their sustained clonal growth.

Conclusions. The studies of the nutritional requirements of somatic human cells by Eagle and associates, and ourselves clearly demonstrate the following:

1) Established, but probably also primary euploid, cell cultures of human origin have a basic and natural requirement for 12 amino acids, 1 half amide (L-glutamine), 9 vitamins, glucose, 5 ionic species and factors, yet unknown, which are carried by, or are part of, serum protein. These are the minimal essential growth factors; they initiate and maintain massive growth of heavily inoculated cell cultures of various origins; omission of any one of them in the Basal Growth Medium produces death of the mass culture.

2) Small inocula, consisting mainly of single cells, of these cell cultures have additional molecular requirements for sustained growth in vitro such as non-essential amino acids or pyruvate. These "anomalous" requirements of small inocula, which we define as quasi-essential growth factors, are either: a) metabolites easily lost from the intracellular pool; b) compounds which facilitate the biosynthesis of certain metabolites; c) compounds which increase the retention of metabolites by the cell; or d) compounds which inhibit cytotoxic materials such as proteolytic enzymes excreted by the cells, or metallic ions. These molecular quasi-essential growth factors or their active substitutes are provided by complete serum and lost upon serum dialysis.

The nature of the supplements to the Basal Growth Medium which are active in promoting sustained growth of single somatic cells suggests that they are metabolites, and they or their precursors are either easily lost from single cells or have a small biosynthetic rate. Let us assume (see Figure 2) that B is a compound which is synthesized from an essential amino acid (suplied externally), A, at rate k_1, and is converted into C, a building block of proteins and of cell structure, at rate k_3. If B leaks from the cell into the external medium at rate k_2, synthesis of C and therefore of cell growth and multiplication is accomplished only if the

biosynthetic rate k_1 exceeds the rate of removal of B from the intracellular pool into the external medium. The rate of leakage of B (per cell) is obviously a function of the surface area (per cell) exposed to the medium, and of the rate of accumulation of B in the external medium. Because the free surface area per cell in monolayer cultures decreases with increasing cell density, due to the cell reaggregation, and because the accumulation rate of B in the media increases with increasing cell density,

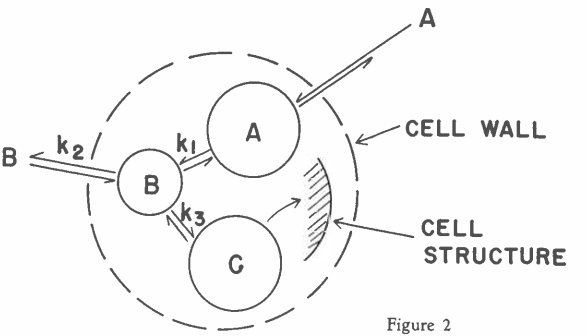


Figure 2

the rate of leakage increases with decreasing cell density in the inocula in a medium lacking B (dilution effect). However, if B is supplied with the nutrient fluid, leakage of B is reduced or suppressed because of concentration effects, and the intraceullar B pool maintains a level adequate for continued synthesis of C and therefore for sustained growth of isolated single cells.

Mutation is likely to affect both essential and quasi-essential nutritional requirements of somatic cells. A mutant affecting an essential requirement has been reported by DeMars (oral communication) in strain Hela. While the majority of cells in a Hela strain population require l-glutamine for growth, the mutant is able to grow on glutamic acid alone and thus is able to synthesize glutamine from its precursor. Puck, on the other hand, isoluated in strain Hela, which does not require external supply of inositol in mass culture, a mutant, Sl, which has a demand for l-inositol, but only when grown in small inocula, ("Permeability mutant"?).

Obviously selective techniques involving defined media are designed for rapid selection of rare biochemical mutants in large cell populations. They are, therefore, useful only for mutations affecting the essential nutritional requirements which express themselves in mass culture.

The identification of the quasi-essential molecular requirements of single heteroploid cells (non-essential amino acids, pyruvate and ovalactate) reported earlier makes available now an operational selective growth medium for a number of heteroploid human cell strains (D-98, Hela). We call it operational because in its present state its application is limited to the selection of mutants affecting only essential molecular requirements. (The macromoleucular requirements of somatic cells in serum protein are not defined yet.)

Studies on Radiation Resistance in Somatic Cell Cultures. The study of radiation resistance, its detection, its possible origin by mutation, and its development in human somatic cell populations is of prime importance today. During the past year we began a study of the development of resistance to UV in a large cell population of an established human somatic cell strain (D-98C6bM) which is exposed to a single UV dose at regular time intervals. This selection experiment has been carried out only for about 3 months.

Methods. The "selected" cell culture, D-98C6bM-SUV, is transferred to a new large culture dish at a cell density of about 10^6 cells and treated, 24-48 hours later, with UV for 60 seconds at a distance of 21". (The general methods of UV treatment were outlined in last year's report.) The next transfer and treatment is carried out after the survivors have grown into macroscopic colonies, that is after 12-14 days. After a given number of transfers and treatments the UV sensitivity of the "selected" line is tested simultaneously with that of a "non-selected" D-98C6bM stock culture grown under the same condiitons as the selected line. The techniques for determining UV sensitivity have also been reported last year.

Preliminary Results. A first UV sensitivity test of the selected D-98C6bM multure was carried out after 4 transfers and treatments. The results of this test are represented in Figure 3. Low plating efficiency and increased variability in colony counts in this experiment do not permit a definite conclusion yet on the development to radiation resistance in the "selected" line. However, the plot of the survival curves show a slight tendency of the selected line towards increased UV resistance, especially in the higher dose range. Obviously, further selection and sensitivity tests have to be performed to obtain a conclusive result. Also methods of a different kind may be employed to select for radiation resistance, methods which eliminate or reduce possible selective disadvantage (reduced growth rate) of rare UV survivors which exhibit increased radiation resistance.

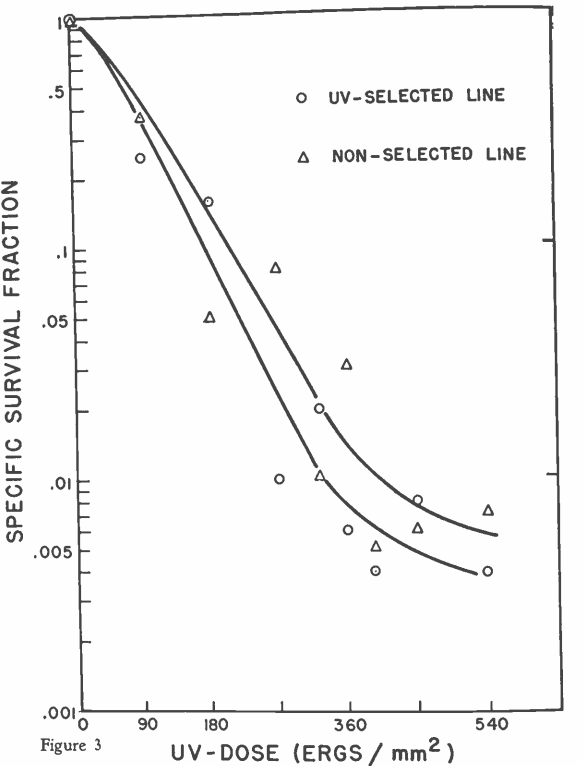


Figure 3

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

Fig. 1 Activity of dialzyed adult horse serum in Basal Growth Medium as a function of time of dialysis

Fig. 2 Model of the dilution effect in Basal Growth Medium

Fig. 3 Sensitivity to ultraviolet irradiation of UV selected cell strain and of non-selected cell strain

THE LEUCINE LOCUS OF SALMONELLA TYPHIMURIUM

Paul Margolin, J. Vandermeulen-Cocito and Elizabeth J. Simrell

The phenomenon of transduction in Salmonella has made it possible to study genetic recombinational events which occur at an extremely low order of frequency. This in turn has permitted the development of linkage maps of mutational sites within a single gene. The purpose of the studies described here has been to attempt to prepare a composite map of the leucine locus of Salmonella in terms of structure and function. The initial results are described below.

For the purpose of developing a linkage map of the locus, it was necessary to accumulate a number of independent leucine auxotrophs (mutants unable to synthesize leucine) in combination with a closely linked marker outside of the leucine locus. In this case mutants in an arabinose locus (ara) were available as the outside marker. Fifteen such leucine auxotrophs, linked with ara$^-$, were obtained. The ara$^-$ marker was replaced by ara$^+$ in subcultures of these auxotrophs by means of transduction. The H4 mutant of PL22 bacteriophage was used for these transductions. This mutant is frequently unstable with respect to establishing lysogeny, so that non-lysogenic ara$^+$ recombinants could be selected. In this way the leucine auxotrophs were made available as both double mutants (elu ara$^-$) and single mutants (leu ara$^+$). Phage preparations were then made and three point tests carried out using two independent leucine mutants and the arabinose marker. Reciprocal transductions involving various pairs of these auxotrophs were made. The ratios of the frequencies of various types of recombinants were then used to determine the relative positions of the sites of the two mutants involved, although the distances between them could not be determined accurately. In this manner a map of the linkage order of the alleles in the leucine locus has begun to emerge. The positions of the sites of additional leucine auxotrophs, from the more than 100 presently available, are now being determined.

A method was developed for detecting the formation of the minute colonies which are indicative of abortive transductions. Abortive transduction has been interpreted to mean a capability of two auxotrophs to complement each other functionally, when in heterozygous condition. This may be considered to be due to the functional independence of the two alleles. In turn, those alleles which do not complement each other may be located at sites associated with the same unit of function. The results thus far indicate that there are four complementation groups at the leucine locus. Each allele can complement all other alleles except those in its own group. So far, group I has 13 alleles, group II has one allele, group III has 16 alleles, and group IV has two alleles. Their order is given below. The sites of the alleles in parentheses appear to be either identical with those above them, or so close that the number of recombinants from transduction are too few to provide valid ratios for deciding relative positions.

I					II	III				IV		
32	124	120	10	125	129	127	126	123	131	128	21	ara
	(121)					(130)	(122)					
						(120)						
27, 33, 46, 47, 48,						25, 26, 34, 35, 36,						
49, and 132*						43, 44,133, and 134*						

*These alleles belong to the complementation group beneath which they are placed, but their exact positions have not yet been determined.

Indirect methods are used to gain knowledge of the structure of the gene. One approach being used in this project consists of determining the sensitivity of regions of the leucine locus to the activities of various mutagenic agents. At present, tests are being made of the effectiveness of UV and 2-aminopurine in inducing reversions to prototrophy of the various leucine auxotrophs. The results of some of the tests are presented in table 1.

Table 1

Reversion to prototrophy, spontaneous and induced by ultraviolet irradiation or treatment with 2-aminopurine.

Leucine mutants

	10	21	25	26	27	32	36	39	120	121	122
Spont.	+	x	x	x	x	+	+	?	+	+	+
U.V.	−	x	x	x	x	−	−	−	+	−	+
2-aminopurine	−	+	−	+	−	−	+	−	+	−	+

Leucine mutants

	123	124	125	126	127	128	129	130	131	132	133
Spont.	+	−	+	+	+	+	+	+	+	+	+
U.V.	?	−	+	−	+	−	+	+	−	x	x
2-aminopurine	−	−	+	−	+	−	−	+	−	−	−

+ = reversions
x = not tested
− = no reversions
? = uncertain

As has been previously demonstrated, specificity with respect to mutation, either spontaneous or induced by various agents, resides in the allelic site. The frequencies of induced reversions is also being determined. Most of the tests of the effectiveness of 2-aminopurine were done by means of a spot test screening technique which does not appear to detect very low frequencies of reversion induction. In spite of this, eight of these 22 spontaneous mutants were detected as being induced to revert by 2-aminopurine.

An experiment was carried out to establish a procedure for obtaining frequencies of reversions induced by 2-aminopurine and to determine if prolonged growth in the presence of the mutagen was necessary. A suitable dilution of a fully grown, washed culture of leu 127 was sus-

pended in a water solution of 2-aminopurine (.6 mg/ml). The same number of cells was added to a solution of 2-aminopurine in synthetic medium supplemented with leucine. At two and eight hour intervals these were assayed for population desity and plated to determine reversion frequency. Table 2 presents the results.

Table 2

Survival and reversions of leu 127 following treatment with 2-aminopurine. Reversion freuencies represent the number of mutations induced per 10^9 surviving cells. Note that treatment in the growth medium does not kill and survival in these cases actually represents growth.

	2 hrs.		8 hrs.	
2-aminopurine	Survival	Induced Reversions	Survival	Induced Reversions
in H_2O	6.6%	18000	1.4%	8000
Control—H_2O	27.0%	——	28.6%	——
2-aminopurine in growth medium	320.0% (approx. 1.5 fissions)	1080	320.0% (approx. 1.5 fissions)	2880
Control—growth medium	310.0% (approx. 1.5 fissions)		3000.0% (approx. 5 fissions)	——

It is of interest to note that 2-aminopurine kills in a water solution, but apparently not in the presence of synthetic medium. It is usually believed that incorporation of the 2-aminopurine during growth of the cells is necessary to establish reversions. Little or no growth can be expected to have occurred during the treatment with 2-aminopurine in water. The cells were then plated on enriched minimal agar and presumably the small amount of growth occurring at this time was sufficient to establish the large number of reversions which were found. The advantage of this type of treatment is clear. It eliminates the problem of correcting frequency data for the effect of clonal inheritance of early reversions in growing cultures.

This research was supported by Grant #CY-3773 given by the U. S. Public Health Service.

PSYCHOBIOLOGY SECTION

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

The project of this Section continues along the same lines described last year. The nature of the psychotic in man as well as the effect of animal tissue extracts on the blocking of the LSD reaction in Siamese fighting fish continues to be investigated.

Experiments are being continued on our five non-psychotic human test subjects. This is the fifth year that this same group has been used. We continue, therefore, to have a base line of welltested subjects on which to investigate the efect of drugs like lysergic acid diethylamide (LSD-25) on man. In addition, we are continuing our experiments on the Siamese fighting fish, using the fish for preliminary investigation as heretofore. Work on the fish has advanced considerably by the successful development of a technique for injecting unanesthesized fish. New compounds have been received from Sandoz for quantitative assay on man.

Blocking Psychosis-Producing Doses of Lysergic Acid Diethylamide in Man

The use of tranquilizers has become one of the most important points in clinical medicine. Tranquilizers, such as chlorpromazine, are used widely in mental institutions. The efect of chlorpromazine on the LSD reaction in man is well-known, but in order to block the LSD reaction, chlorpromazine must be given by hypodermic injection. Analysis of our data on the effect of chlorpromazine taken orally up to 50 mg. rather than given by injection indicates that chlorpromazine is essentially ineffective in blocking the psychotic components of the LSD reaction in man. Investigations are under way testing other central nervous system depressants in co-operation with Sandoz Pharmaceuticals in an attempt to find a more suitable LSD blocking agent, i.e., one that would be more effective when taken orally.

In addition to central nervous system depressants, other drugs, especially hormones, may be of importance in the chemistry of mental illness. Analysis of the data found on our five non-psychotic human test subjects obtained with the action of prednisone, which is a member of the corticosteroid family widely used in medicine at present, has shown a rather remarkable reaction to the administration of prednisone. If prednisone is given five or six days ahead of time in doses ordinarily therapeutically used in asthma and arthritis, and the subjects are then given LSD-25 at, or near, the threshold level, all of the usual LSD reactions occur with one exception: in most of the cases, in spite of the presence of a strong physical reaction (as measured by our questionnaire), the subjects report either no anxiety or less anxiety. This fits in with the clinical

observation that patients treated with corticosteroids like prednisone often become euphoric; indeed, although euphoria is considered to be a side effect, very often it pervades the clinical picture. The reduction of anxiety by prednisone in the LSD reaction in many, with the presence of physical reactions, makes it incumbent on us to study the other newer steroids which are now available. It is conceivable that "tranquility" in certain types of mental illness might be achieved by the administration of steroids of the nature of prednisone. It has been spectacular to observe a subject reporting that he has a strong physical reaction to 50 mcg of LSD-25, but no anxiety. Perhaps more emphasis should be placed on a possible endocrine imbalance in the study of the chemical origins of mental illness.

Lysergic Acid Diethylamide Blocking by Tissue Extracts in the Siamese Fighting Fish

An attempt is being made in collaboration with Chas. Pfizer & Co., Inc., to determine the mechanism of blocking of the LSD reaction in the Siamese fighting fish by brain extract. Since some blocking action has been found in nearly all tissues, investigations are proceeding to determine what common factors in all tissue extracts might be involved. The isolation of a substance or substances in a particular system is complicated by the fact that all of our agents must diffuse into the fish, probably through the gills. For this reason we have developed methods of injecting the juvenile forms of the Siamese fighting fish to avoid this diffusion problem. Meanwhile it has been found that small quantities of salts may occasionally produce a certain amount of blocking of the LSD reaction. At other times this effect does not occur, so that unknown factors operate in these experiments. Just how potassium chloride and sodium chloride can produce this phenomenon is not clear. In any event we are scrutinizing more closely two features of our bio-assay system: (1) the chemical nature of tissure extracts, and (2) the variables involved in the blocking reaction.

Effect of Injected LSD on the Siamese Fighting Fish

When the fish are injected with LSD rather than exposed to the drug in the outside liquid, the sensitivity of the fish to LSD increases about 100 fold. We can detect 0.001 mcg of LSD. If the dose per kilogram required to observe an effect in fish is calculated, it becomes approximately the same as in man, about one microgram per kilogram.

Studies in Schizophrenia

Studies concerning the effect of LSD-25 upon the communication of patients diagnosed as schizophrenic are continuing with the collaboration of Dr. Henry L. Lennard, sociologist at Columbia University. Qantitative methods for the analysis of communication which have been introduced into drug research by Dr. Lennard are currently utilized in assessing changes, over time, in the effect of LSD-25 when the drug is administered to the same patient over several weeks. The data consist of

transcribed tape-recordings of conversations between patient, interviewer, and stablemate. Recordings are made, at intervals, during the course of the experimental series. Both LSD-25 and placebo sessions are transcribed. The transcribed sessions are systematically analyzed for changes in formal and content aspects of communication. The current studies on communication represent a part of the larger research concerned with evaluating the effect of LSD over time, especially in relation to the development of tolerance. If subjects, as hypothesized, developed tolerance to LSD after continued administration of the drug, then such tolerance should be reflected in behavioral changes, and specifically, changes in verbal behavior. A past series of studies has already identified a number of aspects of communication which are changed by single or occasional LSD-25 administration. The question currently explored is, then, whether the direction of these changes is reversed in the course of a long series of LSD-25 administrations. Findings so far indicate that the patterning of communication during the later sessions in the series is much closer to the patterning of communication observed during placebo sessions than to that of LSD-25 sessions. This reversal of the direction of LSD-25 induced changes upon continued administration of LSD appears for variables such as: interaction rate, number of affective propositions, number of questions asked, etc. Additional characteristics of communication are currently being studied to assess further the patterns of change. It is believed that the microscopic study of communication, specifying drug effect, and identifying the nature of the tolerance phenomenon is ading a new dimension to experimental psychiatric research.

MLD produces cross-tolerance in schizophrenic patients apparently in the same way that it does in non-psychotic individuals. This demonstrates the existence of mechanisms in schizophrenia that are more easily studied in non-psychotic subjects.

Production of Cross-Tolerance in Non-Psychotic Subjects

It is of importance to discover what types of molecules can produce tolerance to large loses of LSD. It has previously been shown that MLD produces cross-tolerance very effectively. Of particular importance are drugs which can produce cross-tolerance without symptoms while the drugs are being taken. We have now investigated other derivatives of LSD. These, in the order of effectiveness of cross-tolerance production, are:

	Effectiveness	Corrected for Dose
MLD	23.5	20.6
OML	5.0	.46
UML	2.3	.033
BOL	1.95	1.7
DAM	1.6	.11
LAE	1.3	.08

This project in research has been made possible by a grant from the Josiah Macy, Jr., Foundation.

ELECTRON MICROSCOPY

Berwind P. Kaufmann

A program of study of fine structure of cellular components has been initiated under the terms of a great (RG-5336) from the National Institutes of Health. An RCA EMU-3 electron microscope has been purchased and installed. It is equipped with ultrahigh magnification accessories that permit the preparation of photographs (electron micrographs) at an initial direct magnification of about 200,000 X, and at a final useful working magnification, by enlargement from the original negative, of 1,000,000 to 2,000,000 X. It is apparent that under these conditions a wealth of fine detail, lying far beyond the resolving powers of ordinary light microscopes, can be visualized. The information obtained with the instrument promises to extend our horizons to the finer details of cell structure and broaden our concepts of the nature of the materials of heredity.

Installation of the microscope and adjustment of its extremely sensitive electrical circuits have consumed a good share of our working time during the past eight months. After all this preparation, the microscope now seems to be in good working conditions and an extensive research program has been initiated. We are currently studying the fine structure of chromosomes during somatic and meiotic mitoses, wtih a view to elucidating several important problems about lateral and linear patterns of organization, and are starting a series of investigations to determine the differences between progenitor types and mutants among mammalian cells grown in tissue culture. The studies with mammalian cells will be carried out in cooperation with Dr. Herman Moser, whose program of research is reported in another chapter of this Annual Report. Dr. Harold Mazzone joined this cooperative program in January, 1959; his presence as a staff member enables us to extend the analytical procedures into the related areas of biochemistry and cytochemistry.

Use of the microscope in collaborative projects involving regular staff members and summer investigators of the Biological Laboratory was begun during the past summer. Prof. Ernst Caspari of Wesleyan University engaged in a study, in collaboration with Dr. Helen Gay of Carnegie Insttiution, of the changes occurring in the cells of the tests sheath of the larva of the meal moth, Ephestia. Techniques of fixing and imbedding the testes were perfected, so that fine structural detail would be discernible in the micrographs. A preliminary survey was made of nuclear and cytoplasmic changes. Particular attention was given to changes in number and form of pigment granules and mitochondria at different stages of development and in different mutants. This project will be continued during the coming summer.

STUDIES IN NATURAL AND LABORATORY POPULATIONS OF DROSOPHLIA

Alexander Sokoloff, Ellen McMullen, Gloria Gillies and Virginia Hollely

With the aid of grant #4501 from the National Science Foundation, we have undertaken to study several interrelated population problems. The data collected so far are in the process of analysis so that we cannot give concrete results. This report, therefore, is limited to a statement of the aims of the investigation.

1. Phenotypic variation in natural populations of Drosophila pseudoobscura.

The phenotype or appearance of an individual is the result of an interplay of the organism's genetic make-up and variations in the environment in which this organism develops. Thus, in insects of the same genetic make-up (other conditions being equal) individuals reared at a low temperature will tend to be larger than those reared at higher temperatures. On the other hand, individuals starved during their developmental stages will be smaller than those provided with sufficient food.

The first aim of this study, then, is to get a measure of the degree of variability of populations reared under different climatic conditions. To this end, material has been collected in ten different localities throughout the distribution of the species. Some localities have been sampled over two successive years to compare the average size of individuals in those localities from one year to the next. Throughout this, and the following studies, the characters measured to give an estimate of size are wing length and width, and length of posterior tibia.

2. Genetic variation in population of D. pseudoobscura.

Females collected in the ten different localities have been isolated to start stocks reared under controlled conditions. From these F_1 stocks, a certain number of males and females is measured, and comparisons are being made of flies from stocks collected within and between localities.

There is a possibility that these F_1 data may not give a true picture of the genetic variation of the samples collected in the various localities. Even though the F_1's have been reared under optimal conditions, their mothers have not. Since these females were reared under different environmental conditions, they may pass this environmental influence to their offspring by way of the cytoplasm.

However, the F_1 progeny have been reared under optimal conditions and temperature conditions have been controlled. Hence, any differences we may detect in the F_2 when different localities are compared, must be attributed to genetic differences.

Females from half of the F_1 stocks from a given locality have been mated at random with males from the remaining stocks of that locality. In this manner the genetic variability is greatly increased, and the sample may be said to be representative of the population from which it was derived.

3. Genetic variation in laboratory populations of D. pseudoobscura.

Through the courtesy of Professor Th. Dobzhansky and Dr. Chozo Oshima, Columbia University, we have obtained samples derived from flies maintained in population cages. These populations of flies are characterized by having different inversions in the III chromosome. Two populations are homozyous for the inversion called Chiricahua (CH/CH). Two are homozyous for the Arrowhead (AR/AR) inversion, and three are heterozygous for these inversions (AR/CH).

Since the flies were reared under optimal conditions, any differences in these samples are surely genetic. Perhaps a correlation may be found between some of the characters being measured and these inversions.

4. Genetic variation in laboratory populations of D. melanogaster.

For a number of years Dr. James C. King of the Biological Laboratory, Cold Spring Harbor, has maintained two population cages of D. melanogaster. One was started with a sample of Oregon R flies, a strain that has been maintained in the laboratory for over a quarter of a century. The other was started a few years ago with a sample of wild-type flies collected in a grocery store at Syosset, Long Island.

Samples from these cages have been obtained, and the material reared under optimal conditions for comparison purposes.

A similar study is being carried out with samples obtained from Dr. Bruce Wallace also of the Biological Laboratory. For many years he has maintained a number of inbred and outcrossed stocks. Since Teissier has shown that populations of D. melanogaster from France and Japan differ in the body characters he measured, it is of interest to compare the situation in stocks maintained in the laboratory.

5. The problem of competition.

A previous study by the senior investigator was designed to test intra-and interspecies competition in the pseudoobscura group of Drosophila at two temperatures. The data corroborated the findings of a number of investigators: (1) When flies are reared at low temperatures and optimal conditions of food, flies tend to be heavier than those reared at higher temperatures. (2) When food becomes scarce (a) there is a gradual decrease in size of the flies until a minimum size is obtained. (b) when competition becomes severe, fewer individuals survive as density increases. The study also corroborated Darwin's idea that the closer the species are related, the keener will be the competition.

A salient finding from that study was that when competition conditions arise, the diference in body weight of the flies attributable to temperature disappears: Flies reared at different competition densities will weigh the same, for a particular density, whether reared at 16° or 24°C.

Unfortunately, weight is not a very good criterion for determining flies, especially females reared under natural conditions, have developed under competition.

In order to be able to utilize the data on body size obtained from flies collected in nature as a measure of severity of competition, an experiment was carried out with F_2 D. pseudoobscura obtained from the Grand Canyon. The same medium, densities, and temperatures were used, but now the data consist of measurements of wings and legs as criteria of body size.

It will be possible to tell whether the material collected these two last summers has been experiencing competition under natural conditions, and, if so, to what degree.

6. Competition in laboratory populations of D. pseudoobscura in population cages infested with mites.

This is merely an extension of the competition study. It is of interest because these population cages are infested with a mite which not only feeds on the same medium as Drosophila (hence interspecies competition obtains), but the mite is also a predator on larvae.

7. A character appearing in several species of Drosophila as a result of treatment.

In the course of mounting wings of D. pseudoobscura first fixed in 95% alcohol and then transferred to paraffin oil, it was noted that a certain proportion of wings exhibited the following reaction: The ventral bristles on the first longitudinal vein fold back, sometimes exposing the dorsal bistles throughout their length. This reaction does not affect the width measurements of the wing, unless the wing curls up, as it does in a small percentage of cases.

When populations of D. pseudoobscura derived from different localities were compared, it was noted that in some localities the frequency of the character was low, and in others higher. Furthermore, the character in F_1 samples obtained from these localities correlated in frequency with the parental samples.

Later, the same behavior was observed in wings derived from Dr. Wallace's and Dr. King's populations of D. melanogaster.

Samples obtained periodically from the Ore R and Syosset population cages gave two distinct curves. Genetic studies in relation to this character have been made. The method of transmission remains obscure: F_1 flies derived from matings in which both P_1 possess the character exhibit this character more frequently than when at least one parent does not exhibit the character. However, in the F_2, this effect appears to be nullified.

THE SECOND EDITION OF "THE MUTANTS OF DROSOPHILA MELANOGASTER"

by C. B. Bridges and K. Brehme-Warren

Katherine Brehme-Warren

As reported in last year's annual report of the Long Island Biological Association, this reference book for research workers in Drosophila genetics was originally published in 1944, five years after the death of Dr. Bridges. It was written from brief memoranda left by Dr. Bridges and greatly amplified by Dr. Brehme from the literature and from unpublished data. The second edition constitutes an extensive revision of the earlier publication, both from the point of view of addition and of correction on the basis of new research data. The first (1944) and a reprint (1950) edition are now completely out of print. The Department of Publications of the Carnegie Institution of Washington, who printed the earlier edition, plans to print a larger edition of the manuscript now in preparation.

This work is nearing completion. The author has covered most of the literature in the genetical journals since 1943; much unpublished material has also come into her hands. More than 1000 new mutants have thus been described, and most of these represent new gene loci or new chromosomal aberrations. Reoccurrences of gene and chromosomal mutations are summarized rather than described in detail.

The help of a number of consultants has greatly facilitated the work. As previously reported, a biographical sketch of C. B. Bridges has been written by Dr. Jack Schultz and will apear at the beginning of the book. An appendix by Dr. Irwin Oster on Marker and Tester Stocks has been prepared and will appear over his signature; Dr. Oster has described these widely used stocks with an explanation of their use and a critique of reliability in each case. The section on Linkage Data and Standards has been rewritten by Prof. Maurice Whittinghill from his wide experience in studies of crossing-over. The location upon the salivary gland chromosome map of all mutations which have been so located has been drawn up by Prof. E. B. Lewis, and was exhibited in preliminary form at the 10th International Congress of Genetics at Montreal in August, 1958; this information will be added to the revised chromosome maps. The section on Rank (or valuable qualities) of the mutants has been rewritten with the help of Prof. H. J. Muller, who has contributed in many other ways as well to the content of the manuscript. Sections on pseudoalleles, isoalleles and reversions have been written by the author in consultation with Dr. B. P. Kaufmann and Prof. C. P. Oliver. The section on wild stocks has been revised with the help of Profs. P. T. Ives and E. B. Lewis.

Problems of nomenclature have been numerous since the death of Dr. Bridges; these were discussed in detail at the Montreal Congress at

a meeting called by Dr. Warren, with Dr. Oliver presiding. As far as possible, the thoughts expressed at this meeting will be incorporated in the book.

It is the author's intention to have every part of the book criticized and evaluated by as many authorities as possible. In this she has had the cordial cooperation of all of the many hundreds of investigators who use Drosophila as their experimental material.

The work is supported by a research grant, (#G-5739), from the National Science Foundation.

CYTOTOXIC EFFECT OF SERUM FROM SCHIZOPHRENIC PATIENTS BY THE CLONE CULTURE METHOD

Irwin Fand and Hermann Moser

The plating procedures for single human cells described in last year's report by Moser represent a very sensitive method for the assay of growth inhibitors and cytotoxic agents human tissues. Thus during the past year the co-author suggested that this technique be applied to determined quantitatively the serum toxicity claimed by Fedorov to be associated with acute mental illnes.

Results obtained from experiments in which serum, in 10-15% concentration, was added to Eagle's Basal Medium, reveal a consistently higher anti-growth action in the clone culture from bloods of seven acutely-ill, male, hospitalized, schizophrenic patients (age range 25-58) as compared with seven controls (age 25-32).

Adequate checks were applied in population selection, the fasting blood sample being drawn from control subjects in good general health, with normal nutrition, under medication, and showing no sign of intercurrent infection.

A strain of modified human adult sternal narrow cells (D-98C6b) was plated out in low density (200-400 cells per plate), and the effect of human serum-supplemented growth medium was assayed in terms of absolute numbers of macroscopic colonies appearing after 16 days in vitro. Human serum supplemented medium was applied to the culture during the initial 2 feedings, the protein ingredient was substituted with adult horse serum. Statistical tests of significance applied to the data demonstrated that toxicity ranged from 48-84% in the experimental sera compared to the control sera.

Since the measure is quantitative, the data suggest the method might be applied in differential diagnosis and for serial control. Studies are planned to determine the degree of specifity of the cytotoxic effect of serum in mental disease.

This research was made possible by a grant from the New York State Department of Mental Hygiene.

CHEMICALLY INDUCED REVERSIONS AMONG TRYPTOPHAN MUTANTS IN SALMONELLA TYPHIMURIUM

A. Schalet

During recent years the work of Demerec and his collaborators has demonstrated specific patterns in the mutational response of different genes to the action of various mutagens. Furthermore, the basis for the differential response of a locus has been shown to be intimately related to the individual mutational sites within the gene. A purpose of the present work was to extend these observations to the action of additional mutagens upon the tryptophan D locus in Salmonella typhimurium. Extensive genetic and biochemical analysis concerning the function of this locus has provided some of the basic information necessary for an understanding of the relationship between gene structure and mutability.

One method of attacking this problem is to determine whether there is a relationship between the linear order of non-identical alleles within a locus and the ability of each allele to respond to various types of mutagens. For this purpose an excellent test system has been provided by the recent work of Balbinder who has studied the arrangement of 12 alleles within the tryD locus. In addition, his genetic analysis of revertants involving a number of tryD alleles has confirmed the accuracy of the provisional phenotypic discrimination between those revertants presumed to be associated with genetic changes at the site of the original mutation and revertants presumed to involve mutations at a suppressor locus. The study reported here was concerned with 10 alleles of the tryD locus and at least 8 suppressor genes.

Four chemical mutagens have been tested. Three of these, ethyl sulfate, ethyl methanesulfonate, and bromoethyl methanesulfonate, belong to a large class of chemicals which can function as alkylating agents under physiological conditions. A number of alkylating agents, including some alkyl methanesulfonates, have been studied extensively because they can act as tumorinhibitors. Other alkyating compounds exhibit carcinogenic activity. Triazine and diepoxybutane, two alkylating agents already proven to be potent mutagens in Salmonella, have been used clinically in cancer chemotherapy. So far is known, the three alkylating compounds used in the present work have not been used previously in mutagenic studies with bacteria. As an additional test chemical, $MnCl_2$ was chosen. Although previously shown to be mutagenic in Salmonella, $MnCl_2$ had not been tested with tryD auxotrophs.

The results of some tests with the 4 chemicals are presented in Table 1 together with data of Balbinder for ultra-violet-induced mutations. It is well known that considerable variations in results are obtained with similar treatments in different experiments. This variability can be minimized when treatments with different mutagens are applied on the

TABLE 1

FREQUENCIES OF SPONTANEOUS AND INDUCED REVERSIONS IN TEN TRYPTOPHAN D AUXOTROPHS OF SALMONELLA TYPHIMURIUM

	Mutations per 10^9 Survivors[x]											
	Spont.		E.S.		E.M.		B.M.		$MnCl_2$		UV*	
Mutant tryD	Rev.	Sup.	Rev.	Sup.	Rev.	Sup.	Rev.	Sup.	Rev.	Sup.	Rev.	Sup.
-1	0.30	0.14	2400	11500	3500	6500	680	920	500	1950	1334	7986
-10	.44	0.36	0	6000	0	13000	0	8500	152	+	10	511
-35	.45	1.64	20	1400	6?	1400	0	5000	20	9300	174	2154
-9	.04	0.00	0	0	0	0	0	0	0	0	0	0
-6	.60	0.16	0	12?	0	0?	0	50	500	280	44	414
-7	.23	1.96	6	26000	0	19000	0	3000	61	6000	0	29304
-66	.66	0.50	0	+	0	+	0	+	59	6000	3?	213
-55	.80	0.83	0	0	0	0	0	0	22	3000	11	0
-69	.77	0.02	2300	+	3500	+	3300	+	6600	+	12	0
-42	.06	0.00	26	0	18	0	0	0	5	0	24	0

Spont.= spontaneous; E.S.= Ethyl Sulfate; E.M.= Ethyl Methanesulfonate; B.M.= Bromoethyl Methanesulfonate; Rev.= Reversion; Sup.= Suppressor.

* Data of Balbinder from Carnegie Institution of Washington Year Book 58, and personal communication.

\+ Mutations induced but frequency not determined.

? Tentative values based on small number of colonies indicates order of magnitude of induction.

x Induced frequencies usually reproducible within a factor of about 2 for treatments yielding percentages of survival differing by no more than a factor of about 2. Exceptions listed in table are figures for $MnCl_2$ treatment of tryD-1 and -6; listed values are 5-15 times higher than duplicate experiments.

same day to bacteria from the same culture. Such a procedure was followed in most of the treatments with the chemical mutagens used here. Of course, the ultraviolet data obtained by Balbinder came from independent experiments. However, in at least two instances in which ultraviolet treatment was applied to one sample of a culture at the same time that other samples were undergoing chemical treatments, our ultraviolet results were sensibly identical to those of Balbinder. The 3 alkylating agents were mutagenically active at doses produces a great deal of killing. For each of these 3 mutagens, percentages of survival after different treatments varied from about 2 to 25%. In attempting to evaluate the meaning of the differences noted in induced mutation frequencies, this variability has been kept in mind.

The figures in Table 1 show that all 4 chemicals were active in producing reversions at some sites within the tryD locus. Suppressor mutants

were also induced by all 4 chemicals. Although the data indicates some examples of differences in the response of particular mutants to the action of the various mutagens, in general the mutagenic potencies and patterns of the 3 alkylating agents show more resemblance to one another than to the potencies and patterns of $MnCl_2$ or ultraviolet. This result is consistent with the concept that the mutagenic action of these chemicals is connected with their alkylating properties.

Striking differences in the response of the same mutant to different mutagens are illustrated by two examples. TryD-69 mutated at comparatively high frequencies in response to treatment with all 4 chemical mutagens, while ultraviolet appeared to be capable of eliciting only a very weak mutagenic reaction. Mutations of the suppressor of tryD-55 were readily induced by $MnCl_2$, but this mutant appeared to be stable with treatment by the other mutagens.

The reponse of different mutants to the same mutagen presents good examples of specific reactivity. In this regard the more critical evidence was provided by those tests in which the response of at least 2 mutants was measured simultaneously. Thus tryD-7 suppressor produced a high frequency of mutations after treatment with all 5 mutagens, whereas the tryD-7 mutant was stable to ultraviolet and the alkyl methanesulfonates, but clearly mutated with $MnCl_2$ treatment and possibly responded to ethyl sulfate. On the other hand reversions of tryD-1 and suppressor of tryD-1 were clearly induced by all 5 mutagens.

The arrangement of 9 tryD mutants in Table 1 is according to the map order determined by Balbinder. TryD-7 and tryD-66 may represent independent occurences of mutations at the same site. TryD-55 and tryD-69 have not been located with respect to one another. The exact location of tryD-42 has not been determined. Of possible significance is the observation that compared to the other mutants, the two alleles at or near the extremes of the map show a greatly heightened mutagenic response to the alkylating agents.

It should be noted in connection with the $MnCl_2$ results that induced mutation frequencies for saline pretreated cells were greater, by factors of from about 7 to about 100, than the frequencies found for cells pretreated with water. This enchancement of the mutagenic effect of $MnCl_2$, similar to the situation in E. coli, was found for mutations of tryD-10, suppressor of tryD-10, tryD-7, suppressor of tryD-7 and tryD-69.

This work was supported by Grant #CY-3773 from the U. S. Public Health Service. The samples of ethyl methanesulfonate and bromoethyl methanesulfonate were kindly supplied by the Institute of Cancer Research, Royal Cancer Hospital, London.

BERNHEIMER, ALAN W., New York University College of Medicine, New York, N. Y. Using phase contrast microscopy, studies were made of the changes produced in living polymorphonuclear leucocytes by growth products of hemolytic streptococci. Miss Lois Schwartz assisted in the investigation. The purpose of this work was to determine which of the known products of streptococcal growth are visibly cytotoxic in vitro. This information was then to be used to reveal, if possible, the existence of undiscovered streptococcal toxins in a test cell, the mammalian leucocyte. Streptolysins O and S were found to be leucocidal, each causing marked cytological changes which were documented by photomicrographs. In contrast, erythrogenic toxin, streptococcal proteinase, hyaluronidase, diphosphopyridine, nucleotidase, and desoyribonucleases B and C, were found to have little or no effect. Preliminary observations indicate that desoxyribonuclease A, or possibly a substance contaminating the enzyme preparation, is capable of injuring leucocytes. With one possible exception, the examination of crude concentrates prepared from a variety of streptococcal strains, failed to reveal the existence of additional leucotoxic agents. This exceptional strain requires further study.

Miss Jeanette Winter investigated the applicability of starch gel electrophoresis to the separation of streptococcal proteins. Efforts were directed toward finding a protein-staining reagent of maximum sensitivity, and to this end a number of dyes including representatives of the azo, quinoneimido, phenyl methane, and natural indigo dyes were compared with Amido Black. Of twelve dyes tested, trypan blue was found to have the same order of sensitivity as Amido Black and possessed good storage qualities as well. Indigo carmine was capable of staining proteins but lacked the desired sensitivity while the others were even less sensitive.

Dr. Harriet Bernheimer lyophilized a large number of pneumococcal strains that are being used to study the genetics and biochemistry of capsular transformations.

CASPARI, ERNST, Wesleyan University, Middletown, Conn., and KIKKAWA, HIDEO, Osaka University, Osaka, Japan. Strain differences in amylase activity in Ephestia. Ephestia larve from different strains were homogenized in distilled water using 0.3 ml water per mg larvae. Two ml homogenate were added to 4.0 ml of a 0.1% starch solution and kept at room temperature. After 30, 60, and 90 minutes, 2.0 ml aliquots were removed, the reaction then stopped by addition of 2.0 ml trichloroacetic acid, and the mixtures tested for remaining starch with 0.1 ml Lugol's solution. For comparison, Drosophila from strains with known high and low amylase activity were homogenized and treated in the same way. Controls were run on starch without homogenate and on enzyme homogenates without starch. Results are charted as follows:

Strain	EBII	ER	Ewa	Eaa	D High activity	D Low activity	6 Enzyme controls	Starch control
90 min. Starch reading	1.0	2.0	4.0	2.0	2.5	3.0	0.0	4.0

It is seen that in Ephestia the great strain differences in amylase activity as follows: BII (wild-type) has the highest activity, wa (white-eyes) the lowest; the other two strains are intermediate. The difference between BII and wa is larger than that between high and low activity Drosophila strains. This variability between strains appears remarkable, since starch is supposed to be the main source of food for the meal moth, Ephestia. Pupae of all Ephestia strains showed much lower activity than the larvae. The difference between strains BII and wa could be demonstrated even in pupae by use of a more dilute starch solution, 0.2%. The disappearance of the amylase activity in Ephestia seems to occur rather suddenly at the time when the animals stop feeding, since full-grown larvae have high activity and prepupae show the low activity characteristic of pupae.

FOX, MAURICE S., Rockefeller Institute for Medical Research, New York, N. Y. E. coli requiring diaminopimelic acid will grow in high surcrose broth in the absence of this amino acid. The ability to grow and the morphology of these bacteria are partly determined by the concentrations of magnesium and manganese in the growth medium. Using microscopic criteria, the optimum concentrations for stablility of the forms that grow out under these conditions were found to be about 0.007 M for manganous sulfate and about 0.15 M for magnesium sulfate. Under these conditions, one of the strains grew out in very clumpy masses of about a thousand bacteria. Another strain, blocked at a different step on the biosynthetic pathway, formed very large, bulbous forms of several hundred times the volume of growing coli. Unsuccessful attempts were made to recombine these two strains by mixing them under various conditions and looking for a genetic loss of the diamino-pimelic acid requirement. In addition, unsuccessful attempts were made to obtain a loss of this metabolic requirement by treating these bacteria with desoxyribonucleate from wild-type E. coli.

FRASER, DOROTHY K., University of Illinois, Urbana, Ill. Genetics of phage Pl. Phage Pl. has been shown to give transduction in strains of coli and Shigella (Lennox, E. S., 1955, Virology 1, 190), and to transduce the lactose locus from coli K12 into the normally lactose-negative Shigella, resulting in an unstable Lac^+ strain which yields high-frequency transducing phage (Luria, et al., 1958). For the further analysis of the transduction process with P1 by the use of phage genetic markers, basic information was needed about the genetics of P1. The previously known characters vir (virulent) and k (ability to grow

on K12 were used. A third, c (clear plaques on K12), proved to be unsatisfactory. A new mutant character, h (ability to grow on Sh/P1), was isolated. Crosses were made by mixed infection of Shigella cells and recombination was shown to occur between these loci in the phage yield. A tentative map was constructed. Among the cells surviving such a mixed infection, some were shown to carry both vir^+ and vir prophages in an unstable form of double lysogeny which segregates to give cells carrying the vir^+ prophage alone.

GRACE, THOMAS D. C., Rockefeller Institute for Medical Research, New York, N. Y. During a previous study, a polyhedral virus was found in cultures of ovarian tissue grown in vitro after these cultures had been subjected to a "physiological shock" brought about by an extreme and abrupt change in the medium used. It was not possible at that time to determine whether the virus induced in vitro would produce symptoms of disease in larvae. Efforts were made this summer to determine whether this would happen. Some difficulty was experienced in obtaining larvae free of the disease, but eventually, about 100 disease-free larvae were reared from eggs. Four to seven days after injection or oral feeding of the virus to the larvae, typical symptoms of a polyhedral virus disease became apparent in 24 of the treated larvae (60%). The 24 larvae died within three days after the disease became apparent. None of the other larvae showed symptoms and eventually completed their development. The low incidence of disease (60%), and resultant death was probably due to the fact that the amount of virus injected was very small. In concurrent experinemts in which larvae were injected with virus obtained from diseased larvae, all acquiring the virus died.

Some preliminary experiments were also carried out in which crystalline DDT was added to cultures of ovarian tissue of the cynthia moth, (Samia walkeri), grown in vitro. Apparently, the DDT crystals had no effect on cell growth and migration, or on the contractility of the ovarian muscles. Some phases of the work on induction of polyhedrosis were supported, in part, by a Lalor Foundation Summer Research Fellowship.

GRANICK, S., Rockefeller Institute for Medical Research, New York, N.Y. The summer was spent in completing a manuscript for publication, and writing a review on chloroplast inheritance, structure, and function.

HARTMAN, PHILIP E., and SERMAN, DRASKO, Johns Hopkins University, Baltimore, Md. Previous studies demonstrated that mutants of locus hisD of Salmonella were of three functional types, designated Da, Db, and Dab. More accurrate mapping of these mutants was accomplished during the summer. The results to date show that locus D may be divided into two functional subunits of approximately equal size. All mutants which are functionally of the Da class map within one region now also designated Da. All Db mutants so far examined map in the second re-

gion, Db. The Dab mutant, which show no complementation with either subunit, are scattered within the Db region and to the right of all Db mutants so far tested. Locus D appears to be, in terms of recombination, of a size equal to the loci adjacent to it (loci C and G). Thus, all three loci appear to be equal in size, making the subunits of locus D half of the recombinational length of each of the loci. All evidence is consistent with the interpretation that the three loci are truly adjacent.

HAUGAARD, NIELS, University of Pennsylvania School of Medicine, Philadelphia, Pa. The studies carried out this summer were concerned with the properties of an enzyme complex from E. coli which oxidizes d- and l-lactic acids. Sonic extracts were prepared from E. coli, strain B, and particulate preparations were obtained by high-speed centrifugation. The particles have been found to oxidize d- and l-latic acids to pyruvate in the absence of added cofactors (Haugaard, in press). It is of particular interest that the relative activity of the enzyme complex toward the two lactate isomers varies from one preparation to another and is dependent on the growth conditions of the bacteria from which the enzyme preparations are made. Enzyme particles prepared from bacteria grown in the presence of d- and l-lactate oxidize d- and l-lactic acid at about equal rates, while preparations obtained from cells grown in the presence of glucose oxidize l-lactate at a much lower rate than d-lactate.

Further studies were carried out on the reaction mechanization of this enzyme complex. It was found that aging or treatment of the particles with xylene caused a destruction of the electro carrier system with little decrease in d- or l-lactic acid dehydrogenase activity when determined in the presence of methylene blue. Neither d- nor l-lactic acid dehydrogenase activity was affected by the addition of diphosphopyridine nucleotide. The studies indicated that the stereospecificity resides in the structure of the protein component(s) of the lactic acid dehydrogenase(s) of the bacterial particles.

HOTCHKISS, ROLLIN D., and POWERS, SUSAN, Rockefeller Institute for Medical Research, New York, N.Y. Growth of pneumococcus was studied in several media in the presence of a variety of metabolite analogs and inhibitors. The purpose was to seek metabolic systems capable of clearly defined inhibition, resulting at the same time in specific metabolite accumulation and growth suitable for mutant selection. In addition, the interaction between growing or killed pneumococci and acrifiavine dyes was investigated in some detail under a variety of conditions.

KIKKAWA, HIDEO, Osaka University, Osaka, Japan. The strain of D. melongaster, KSL, obtained from Sweden, was originally selected on the basis of its small number of sternopleural bristles. Later it was found to be highly resistant to parathion. Using strain KSL along with some marked, sensitive strains, a genetic analysis was made of the parathion resistance inheritance. A major dominant gene was found to be

responsible for parathion resistance and is present at locus 64 on the second chromosome. A maternal effect was also noted. Using strain KSL females and sensitive-strain males, the F_1 generation was more resistant to parathion than to those of the reciprocal mating. These results are in accordance with those previously determined in Japanese resistant strains such as Hikone and WMB. The parallel condition found in inheritance in various populations of D. melanogaster suggests that parathion resistance may be brought about by a similar mechanism.

LURIA, S. E., and BOICE, LUBELLE, University of Illinois, Urbana, Ill. Transduction with phage Pl. A variety of experiments were done to test some hypotheses recently put forward concerning the mechanism of transduction (Adams and Luria; Luria et. al, 1958).

1. Transduction of lactose utilization by a virulent mutant of phage Pl.—Phage Pl vir k (virulent, grown on K-12 Lac^+) transfers Lac^+ at a low frequency to E. coli Lac_1^-. Cells transduced at low multiplicities of infection (about 0.1) are stably lactose positive and sensitive to phage Pl vir k. Addition of non-transducing phage (m.o.i. 1.5) did not increase the transduction frequency.

2. Attempts to prepare a Pl lysate transducing Gal^+ at high frequency.—Many sectored colonies were obtained on EMB galactose agar by transducing GAL^+ into a galactose negative (Gal_1^-) K-12 strain with phage Pl grown on S. dysenteriæ. The sectored colonies carry a defective prophage. The do not, however, contain Gal^- cells; the segregating character and its association with the defective prophage have not been identified. No HFT lysates for Gal^+ could be obtained.

3. Transduction of try^+ into B/r/l, tr to Pl.—Transduction using phage Pl grown on S. dysenteriæ Sh gave a variety of colonies containing cells with different abilities to grow with limiting amounts of tryptophane. No defective lysogenic, nor heterogenotes, were obtained.

S. E. Luria wrote two articles on "Viruses as Determinants of Cellular Functions" and on "Viruses: a Survey of Some Current Problems". IuBelle Boice completed an M.S. thesis (Univ. of Illinois).

MARAMOROSCH, KARL, The Rockefeller Institute for Medical Research, New York, N.Y. A study was made of a beneficial effect of aster yellows virus on a nonvector insect. Corn leafhoppers are ordinarily unable to survive on healthy aster plants for more than 2 days. When such insects were confined, however, to aster plants infected with the eastern strain of aster yellows virus, they survived almost as well as on corn plants. Feeding on diseased plants resulted in the acquisition, but not in the transmission of aster yellows virus. It also conditioned the insects so that they were later able to feed also on healthy aster plants. This acquired ability was retained for very long periods, probably for the rest of the insects' lives. The conditioning effect was destroyed by exposing conditioned insects to 37° C for 10 days. Because this temperature was found to destroy the aster yellows virus in living aster leafhoppers, it was assumed that it also destroys the virus in corn leafhoppers. This

assumption was confirmed by the failure to recover the virus from the hemolymph of heat-treated corn leafhoppers. Recovery was made from untreated conditioned controls by the insect injection technique.

It was therefore assumed that the acquisition and retention of the aster yellows virus by corn leafhoppers benefited the insect by increasing its plant host range. It seems likely that the virus multiplies to a certain degree in corn leafhoppers and in some way renders the previously unpalatable food digestible. Breeding tests were begun to find whether or not the beneficial effect can be detected in the progeny of insects that acquired virus.

Part of the summer was spent in preparing papers for the XV International Congress of Zoology in London and for the VII International Congress for Microbiology in Stockholm.

MELECHEN, NORMAN E., St. Louis University School of Medicine, St. Louis, Mo. "Acriflavine-resistance" of a mutant of T2H, isolated (and kindly supplied) by Dr. J. Tomizawa, was tested for its possible usefulness as a marker in superinfection experiments. A system was desired which would permit the selection, from among a large excess of infected bacteria and the phage which they yielded, of a few superinfected bacteria and their phage yields. This mutant character (ac) confers increased resistance to acriflavine upon the phage-synthesizing ability of infected E. coli. It was looked for as an indicator of superinfection in a population of bacteria previously infected by "non-resistant", wild-type phage (ac^+). Many observations, which the following largely corroborate, were made by Tomizawa.

Various methods of discriminating ac and ac^+ phage and infcetive centers were assayed: E. coli H were grown in a tris-salts-glucose medium and were infected with either ac or ac^+. Various concentrations of acriflavine were added to aliquots of such infected cultures. At various times the cultures were subjected to cyanide or chloroform lysis and the yields of phage determined. From both ac and ac^+ infected cultures, the yield decreased essentially logarithmically with increased dosage to a level of about 10^{-4} of the progeny yields of untreated cultures. The untreated ac and ac^+ controls gave similar yields. The survival value of 10^{-4} was reached with $3\mu g/ml$ for those infected with ac. Sensitivity tests of individual free phage and infective centers were also performed on plates containing various concentrations of acriflavine added to the regular plating medium. The plaque size obtained from both ac and ac^+ infective centers and free phage was very dose-independent. No concentration of acriflavine could be found which would allow discrimination of ac from ac^+ and, at the same time, allow the ac phage or infective centers to be assayed with practical efficiency. Bacteria simultaneously infected with proportionately high multiplicities of ac^+ to ac did not show acriflavine resistance in the proposed experimental setup. Analogous experiments were performed using a selected acriflavine resistant E. coli H. The results obtained al-

lowed no better discrimination than those already described. The ac-ac$^+$ system, therefore, seemed inapplicable.

STAHL, FRANKLIN W., University of Missouri School of Agriculture, Columbia, Mo. During the summer a review, "Radiobiology of Bacteriophage", was written for inclusion in Vol. II of "The Viruses" (Stahley and Burnet, eds.) to be published by Academic Press.

TING, ROBERT C., University of Illinois, Urbana, Ill. Some properties of chloramphenicol resistant mutants of Salmonella typhimurium. In an extension of work on the effect of chloramphenicol on lysogenization and curing of S. typhimurium T, we have studied a chloramphenicol resistant mutant, T cal^r which was obtained by the gradient plate method. T cal^r resists 100 gamma/ml chloramphenicol in agar and 40 gamma/ml in broth. When young cells of T are infected with phage P22 c$^+$ and exposed within a few minutes to 50 gamma/ml chloramphenicol, many infected cells are cured and give rise to phage-sensitive colonies upon removal of the drug. When chloramphenicol is added 10 minutes after infection, the result is, instead, a shift from the lytic to lysogenic response. Chloramphenicol treatment up to 200 gamma/ml does not affect the outcome of infection of the T cal^r cells. When T cal^r cells are used as plating bacteria for phage c$^+$, a change of plaque morphology on Bresch indicator agar is observed; the plaques have clear centers. This suggests that T cal^r mutant was less readily lysogenized than T. In fact, at phage/cell ratios of 4.5-5, less than 5% of the infected T cal^r are lysogenized, in comparison to 80-85% lysogenization with T cells. The adsorption of phage c$^+$ and its latent period in T cal^r are the same as in T. The burst size in T cal^r is only $\frac{1}{3}$ that found in T. The mutation appears to provide a phage-producing mechanism less sensitive to various external or internal agencies that can shift the phage cycle towards lysogeny.

WATSON, G. S., Australian National University, Canberra, Australia; and CASPARI, ERNST, Wesleyan University, Middletown, Conn. Population genetics of cytoplasmic factors. Considerations were started which will lead to a statistical investigation of the behavior of cytoplasmic hereditary factors in populations. Two of the most frequently occurring cases were considered, cytoplasmic male lethality, which has been found in several species of Drosophila, and cytoplasmic pollen sterility, as frequently found in plants, e.g., maize.

In both cases the proportion of the two cytoplasms in the population will remain constant, unless changed by selective effects of cytoplasmic mutations. This may explain why characters affecting the viability and fertility of males are so frequently encountered in cytoplasmic inheritance. If genes are present in the population which restore the pollen fertility of cytoplasmically pollen-sterile plants ("restorer genes" of D. F. Jones) selection will occur in favor of the "restorer genes", so that the alleles permitting phenotypic expression of the cytoplasm causing pollen sterility will be eliminated from the population. There may therefore occur cyto-

plasmic differences inside a population which are not expressed phenotypically in the population, but will become apparent after crosses with different populations. This situation is frequently found in investigations dealing with cytoplasmic inheritance. The considerations show that cytoplasmic differences resulting in pollen sterility or male lethality will not act as primary mechanisms of sexual isolation which might give rise to the emergence of reproductively isolated populations.

COURSE ON BACTERIAL VIRUSES

June 16-July 5, 1958

Instructors: F. W. Stahl, California Institute of Technology; S. E. Luria, University of Illinois.
Assistant: C. M. Steinberg, California Institute of Technology.

The intensive three weeks' course on bacteriophages was given for the fourteenth time in the summer of 1958. The laboratory portion of the course was taught along the lines followed in recent years. A few changes were made in order to maintain the up-to-date standards for which the course is noted. A grant from the U. S. Public Health Service made it possible to invite a number of noted speakers to address the class as well as other interested persons. In contrast to the custom of previous years, the speakers were encouraged to address their remarks primarily to the students in the course. Their cooperation in this respect, coupled with our good fortune in being able to arrange a speaking schedule with a logical sequence of topics, made this part of the course of considerable value to the students.

The speakers and their topics were:

Roger B. Herriott, Johns Hopkins University—Phage structure and properties of phage protein.
Bruce Ames, National Institute of Health—Chemistry of the germinal substance of bacteriophage.
Charles A. Thomas, Johns Hopkins University—Organization of phage DNA.
Allan Garen, Massachusetts Institute of Technology—Early steps in infection by phage.
Cyrus Levinthan, Massachusetts Institute of Technology—Genetics of bacteriophage.
Frank Stahl, California Institute of Technology—Radiation genetics of bacteriophage.
Francois Gros, Pasteur Institute, Paris, France—Mechanism of amino acid incorporation.
A. D. Hershey, Carnegie Institution—Chemistry and/or radiobiology of vegetative phage.
J. D. Watson, Harvard University—The structure of DNA and a hypothesis for the method of its duplication.
Myron Levine, Brookhaven National Laboratory—Lysogenization.
Alan Campbell, Carnegie Institution—Transduction and defective temperate phage.

The eighteen students and two auditors enrolled in the course were:

Melvin M. Belsky, Ph.D., Brooklyn College, Brooklyn, N.Y.
D. G. Catcheside, Ph.D., The University, Birmingham, England.
Carlo Cocito, M.D., Rutgers University, New Brunswick, N.J.
Morton Davidson, Grad. Stud., New York University College of Medecine, New York, N.Y.

Leila Diamond, Grad. Stud., Sloan-Kettering Institute for Cancer Research, New York, N.Y.

Maxwell Eidinoff, Ph.D., Sloan-Kettering Institute for Cancer Research, New York, N.Y.

Robert A. Fitts, B.S., Charles Pfizer and Co., Inc., Brooklyn, N.Y.

Edwin B. Horowitz, Grad. Stud., University of Chicago, Chicago, Ill.

Urs Leupold, Ph.D., University of Zurich, Zurich, Switzerland.

John C. Loper, Grad. Stud., Johns Hopkins University, Baltimore, Md.

Louis H. Muschel, Ph.D., Walter Reed Army Institute of Research, Washington, D.C.

Theodore Sall, Ph.D., University of Pennsylvania School of Medicine, Philadelphia, Pa.

Drasko Serman, Grad. Stud., Johnson Hopkins University, Baltimore, Md.

K. K. Takemoto, Ph.D., National Institute of Health, Bethesda, Md.

D. von Wettstein, Ph.D., Forest Research Institute, Stockholm, Swede.n.

G. S. Watson, Ph.D., Australian National University, Canberra, Australia.

Bernard Weisblum, Grad. Stud., State University of New York, College of Medicine, Brooklyn, N.Y.

Jeanette Winter, Grad. Stud., New York University College of Medicine, New York, N.Y.

Auditors:

E. Balbinder, Ph.D., Carnegie Institution, Cold Spring Harbor, N.Y.

Robert C. Baumiller, S.J., Bellarmine House of Studies, St. Louis, Mo.

COURSE ON BACTERIAL GENETICS

July 7-26, 1958

Instructors: M. Demerec, Carnegie Institution; Evelyn Witkin, State University of New York; V. Bryson, Rutgers University; P. E. Hartman, Johns Hopkins University.

This course on selected methods in bacterial genetics research was given for the ninth time, in 1958, to a group of advanced graduate and post graduate 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. In connection with the course, the following special lectures and seminars were given by instructors and other research scientists in the field:

Francis J. Ryan, Columbia University—Natural mutation in bacteria.
Evelyn M. Witkin, State University of New York—Induced mutation in bacteria.
H. E. Umberger, Harvard Medical School—Genetic and physiological control of isoleucine, valine, and leucine biosynthesis in E. coli.
M. Demerec, Carnegie Institution—Use of transduction in genetic studies.
D. G. Catcheside, The University, Birmingham, England—Complementation between alleles in heterocaryone of Neurospora.
Norman H. Giles, Yale University—Complementarity between alleles in Salmonella.
Urs Leupold, University of Zurich, Zurich, Switzerland—Studies on allelism in yeast.
M. Westergaard, University of Copenhagen, Copenhagen, Denmark—Chemical mutagenesis.
S. Suskind, Johns Hopkins University—Immunological approach to enzyme formation.
S. H. Goodgal, Johns Hopkins University—Transformation in Hemophilus.
J. A. Roper, The University, Glasgow, Scotland—Nucleus cytoplasmic interaction in Aspergillus.
S. E. Luria, University of Illinois—Integration phenomena after mating and transduction in bacteria.

The eighteen students and one auditor who attended were:
Robert C. Baumiller, S.J., Bellarmine House of Studies, St. Louis, Mo.
A. P. Bayan, M.S., E. R. Squibb and Sons, New Brunswick, N.J.
D. G. Catcheside, Ph.D., The University, Birmingham, England.
James E. Darnell, M.D., National Institute of Health, Bethesda, Md.
Leila Diamond, Grad. Stud., Sloan-Kettering Institute for Cancer Research, New York, N.Y.
Michael Filosa, Ph.D., Princeton University, Princeton, N.J.
Edwin B. Horowitz, Grad. Stud., University of Chicago, Chicago, Ill.

Urs Leupold, Ph.D., University of Zurich, Zurich, Switzerland.

Timothy Loeb, B.S., Rockefeller Institute for Medical Research, New York, N.Y.

Marian Martinello, B.S., New York University College of Medicine, New York, N.Y.

Julius E. Officer, Ph.D., Fort Detrick, Frederick, Md.

Milton J. Schlesinger, M.S., University of Michigan, Ann Arbor, Mich.

Sondra Schlesinger, B.S., University of Michigan, Ann Arbor, Mich.

Aaron J. Shatkin, Ph.D., Rockefeller Institute for Medical Research, New York, N.Y.

Edward J. Tynan, B.S., Charles Pfizer and Co., Inc., Brooklyn, N.Y.

D. von Wettstein, Ph.D., Forest Research Institute, Stockholm, Sweden.

Bernard Weisblum, Grad. Stud., State University of New York, College of Medicine, Brooklyn, N.Y.

Jeanette Winter, Grad. Stud., New York University College of Medicine, New York, N.Y.

Auditor:

Davidson, Morton, Grad. Stud., New York University College of Medicine, New York, N.Y.

COUSE ON QUANTITATIVE STUDY OF HUMAN CELLS IN TISSUE CULTURES

July 28-August 16, 1958

Instructors: H. Moser, Biological Laboratory; E. H. Y. Chu, Yale University.

Assistants: Leila Diamond, Sloan-Kettering Institute for Cancer Research; Jean Thompson, Huntington, N.Y.; Dr. K. Tomizawa, Biological Laboratory.

This course, offered for the first time, was given to acquaint the student with recently developed methods for quantitative analysis of mammalian cell cultures under defined environmental conditions. The three-week course consisted of selected experiments with cell cultures derived from man and other mammals. The exercises included the determination of clonal growth rates, the determination of the essential nutritional requirements of established cell cultures, the analysis of the effects of ultraviolet irradiation, karyotype analysis of cell cultures (cytogenetics of tissue cultures), preparation of primary cell cultures, and clone isolation. The number of applicants, which exceeded the capacity enrollment, reflected the great interest in the topic.

In addition to the intensive laboratory sessions, ten seminars were presented by investigators active in tissue culture research or in related fields. The speakers and their topics were:

Grace Leidy, Presbyterian Medical Center—Sensitivity to poliomyelitis virus of clonal line of HeLa.

George Yerganian, Harvard Medical School—Viable chromosomal aberration in vivo and in vitro of the Chinese hamster.

John Biesele, Sloan-Kettering Institute for Cancer Research—Some studies with antimetabolites in mammalian tissue cultures.

Ernst Caspari, Wesleyan University—Cytoplasmic inheritance.

J. E. Darnell, National Institutes of Health—Nutritional studies on polio virus HeLa cell systems.

F. Kaudewitz, Max Planck Institut fur Virusforschung, Tubingen, Germany—A new type of mutation delay in bacteria.

Joel G. Flaks, University of Pennsylvania School of Medicine—The induction of enzyme synthesis on phage infection.

T. Grace, Commonwealth Scientific and Industrial Research Organization—Insect tissue culture.

R. I. DeMars, National Institutes of Health—A proposed method of selecting of auxotrophic mutants in animal cells.

H. Bernheimer, State University of New York College of Medicine—Capsular transformations in Pneumococcus.

Mr. H. S. Tubiash, of the United States Department of Agriculture, Greenport, New York, presented a film entitled "The Story of Plum Island Animal Disease Laboratory", and Mr. A. Delong, of the Bellco Glass Company, gave a demonstration on tissue culture glassware.

The fourteen students and six auditors enrolled in the course were:

Marc Beem, M.D., University of Chicago School of Medicine, Chicago, Ill.

Wilma B. Bias, Grad. Stud., Johns Hopkins University, Baltimore, Md.

Ernst Caspari, Ph.D., Weslyan University, Middletown, Conn.

Choppin, Purnell W., M.D., Rockefeller Institute for Medical Research, New York, N.Y.

Flaks, Joel G., University of Pennsylvania, Philadelphia, Pa.

Graber, C. D., Ph.D., Brooks Army Medical Center, Fort Sam Houston, Texas.

Higuchi, Kiyoshi, Ph.D., Fort Detrick, Frederick, Md.

Kaudewitz, Fritz, Ph.D., Max Planck Institut fur Virusforschung, Tubingen, Germany.

Jackson, Peter W., Ph.D., Squibb Institute for Medical Research, New Brunswick, N.J.

Pine, Martin J., Ph.D., Roswell Park Memorial Institute, Buffalo, N.Y.

Sparck, Jorgen V., Ph.D., Tuberculosis Immunization Research Center, Copenhagen, Denmark.

Watson, G. S., Ph.D., Australian National University, Canberra, Australia.

Wheelock, Earl F., M.D., Rockefeller Institute for Medical Research, New York, N.Y.

Wisniewski, Henry J., Ph.D., Milwaukee Health Dept., Milwaukee, Wis.

Auditors:

Banic, S., Ph.D., Institute of Microbiology, Ljubljana, Yugoslavia.

Guidote, Paz C., M.D., Booth Memorial Hospital, Flushing, N.Y.

Hadden, Joanna, B.S., Cold Spring Harbor, N.Y.

Petker, K., M.D., Booth Memorial Hospital, Flushing, N.Y.

Therkelsen, Aage J., M.D., University of Aarhus, Denmark.

Tubiash, Haskell S., M.S., Plum Island Animal Disease Laboratory, Greenport, N.Y.

NATURE STUDY COURSE

June 30-August 1,
August 4-22, 1958

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

Assistants: Gail Geraghty, Northport, N.Y.
Struan Robertson, Northport, N.Y.
Gay Adams, Huntington, N.Y.
Cynthia Hotchkiss, Cold Spring Harbor, N.Y.

The Nature Study Courses extended over a five-week period. These courses are designed to serve two purposes: first, to stimulate children's interest in nature by observation and study of the flora, fauna, and geology of the area around Cold Spring Harbor, N.Y.; second, to fill the needs of those children who want to know more about the environment surrounding them.

For the first time, several new specific subject matter courses were instituted in lieu of the more general nature study presented in previous years. It was felt that children were more likely to return in succeeding years if a variety of subjects were offered. A brief description of the courses follows:

1. General Nature Study. (3 sections). This was an introductory course in nature study for six-and seven-year olds in which a general sampling of all aspects of the subject was presented.
2. Conservation and Ornithology. Emphasis was placed on conservation of our natural resources and wildlife in the Long Island area. Insufficient registration in the ornithology section occasioned it to be placed as a supplement to conservation study. The identification and study of birds, birds' nests and foods, were undertaken.
3. Entomology. This course dealt with the collection, identification, preservation, and study of insects common to Long Island. Of particular note were the live specimens kept in the lab through the larval sage into the period of emergence.
4. Seashore Life. In this course, studies were conducted on marine life, both plant and animal, found on the shore below the low tide mark. Our large salt water aquarium abounded with a collection of fish, crustaceans, mollusks, and algae.
5. Botany. This subject concentrated on the collection and identification of different forms of plant life from algae through the flowering types. The children expressed particular interest in the area of edible wild foods. A representative collection of potted, native ferns was exhibited.

6. Vertebrate Zoology. Each successive week of this course was devoted to the study of a different class of vertebrates, i.e., fish, amphibians, reptiles, birds, and mammalians. Representative living specimens of each of the classes were kept and examined. Extensive collections of fish, owl pellets, and bird feathers were prepared.
7. Advanced Nature Study. This course offered the children an intensive survey of each of the other five electives. Each student prepared an original project under the supervision of the instructors. Reports presented were excellent and served only to astonish us with the children's capabilities.

In general, the response to the change made in the use of subject courses in 1958, was favorable. The enrollment increased this year to 147 as compared to the 1957 figure of 137.

Wawepex again served as home base for the classes. Two additional classrooms on the upper floor were renovated for use, an outside exit was built, and lavatory facilities provided.

Of particular note during the summer was the extremely large number of live specimens held for observation and study. Film showings on two Tuesday evenings were well attended. Other films and filmstrips were used in rainy-day programs. During the last week of the courses all-day trips were made to Jones Beach, Shelter Rock Pines, Tackapausha Preserve, The Botanical Gardens in the Bronx, and to a Syosset pond. Many thanks go to the deserving mothers who functioned as chauffeurs.

The first session closed on August 1, 1958, with an Open House at Wawepex. Here, the collections, special projects and other work of the children were exhibited. Refreshments were served beneath the apple tree on the lawn.

An additional session in General Nature Study was offered this year for those who wished to remain and for those who could not enter the first session because of its closed classes or for various other reasons. This course met on Mondays, Wednesdays, and Fridays, for a three-hour period each of these days from August 4 to August 22, 1958. Mr. Rosenberg and Mr. Heck presented this part of the Nature Study program in two sections, with a total enrollment of 20.

A few of the children took more than once course. The following is a list of students enrolled in Nature Study Courses:

Abramowitz, Joel William
Allardice, Susan
Amsler, Donald
Amsler, Louis
Arthur, D. Richard
Baker, Barbara
Barker, Steven
Barnett, Paul
Baron, Richard
Baron,Baron, Ruth
Bates, Gordon
Bernheimer, Alan W.
Blauman, Ruth Ann
Blecher, Robin
Bloom, Christopher
Broderick, Michael J.
Bryan, Robert
Castellana, R. J.
Caveny, Neola
Collyer, Barry

Coulter, Hillary
Doyle, Gerard
Doyle, Peter
Dudley, Sarah W.
Dull, Stephen R.
Duvernoy, Eugene G.
Eastment, Jeffrey
Eastment, Tony
Egan, Bruce
Egan, Wesley
Elliot, Robert
Emberson, Richard M., Jr.
Emery, David R.
Fennebresque, Kim
Fieldgate, David J.
Franklin, Michael R.
Friedlander, David
Friedman, Andrew
Friedman, Edward
Ghoreyeb, Albert, Jr.
Gould, Arnold
Grant, R. Alexander
Grant, Deborah S.
Hart, Douglas S.
Hellmann, Bruce
Hellmann, Margaret Ann
Herskovitz, Stephen
Hilbert, Guy
Jackson, Melissa C.
Jamieson, John L.
Jamieson, Norman, Jr.
Jeanette, Arthur
Jeanette, Michael
Jensen, James
Jensen, Mary
Johnson, Timothy David
Johnson, Jean M.
Kafka, Robert
Karpen, Daniel
Karpen, Joseph
Karpen, Seth
Kendrick, Eleanor
Knowles, Kenneth E. III
Korwan, Mark
Korwan, William
Kronenberg, Fredi
Krumenauer, James
Krumenauer, Susan
Kurahara, Mark
Kurahara, Wendy Sona
Lamb, Kirk E.
Lazare, Daniel
Lazare, Peter
Levine, Daniel
Levitt, Marc P.
Levy, Paul Allen
Luria, David Daniel
Magnusen, Christian
Magnusen, Nels
Mainhardt, Peter J.
Mason, Ellen
McGreevy, Kathleen
Miller, Terry
Minerd, Barbara
Minerd, Cynthia
Moulton, Suzanne
Muschell, Ruth Jo
Nagy, George
Nash, Nicholas
Nerken, Ira
Nerken, Ruth
Noble, Mark D.
Novak, Michael
Nystrom, Bruce
Olinsky, Franklin
Olitsky, Eve
Olson, Gregory
O'Neil, George D.
Palter, John
Paul, Richard J.
Post, Peter W.
Rankin, Bruce
Rankin, Douglas
Robertson, John L.
Rowe, David
Rowe, Stephen
Schur, Ellen
Shaut, Christopher A.
Sherman, Susan
Simon, Ellen J.
Slack, Nancy
Smith, Gregory
Solbert, Elizabeth A.
Steinberg, Alan

Takami, Bruce C.
Thompson, Michael
Tienken, Joycelyn
Toner, John
Trautmann, Charles H.
Treu, Jesse
Trieste, Floyd
Truesdale, Edward D.
Vuckovic, Ann
Vuckovic, Jill
Wagner, Glenn Arthur
Wagner, Ruby Gay
Walker, Peter Michael
Warren, Constance B.
Warren, Virginia G.
Waters, Dennis Perry
Wattel, Karen
Weber, Bruce
Wehle, Nancy
White, Kathy
White, Paul
Wilson, Candice Jean
Wisoff, Claire Ellen
Witkin, Andrew
Wittenberg, Louise E.
Young, Karen
Young, Kim
Young, Susan
Zimmet, Donald

Session August 4-22

Abramowitz, Joel William
Anderson, Lance
Anderson, Stephen
Blecher, Robin
Caveny, Neola
Claflin, Barbara B.
Eastment, Jeffrey
Glenn, Kevin
Jazombek, Jeffrey
Mason, Ellen
McCabe, Mary
McCabe, Peter
Nash, Nicolas
Novick, David
Paul, Richard J.
Raskin, Adam
Thiele, Nancy K.
Warren, Constance
Warren, Virginia
Wilson, Candice

WORKSHOP IN NATURE STUDY

June 23-July 24, 1958

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

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

The Workshop in Nature Study, offered in 1958 for the third consecutive year, is designed primarily for teachers of the elementary grades who, upon successful completion of the course, are given two in-service credits as authorized by the State Education Department, Division of Teacher Education.

The Workshop was held for two weeks, Mondays through Fridays, with sessions from 9:00 to noon and from 1:00 to 3:00 p.m. As usual, there was an early morning bird walk. The morning sessions were devoted to field trips to the various types of natural habitats on Long Island. Afternoons were given over to lectures, demonstrations, discussions, examinations, and identification of field collections, and also to project work. Fourteen teachers were enrolled.

The purpose of the Workshop is to acquaint teachers with the natural environment of Long Island, and to give such background information and experience for the purpose of improving classroom presentations. The principal means of instruction consisted of visits to explore representative ecological situations. In each habitat, the participants collected specimens of the flora and fauna for subsequent identification and study. Various techniques for incorporating nature study into the school curriculum were presented. Information pertaining to the organization of field trips for class groups was reviewed. Each student kept a nature log for the duration of the course and prepared a term project. Reports and demonstrations were given on the last day of the Workshop.

Certificates were awarded on the fourth of July, 1958, to the following students:

Brevda, Shirley
Haseth, Beatrice T.
Horn, Ruth D.
Jorge, Evelyn R.
Koster, Joan Arden
Kreutziger, Joan L.
Leidner, Rita
Parada, Lydia P.
Potter, Royal F.
Rogers, James
Roth, Leonora
Shaffer, Catharine
Willard, Evelyn W.
Willard, Gates

PUBLICATIONS

SYMPOSIA VOLUMES

*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 Membrances, 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 p..
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	1957	Population Studies: animal ecology and demography, xiv + 437 pp.
Vol. XXIII	1958	Exchange of Genetic Material: mechanisms and consequences xvi + 433 pp.
Vol. XXIV	1959	Genetics and 20th Century Darwinism (in press)

* Out of print

PUBLICATIONS BY STAFF MEMBERS

Abramson, H. A. 1958a. (Editor Neuropharmacology, Vol. IV. New York: Josiah Macy, Jr. Foundation.

1958b. Esquizofrenia seudoalergica. Revista Argentina de Algeria.

1958. Lysergic acid diethylamine (LSD-25): XXIX. The response index as a measure of threshold activity of psychotropic drugs in man. J. Pschol. In press.

Abramson, H. A. and Peshkin, M. M. 1958. First National Seminar of Regional Consultants. Ann. Allergy 16:473.

Baron, B. O., Sklarofsky, B., Fremont-Smith, N., and Abramson, H. A. 1958. Lysergic acid diethylamide (LSD-25). XXVIII. Assay of 2-bromolysergic acid diethylamide by Siamese fighting fish. J. Psych. 46:303-08.

Evans, L. T., Abramson, H. A., and Fremont-Smith, N., 1958. Lysergic acid diethylamide (LSD-25): XXIV. Effect on social order of the fighting fish, Betta splendens. J. Psychol. 45:263-73.

King, James C. 1959. Difference between populations in embryonic development rates. Amer. Nat. 93:171-80.

Sokoloff, A., 1958. Types of bristles on the anterior margin of Drosophlia wings conspicuous as a result of treatment DIS, 171-172.

Weiss, B. Abramson, H. A., and Baron, B. O., 1958. Lysergic acid dieythlamide (LSD-25): XXVI. Effect of potassium cyanide and other oxidase and respiratory inhibitors on the Siamese fighting fish. Arch. Neurol. and Psychiat. 80:345-50.

LONG ISLAND BIOLOGICAL ASSOCIATION

Report of the Secretary

The 35th annual meeting of the Long Island Biological Association was held in the Lecture Hall Cold Spring Harbor, N.Y., on June 24, 1958, with Chairman Nevil Ford presiding. The Secretary reviewed the operations of the Association for the previous year and his report was followed by a statement from the Treasurer on the finances of the organization. Mrs. Franklin as chairman of the Nominating Committee, presented a list of the following names for the Board of Directors: Mrs. David Ingraham, Mr. Gerard Piel, Mr. Richard Storrs, Mr. Amyas Ames, Prof. Th. Dobzhansky, Dr. Rollin D. Hotchkiss, Class of 1962; Mrs. William Smoot, Class of 1960; Mr. Robert V. Lindsay, Class of 1959. All these persons were elected at the meeting. Dr. George W. Corner was elected a Director Emeritus. Chairman Ford introduced the new President, Mr. Walter H. Page, who presented a plan for maintaining a distinguished research staff at the Laboratory. Mr. Page said this could be accomplished through large annual gifts and funds for endowment which would serve to put the Laboratory on a firm working basis and allow for a building program. Dr. Demerec spoke of the needs of the Laboratory in terms of personnel, a new laboratory building, and the funds necessary to carry on the regular services offered by the Laboraory.

During the year, 1958-59, the Board of Directors met twice. At the meeting held at the Harvard Club in New York on November 25, 1958, President Page announced the resignation of Mr. Grinnell Morris as Treasurer and member of the Executive ommittee. A vote was taken to re-elect all officers with Mr. Robert Lindsay replacing Mr. Morris as Treasurer. An Executive Committee was elected for the year as follows: Mr. Page, Mrs. Franklin, Mr. Curtis, Mr. Ford, Mr. Lindsay, Mr. MacDowell and Mr. Nichols. Dr. Demerec reported that Dr. Arthur Chovnick from the University of Connecticut, had been selected to succeed Dr. Bruce Wallace as Director of the Biological Laboratory. Two new projects involving undergraduate students and college teachers were discussed. Arrangements were made to help in the completion of the Mark Adams Fund, a special endowment to aid young scientists in financing their studies at the Laboratory. President Page described plans for continuing the program to obtain funds for the Laboratory.

At the 78th meeting of the Board of Directors held May 28, 1959, also at the Harvard Club in New York, President Page made two important announcements: first, Dr. Arthur Chovnick had become the new Assistant Director of the Biological Laboratory, and second, the National Science Foundation had given the Laboratory $135,000 for rehabilitation of present buildings. President Page pointed out that, in order to obtain research scientists of first rank, an endowment should be established to support them and for this purpose he suggested a Research Guarantee Fund of $2,000,000. He stated further that that the organization was growing to

the extent of having its membership doubled in recent months. A Nominating Committee was appointed to choose new Board members at the 1959 annual meeting. This consisted of the following persons: Mr. Ames, Mrs. Smoot, and Dr. Kaufmann. Announcements of grants from the National Science Foundation will make possible the summer program for undergraduate students and college teachers. Also twelve scholarships for the Nature Study Courses were reported as a donation from the Huntington Federal Savings and Loan Association.

The Executive Committee met on four occasions during the year. The first of these was held in the Lecture Hall at Cold Spring Harbor, N.Y., September 11, 1958. President Page appointed a new Membership Committee with Mr. Crispin Cooke as chairman, and a committee on Endowments with Mr. Nevil Ford, chairman. Most of the discussion was directed toward possible plans for raising funds for the Laboratory. The Executive Committee met a second time in the Lecture Hall on January 11, 1959, when it was announced that the National Institutes of Health had given a five-year grant of $14,000 in support of the summer courses. At the third meeting on March 1, 1959, Dr. Chovnick presented a plan for the Laboratory to become affiliated with the Teachers Insurance and Annuity Association to provide for retirement of full-time employees. This retirement plan was approved to become effective as of April 1, 1959. It was also approved the transfer of funds and securities of the Association from the Hanover Bank to the J. P. Morgan and Co., Inc., in order to facilitate the transactions of the Treasurer. At the final meeting of the year held in the Lecture Hall on April 12, 1959, it was agreed to utilize the services of the Bank of Huntington as a depository for funds belonging to the Association which are used for checks and payments.

The 36th annual meeting of the Long Island Biological Association met in the Lecture Hall on the evening of June 23, 1959, with Chairman Nevil Ford presiding. A report was made by Mr. Ford on the progress of work on the plans for the proposed new building for the Laboratory. Dr. Arthur Chovnick, Biological Laboratory Director, briefly reviewed the research program during the past year and spoke of the use being made of the $135,000 grant received from the National Science Foundations. Mr. Ames for the Nominating Committee presented the following slate for the Board of Directors: Mrs. Franz Schneider, Mr. Joseph Eggert, Jr., Mr. Lloyd Berkner, Mr. H. J. Curtis, Mr. B. P. Kaufmann, Mr. Jesse Knight, Jr., and Mrs. Robert V. Lindsay. These were elected as Directors. President Page proposed votes of thanks to the following: Mr. Franz Schneider for his service as Director, Class of 1959; Mr. Grinnell Morris for his services as Director since 1957, his ten years as Vice President, treasurer, and member of the Executive Committee. These votes of thanks were enthusiastically approved.

LABORATORY PERSONNEL

Abramson, Harold A., Research Psychiatrist
Baron, Myrna, Research Assistant
*Bryson, Vernon, Instructor, Bacterial Genetics Course
*Burtch, Ethel, Typist
Chovnick, Arthur, Assistant Director
*Chu, E. H. Y., Instructor, Tissue Culture Course
Cicchetti, Joan, Stenographer
Cocito, Jeannine, Research Assistant
Coyne, Mary, Technical Assistant
Dean Gladys, Research Assistant
Demerec, M., Director
Diglio, Dominick, Gardener
Franzese, Eleanor, Business Manager
Fremont-Smith, Nicholas, Research Assistant
Gardner, Henry, Maintenance Man
Gillies, Gloria, Research Assistant
Glanville, Edward V., Research Assistant
Granick, Donna, Nature Study Course Instructor
Grein, Patricia, Stenographer
*Hartman, Philip E., Instructor, Bacterial Genetics Course
*Heck, Otto, Nature Study Course Instructor
Hollely, Dorothy, Research Assistant
Hyde, Olive, Administrative Assistant
King, James C., Geneticist
Krauss, Marian, Research Assistant
*Lamoureux, Jill, Nature Study Course Instructor
*Luria, S. E., Collaborator, Bacterial Viruses Course
Margolin, Jean, Research Assistant
Margolin, Paul, Geneticist
Maden, Carol, Research Assistant
Mazzone, H. M., Research Associate
McMillan, Jane, Research Assistant
McMullen, Ellen, Research Assistant
Meissner, Richard, Superintendent of Buildings and Grounds
Moser, Hermann, Geneticist
*Nevole, Nancy A., Technical Assistant
Neviackas, Gwendolyn, Stenographer
Nilsson, Dorothy, Assistant to Business Manager
Pascarella, Carol, Technical Assistant
Prokop, Barbara, Research Assistant
*Reinhold, Robert, Technical Assistant
Rolfe, Wililam, Research Assistant
*Rosenberg, Marvin, Nature Study Course Director
Sams, Joan, Research Assistant
Schalet, Abraham P., Geneticist
Schoonmaker, Doris, Technical Assistant

Scudder, Charles, Maintenance man
Simrell, Elizabeth J., Research Assistant
Sklarofky, Bernard, Psychobiologist
-Sokoloff, Alexander, Geneticist
Stachs, Jean, Secretary
*Stahl, Franklin W., Instructor Bacterial Virus Course
*Steinberg, Charles, Assistant Instructor Bacterial Virus Course
*Sundgaard, Stephen A., Maintenance man
-Tomizawa, Keito, Research Assistant
-Wallace, Bruce, Geneticist; Assistant Director
Warren, Katherine Brehme, author "Mutants of Drosophlia melanogaster".
-*Witkin, Evelyn, Instructor Bacterial Genetics Course
Weisbrot, David Research Assistant
Wooldridge, Clara, Editor Symposium Volume
Zerfass, Arthur, Mechanic

* Summer or temporary

\- Resigned during the year

COLLABORATORS AT CARNEGIE INSTITUTION

Balbinder, Elias, Research Associate
Bocskay, Elizabeth, Stenographer
Carley, Catherine, Switchboard Operator
Fisher, Agnes, C., Secretary to Director
Hershey, A. D., Microbiologist
Jones, Henry H., Photographer
Kaufmann, B. P., Cytogeneticist
Lahr, E. L., Associate in Research
McDonald, Margaret R., Chemist
Peckham, Leslie E., Senior Clerk
Smith, Guinevere C., Librarian
Snyder, Emmy M., Technical Assistant
Streisinger, G., Geneticist
Van Houten, William B., Engineer

Summer Research Investigators

Bernheimer, Alan W., New York University College of Medicine, New York., N.Y.
Bernheimer, Harriet, State University College of Medicine, Brooklyn, N.Y.
Boice, LuBelle, University of Illinois, Urbana, Ill.
Caspari, Ernst, Wesleyan University, Middletown, Conn.
Wrasko, Serman, The Johns Hopkins University, Baltimore, Md.
Fox, Maurice S., The Rockefeller Institute for Medical Research, New York, N.Y.
Fraser, Dorothy K., University of Illinois, Urbana, Ill.
Grace, Thomas D. C., The Rockefeller Institute for Medical Research, New York, N.Y.
Granick, S., The Rockefeller Institute for Medical Research, New York, N.Y.
Hartman, Philip E., The Johns Hopkins University, Baltimore, Md.
Haugaard, Niels, University of Pennsylvania School of Medicine, Philadelphia, Pa.
Hotchkiss, Rollin D., The Rockefeller Insttiute for Medical Research, New York, N.Y.
Kikkawa, H., Osaka University, Osaka, Japan
Luria, S. E., University of Illinois, Urbana, Ill.
Maramorosch, K., The Rockefeller Institute for Medical Research, New York, N.Y.
Melechen, N., St. Louis University School of Medicine, St. Louis, Mo.
Powers, Susan, The Rockefeller Institute for Medical Research, New York, N.Y.
Stahl, F. W., University of Missouri College, of Agriculture, Columbia, Mo.
Tin, Robert C., University of Illinois, Urbana, Ill.
Watson, G. S., Australian National University, Canberra, Australia

ORGANIZATION OF THE ASSOCIATION

President
Walter H. Page

Chairman
Nevil Ford

Secretary
E. C. MacDowell

Vice-President and Treasurer
Robert V. Lindsay

Assistant Treasurer
Mrs. David Ingraham

Laboratory Director: M. Demerec
Assistant Director: Arthur Chovnick

BOARD OF DIRECTORS

To serve until 1963

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

To serve until 1962

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

To serve until 1961

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

To serve until 1960

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

Members Emeriti

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

EXECUTIVE COMMITTEE

Walter H. Page, Chairman

H. J. Curtis
Nevil Ford
Mrs. George S. Franklin
E. C. MacDowell
Joseph R. Eggert, Jr.
William B. Nichols

FINANCE COMMITTEE

Robert V. Lindsay, Chairman

Hoyt Ammidon
Joseph R. Eggert, Jr.

BUILDINGS AND GROUNDS

William B. Nichols, Chairman

B. P. Kaufmann
Franz Schneider

SCIENTIFIC ADVISORY COMMITTEE

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

FORMER PRESIDENTS, LABORATORY DIRECTORS, AND BOARD MEMBERS

Presidents

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

Laboratory Directors

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

Directors

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

Jones, Mrs. O. L. 1907
Jones, W. E. 1903-06
Kahn, Mrs. O. H. 1924
Leffingwell, R. C. 1924-32
Levermore, C. H. 1896
Lloyd-Smith, Wilton 1928-40
Low, Seth 1890-1902
Lucas, F. A. 1905-17
Lusk, Graham 1909-17
MacCracken, H. M. 1890-1905
Mather, Frederic 1890-1900
Matheson, W. J. 1901-22
Mayr, Ernst 1950-58
Mayer, A. G. 1903-17
Merle-Smith Mrs. Van S. 1931-50
Mickleborough, John 1890-1917
Mills, D. H. 1926-52
Montant, A. P. 1902-09
Morgan, T. H. 1924-28
Morris, Grinnell 1947-51
Newberry, J. S. 1890-93
Nichols, Acosta 1927-45
Nichols, J. W. T. 1910-17
Noyes, H. F. 1902-21
Osterhout, W. J. V. 1927-41
Overton, Frank 1924
Page, Arthur W. 1924-58
Page, Mrs. Walter H. 1950-58
Palmer, L. M. 1899-1913
Parshley, H. M. 1924-33
Peabody, Julian 1911-17
Perkins, A. C. 1890-92
Ponder, Eric 1937-41
Pratt, H. I. 1929-30
Prime, Cornelia 1909-17
Raymond, J. H. 1890-1900
Redmond, Roland 1942-52
Roosevelt, John K. 1927-56
Rumsey, Mary H. 1924
Schiff, J. M. 1931-50
Schiff, M. L. 1924-31
Schneider, Franz 1951-59
Scott, Donald 1911-17
Seamans, C. W. 1906-15
Shapley, Harlow 1943-51
Smith, H. C. 1913-17
Stewart, J. H. J. 1893-1917; 1924-26
Stimson, H. L. 1925-36
Stockard, C. R. 1924-39
Stoddard,Howland B. 1951-55
Stratford, William 1890-1917
Straubenmuller, Gustav 1911-17
Strauss, Albert 1914-17
Stutzer, Herman 1911-23
Swingle, W. W. 1924-44
Taylor, H. C. 1926-42
Thompson, Edward 1903-17
Tiffany, L. C. 1892-1917
Urey, H. C. 1934-49
Vanderbilt, W. K. 1924-43
Walter, H. E. 1924-43
Webb, Alexander 1890-1902
Weld, F. M. 1914-17
Wetmore, C. W. 1902-07
White, Mrs. Alexander M., Jr. 1951-58
White, S. V. 1890-1905
Williams, T. S. 1910-30
Wilson, E. B. 1903-17
Wood, Willis D. 1926-52
Woodbridge, C. L. 1894-1901
Woodward, J. B. 1890-96
Woodward, R. B. 1890-1914

FOUNDERS

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

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

PATRONS

Contributions of at least $500

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

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. Wiliams	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 experiation 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.

BY-LAWS

ARTICLE I

Members

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

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

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

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

Members of all classes shall have equal voting powers.

ARTICLE II

Meetings

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

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

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

ARTICLE III

Directors

Sec. 1. The Board of Directors shall consist of twenty-eight members, who shall be elected as provided in the Certificate of Incorporation. Nine Directors shall constitute a quorum. The Board of Directors shall have general control and management of the affairs of the Association. It shall elect the officers and, except as otherwise herein provided, appoint all other employees, prescribe their duties and fix their compensation and tenture 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 years at such place and time as may be stated in the call. Special meetings of the Board, and meetings of the Executive Committee, may be called by the President at any time upon reasonable notice, and shall be called by him upon the written request of any three Directors.

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

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

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

ARTICLE IV

Officers

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

ARTICLE V

Duties of Offices

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

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

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

Sec. 4. The Secretary shall keep a list of the names and addresses of members, have the custody of the seal,the records and file of the Association, give due notice of all meetings, keep the minutes of the Board of Directors and Executive Committee, and attach the seal, together with his signature, to all instruments requiring sealing which shall have been executed by the President or Vice President. The Assistant Secretary shall perform the duties of the Secretary in case of the absence or inability of the Secretary.

Sec. 5. The Treasurer shall receive, collect and hold, subject to the order of the Board, all dues, subscriptions, donations and other revenue

of the Association. He shall deposit all funds, in the name and to the credit of the Association, in such financial institution or instiutions 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 Directors or such other officer as the Board of Directors or the Executive Committee shall designate. Funds not required for current operations shall be invested and reinvested in such manner as the Board of Directors or Executive Committee shall determine.

Sec. 6. The Laboratory Director shall be responsible executive officer of the Association in matters pertaining to the immediate conduct of the Laboratory and such other of the activities of the Association as may be assigned him. He shall prepare and issue the general announcements, after their approval by the Board of Directors, provide for the needs of instructors, investigators and students at the Laboraory. 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 Eexcutive 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 ot time of his receipts and disbursements.

ARTICLE VI

Seal

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

ARTICLE VII

Amendments

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

ARTICLE VIII

Women's Committee

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

ARTIVLE 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 purpose to such members of the Association and to impose such restrictions in the covenants as in the opinion of the Board will result in a residential development attractive to scientists and promote the best interests of the Association.

June 1959