ANNUAL REPORT

of

THE BIOLOGICAL LABORATORY

1955-1956

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-Sixth Year

1955-1956

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

TABLE OF CONTENTS

Security for Scientists	5
Report of the Director	7
Reports of Laboratory Staff	15
Bibliography	39
Reports of Summer Investigators	41
Course on Bacterial Viruses	48
Course on Bacterial Genetics	50
Nature Study Course	52
Symposia Publications	55
Summer Research Investigators	56
Laboratory Personnel	57
Report of the Secretary	59
Report of the Treasurer	61
Organization of the Association	68
Officers	68
Board of Directors	68
Committees	69
Former Officers and Board Members	70
Members	73

SECURITY FOR SCIENTISTS

A short time ago I read the sobering report on atomic radiation prepared by the National Academy of Sciences. Dr. Warren Weaver of the Rockefeller Foundation concluded the genetics section in this way: "We badly need to know much more about genetics . . . This requires serious contributions of time, of brains, and of money. Although brains and time are more important than money, the latter is also essential; and our society should take prompt steps to see to it that the support of research in genetics is substantially expanded and that it is stabilized."

Last year a distinguished scientist on the staff of the Biological Laboratory resigned to join a privately endowed foundation.

He did not seek a larger salary. He sought security to pursue his researches without interruption, security for himself and his family.

A year has passed, and this scientist has not yet been replaced.

Why? Because under its present financial organization it is difficult for the Biological Laboratory to offer security to any member of its staff. Its sights cannot be raised beyond the single year of a government or private grant.

This troubles us because stability for continued genetics research is the key problem facing the Biological Laboratory.

The Biological Laboratory has had a distinguished history for more than 66 years. It is an acknowledged leader in three areas: pure biological research; the education of scientists; the promotion of international scientific understanding.

The Biological Laboratory has two major sources of income: contributions from members of the Long Island Biological Association, barely sufficient to pay operating costs and plant maintenance; and grants from government or private foundations to pursue research and other projects.

Because of the Laboratory's international reputation, because of the standing of its scientists among their peers, no real problem is met in securing grants for the research projects planned by the staff. Our educational projects—the summer courses for scientists, the annual symposia—have long since proved their value.

Yet scientists cannot work and plan most effectively under the limitations of a year's grant. Annually, the question of renewal must arise. The scientist can only look ahead in terms of his work, himself and his family for 52 weeks. This is a serious problem.

To continue functioning effectively, the Biological Laboratory needs a modest endowment fund. Estimates indicate that $500,000 in research

capital will be sufficient to guarantee the salary of three key scientists for a period of fifteen to twenty years. The Laboratory would plan to expend both income and principal of this research fund. The fund might even be stretched for a longer period, since grants will continue to be readily available for all research projects, and some portion of grant funds can be used for salaries.

If the essential needs of its principal investigators are provided, the Laboratory should have no difficulty in obtaining research grants totalling two to five times the amount which would be spent on the salaries of the principal investigators.

Our key scientists need tenure. They need security. Under such conditions they can continue the outstanding work which has given the Biological Laboratory a secure and unique place among research institutions.

Despite its limitations of small staff and budget, the Biological Laboratory has already made a fundamental contribution to human betterment. Its future effort depends on adequate research capital so that man's knowledge of himself and other living organisms, and their adaptation to their environment may be broadened.

AMYAS AMES, President
Long Island Biological Association

REPORT OF THE DIRECTOR

Ever since the Biological Laboratory was organized in 1890, as a department of the Brooklyn Institute of Arts and Sciences, residents of Long Island and particularly those in the vicinity of Cold Spring Harbor have taken a prominent part in its affairs. With the formation of the Long Island Biological Association, which in 1924 took over responsibility for the Laboratory, the role played by the community in Laboratory concerns was considerably augmented. As officers and as members of the Board of Directors, citizens of this community have participated in the formulation of policies. They have supported important changes in the Laboratory's program, once when the schedule of summer teaching and research was supplemented by the addition of year-round research, and again when the pioneer series of scientific conferences known as the Cold Spring Harbor Symposia on Quantitative Biology was instituted. These new activities increased our financial obligations, and members of the community have given and are continuing to give significant service in helping to solve the many problems of financing the Laboratory.

Wars have given rise to marked changes in research activity, in this country as well as elsewhere, and the program of the Laboratory has undergone considerable modifications in the process of adjusting to changed conditions. The shift of population away from New York City has also brought about changes in our community. Therefore, on the initiative of Mr. Amyas Ames, president of the Association, an illustrated pamphlet has been prepared to present the story of the Laboratory for the information of those who may be interested in its history and activities. Copies of the pamphlet have been sent to all members of the Association.

RESEARCH

The research of the Laboratory staff is carried on in three sections, namely, Population Genetics, Microbial Genetics, and Psychobiology. Detailed progress reports of the work in each section are presented later in this Report by the members in charge of the various projects. Here I will summarize only briefly.

Dr. Bruce Wallace and his collaborators have continued studies of irradiated populations of fruit flies (Drosophila melanogaster). They are using new experimental techniques to extend their analyses of the genetic effects of irradiation on the fitness of populations exposed to radiation for various periods of time, some for as long as 150 generations. One technique is a modification of the routine analysis of chromosomes (the carriers of genes, the units of heredity). Certain chromosomes are known to have a detrimental effect on individuals that carry them in the "homozygous" form, that is, when both members of the pair of chromosomes are the same. This new technique will enable Dr. Wallace to determine, more

precisely than has yet been possible, the effect produced when individuals possess such chromosomes in the "heterozygous" state, that is, when the two members of the chromosome pair are different. Preliminary results indicate that the effect of these chromosomes in heterozygous individuals may not be the same in different populations—in other words, that the functioning of a specific genetic entity depends on the make-up of the whole genetic system. Another new technique, recently introduced, yields more information than earlier tests regarding the components that measure the fitness of populations. In the past, relative fitness was estimated on the basis of egg hatching and larval mortality, whereas present observations include also egg production and the fertility of adult flies.

Continued selection of Drosophila flies for resistance to DDT, carried on by Dr. James C. King and his collaborators, has produced two highly resistant lines, one with an LD50 (the measure of resistance) about sixteen times that of the control and another with an LD50 about twenty times that of the control. Genetic analysis of the more resistant strain shows that a part—although not all—of the factors influencing resistance are located in one chromosome (the X chromosome). Numerous crosses between strains of similar and different levels of resistance have shown clearly that the LD50 of the first-generation hybrids can be predicted as the geometric mean of the two parental LD50's. This fact is interesting because of the logarithmic relationship between dose and percentage of mortality, and because the geometric mean of the two quantities is merely the antilog of the average of their logarithms. The evidence becomes stronger, as the data accumulate, that resistance in Drosophila flies is built up through the consolidation of a large number of independent genetic factors.

Dr. Ellis Englesberg and his collaborators have continued the study of "gain mutations"—that is, the acquisition by bacteria of apparently new biochemical functions—and have initiated a study involving the isolation of mutants of Salmonella that are blocked in the utilization of certain important organic acids.

Dr. P. D. Skaar, using bacteria (Escherichia coli), has continued the work of previous years and has sought to unravel the details of the hybridization process. In collaboration with Harriet Davidson, he has determined by means of the transduction method that four tryptophan genes are located close together on the bacterial chromosome.

Dr. H. A. Abramson and his collaborators have continued their studies of the biological effects of LSD-25 (lysergic acid diethylamide), a compound which affects the nervous system and which, when administered in extremely small amounts, can produce in normal people states that resemble schizophrenia. After tests had been made with many different kinds of animals, the Siamese fighting fish and the "mystery" snail were found suitable for conducting bioassays in which extremely small concentrations of LSD-25 need to be determined. Studies of the reaction

of the compound with blood serum indicate that LSD combines with one component of the serum, which may be a lipoprotein. In order to facilitate studies on man, cooperative relations have been established with the Central Islip State Hospital and with Brookhaven National Laboratory.

During the summer of 1955, particularly active research was carried on with microorganisms—bacterial viruses and bacteria. A group from the University of Illinois (Urbana, Illinois), consisting of S. E. Luria, E. S. Lennox, Dorothy K. Fraser, M. Levine, and Rose M. H. Boicourt, studied transmission of serological properties in hybrids of two genera of bacteria (Shigella and Escherichia), transfer of genetic properties from one bacterium to another by virus, and genetics of bacterial viruses. A group from the University of Rochester (Rochester, New York), which included A. H. Doermann, Martha C. Chase, R. S. Edgar, and R. H. Epstein, carried on genetic analyses of bacterial viruses; and R. D. Hotchkiss and his assistants, of the Rockefeller Institute (New York City), studied the role of nucleic acid (DNA) in the transfer of genetic properties in bacteria. F. E. Wassermann, of the New York University College of Medicine, studied the kinetics of release of bacterial virus; and Alan W. and Harriet P. Bernheimer, from the same institution, worked on the preparation of a large number of stock cultures of various bacteria.

Several scientists used other materials than microorganisms for their studies. S. Granick, of the Rockefeller Institute, and his assistant worked out a method for preserving chlorophyll in thin sections of material prepared for study with the electron microscope. D. D. Miller, of the University of Nebraska (Lincoln, Nebraska), collected flies on the grounds of the Laboratory and in the vicinity of Cold Spring Harbor, for use in his studies of differentiation between strains belonging to one species, which is one of the primary steps in evolution.

CHANGES IN STAFF

Dr. Vernon Bryson has resigned from our scientific staff to become a professor at Rutgers University, New Brunswick, New Jersey, and Assistant Director of the Institute of Microbiology there. Bryson became associated with the Laboratory in 1943, when he joined our staff to work on a war research project dealing with analysis of the physical properties of aerosols. After the war ended he took a prominent part in research on the genetics of bacteria and particularly in studies of the origin of bacterial resistance to various drugs. In September, 1955 he took a leave of absence to work with the National Science Foundation in Washington as Program Director for Genetic and Developmental Biology.

In June, 1956 Dr. Hermann Moser joined our staff as a member of the Microbial Genetics section. Previously he had held a Carnegie Institution Fellowship at the Department of Genetics, where he studied genetic changes occurring in growing populations of bacteria. He will attempt to develop methods for the study of mutations occurring in mammalian cells when they are grown in cultures like bacteria.

TEACHING

The Nature Study Course was taught in the summer of 1955 by Dr. John A. Gustafson, of the State University Teachers College, Cortland, New York (in charge of the course), Mr. Marvin Rosenberg, of North Syracuse Central High School, North Syracuse, New York, and Mrs. Jill A. Lamoureux, of Port Washington, New York. They were assisted by Mrs. Shayna Rosenberg and Miss Donna Granick. This course is designed to stimulate interest in nature among young people of the community, by improving their observation of the many animals and plants in their environment, by teaching them how to find the answers to questions raised by their observations, and by helping them to realize that careful and accurate study of the small incidents we can all observe contributes greatly toward expanding our knowledge of natural phenomena. The children were separated into eight groups according to age, and classes ranged in size from eight to twenty students. One hundred and twelve children attended the course.

For the eleventh consecutive year a three-week course was offered in techniques and problems of research with bacterial viruses. It was taught by Dr. A. H. Doermann, of the University of Rochester. Eighteen students and four auditors were enrolled.

The course in bacterial genetics was given for the sixth year and was conducted by M. Demerec, V. Bryson, E. M. Witkin, P. E. Hartman, and P. D. Skaar, in collaboration with S. W. Glover, P. FitzGerald, and H. Moser. There was an enrollment of seventeen students and three auditors.

During the summer of 1956 two new courses were offered in addition to the three mentioned above. One, on the genetics of filamentous fungi, was taught by Professor G. Pontecorvo and Dr. Etta Käfer of the University of Glasgow, and, like the bacterial viruses and bacterial genetics courses, was designed for research workers and advanced graduate students. With this course our coverage of the genetics of microorganisms was considerably extended. It was attended by eighteen students and one auditor.

The other new course, Workshop in Nature Study, was given by Dr. Gustafson and Mr. Rosenberg. It was organized so as to familiarize elementary and secondary school teachers with the natural environment of the Long Island area. The New York State Education Department has authorized the Laboratory to issue credit to teachers who have successfully completed the required work. Thirteen students were enrolled.

LECTURES

As a part of the program of courses, invited speakers presented a series of seminars which were open to all members of the Laboratory. These seminar schedules are listed in the reports of the courses. In connection with the Nature Study course, several films were shown in the evening.

In addition, a regular schedule of scientific lectures was arranged during the summer of 1955, in cooperation with the Department of Genetics of the Carnegie Institution.

On April 4, 1956, the Laboratory invited members of the Long Island Biological Association and their friends to attend a lecture by Mr. Henry A. Wallace, former Vice-President of the United States, entitled "Reflections on Hybrid Vigor Based on Observations Made with Chickens and Corn."

Our lecture hall was used by several local organizations during the year. A meeting of the Long Island Chapter of the Nature Conservancy was held on the evening of November 4, 1955; three Audubon Society lectures were scheduled early in 1956 under the sponsorship of the parents of the Cold Spring Harbor Nursery School; and on May 24, 1956 the League of Women Voters met for a workshop meeting, which featured a lecture on the peaceful uses of atomic energy.

SYMPOSIUM

The twenty-first Cold Spring Harbor Symposium on Quantitative Biology met from the 4th to the 12th of June, 1956. The subject this year was "Genetic Mechanisms—structure and function." The purpose of the meeting was to bring together, in the unique atmosphere of the Laboratory, a group of leading scientists who are engaged in various phases of research relating to fundamental problems of heredity. Chemists, physicists, and biologists attended the conference. In particular, an effort was made to include both geneticists who investigate the mechanisms of inheritance and experimental embryologists who study problems of differentiation concerned with the development of fully grown individuals from single-celled ova.

The program was organized by a committee consisting of E. W. Caspari, Wesleyan University; M. Demerec and B. P. Kaufmann, Carnegie Institution; A. E. Mirsky, Rockefeller Institute; and Jack Schultz, Institute for Cancer Research.

The Symposium was attended by over 300 scientists. About fifty of the participants came from foreign countries, including Canada, Great Britain, France, Germany, Sweden, Netherlands, Belgium, Denmark, Yugoslavia, Italy, Switzerland, Brazil, Chile, South Africa, Australia, India, Japan, and Iraq. The Laboratory was able to finance the expenses of the conference with funds obtained from the Carnegie Corporation of New York, the Association for the Aid of Crippled Children, the National Science Foundation, the U. S. Public Health Service, and the Alfred P. Sloan Foundation.

SPECIAL EVENTS

On Sunday, September 18, 1955, more than two hundred and fifty members and friends of the Association attended a demonstration and tea

in Blackford Hall followed by a talk in the Lecture Hall. This open-house demonstration, which has become an annual fall event, is held so that members may learn informally about current research at the Laboratory, and become more closely acquainted with the work they help to support. The scientific exhibits included projects of the regular staff of the Laboratory and staff members of the Department of Genetics of the Carnegie Institution. The brief lecture, entitled "Report from the 'Atoms for Peace' Conference," was given by Dr. Bruce Wallace and described his experiences as a participant in that conference when it was held in Geneva, Switzerland. The serving of tea and refreshments by members of the Women's Committee was efficiently organized by Mrs. Edward S. Blagden, Vice-Chairman of the Committee.

SCHOLARSHIPS

The funds available for scholarships in 1955 were distributed among the following summer investigators and students, to help toward the living expenses of the former and the tuition fees of the latter:

John D. Jones Scholarships.—Two research workers: Dr. Edward D. DeLamater, University of Pennsylvania; Dr. Dwight D. Miller, University of Nebraska. Nine students: Robert F. Acker, Iowa State College, Ames, Iowa; Dr. R. C. Clowes, The Wright-Fleming Institute of Microbiology, London, England; E. Virgil Howell, University of Utah, Salt Lake City, Utah; Dr. D. Kanazir, Institute of Nuclear Sciences "Boris Kidrich," Belgrade, Yugoslavia; Dr. Hermann Prell, University of Marburg, Germany; Richard W. Sames, Indiana University, Bloomington, Indiana; Stephan R. Taub, University of Rochester, Rochester, New York; Walter Vielmetter, Max-Planck-Institut fur Virusforschung, Tubingen, Germany; and Alfred M. Wallbank, Michigan State College, East Lansing, Michigan.

Dorothy Frances Rice Fund.—Student: Constance T. Thomas, Carnegie Institution.

Temple Prime Scholarship.—Student: Dr. Hermann Prell, University of Marburg, Germany.

BUILDINGS AND GROUNDS

For several years it had been evident that the living accommodations available at the Laboratory were not adequate to take care of our requirements during the summer months. The expanded teaching program and increased attendance at the Symposia had brought about an acute shortage of rooms and small apartments. To satisfy this need, the Executive Committee authorized the construction of a motel-like summer residence, consisting of ten rooms and seven baths. Construction began in the fall of 1955, and the building was ready for occupancy during the 1956 Symposium. Planning and supervision were very efficiently carried out by Mr. Robert K. Thurston, superintendent of buildings and grounds. Equipped

with new furniture, the "Motel" is at present one of our most desirable summer residences.

During the year the following major repairs were accomplished: new roofs were put on the Davenport and Nichols laboratory buildings; a ventilating fan was installed in the kitchen of Blackford Hall; air-cooling units were placed in the Davenport Laboratory room used for summer courses; the cooling unit of the cold room in Jones Laboratory was replaced; and remodeling of Jones for use in research with microorganisms was completed.

FINANCES

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

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

ACKNOWLEDGMENTS

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

For research of the Population Genetics section: the Atomic Energy Commission and the Office of the Surgeon General of the Army.

For research of the Microbial Genetics section: the Army Chemical Corps, the Office of Naval Research, the National Tuberculosis Association, and the Atomic Energy Commission.

For research of the Psychobiology section: the Josiah Macy, Jr. Foundation and the Geschickter Fund for Medical Research.

For the Symposium: the Carnegie Corporation of New York, the Association for the Aid of Crippled Children, the National Science Foundation, the U. S. Public Health Service, and the Alfred P. Sloan Foundation.

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

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

I wish to express thanks to the following members of the Long Island Biological Association for entertaining our guests at dinner parties during the Symposium: Mr. and Mrs. Amyas Ames, Mr. and Mrs. Edward S.

Blagden, Dr. and Mrs. Crispin Cooke, Mr. and Mrs. George Crocker, Mr. and Mrs. Richard S. Emmett, Mr. and Mrs. Nevil Ford, Mrs. George S. Franklin, Dr. and Mrs. Edwin J. Grace, Mr. and Mrs. R. Graham Heiner, Mrs. Percy H. Jennings, Mrs. Burton J. Lee, Sr., Mrs. George Nichols, Mr. and Mrs. William Nichols, Mr. and Mrs. Arthur W. Page, Mr. and Mrs. Walter H. Page, Mr. and Mrs. Francis T. P. Plimpton, Mrs. Lansing P. Reed, Mr. and Mrs. Franz Schneider, and Mr. and Mrs. A. M. White.

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

We wish to acknowledge, too, the generous support in terms of time and effort given the Laboratory by Mr. Herman Hartmann and Mr. Charles W. Bechtold who arranged for the purchase and delivery of all furniture in our new building.

We are grateful to Mrs. Van S. Merle-Smith for donating to the Laboratory a Webster Dictation Wire Recorder.

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

M. DEMEREC
Director of the Laboratory

REPORTS OF LABORATORY STAFF

STUDIES ON IRRADIATED POPULATIONS

B. Wallace, C. V. Madden, G. Cosillo,
E. McMullen, M. Matson, and H. Gardner

The work with irradiated populations of Drosophila melanogaster has continued during the past year with emphasis placed on extending our techniques for measuring fitness of populations. The true fitness of a population may never be known because experimental studies themselves interfere with the normal existence of the population. Nevertheless, for populations of individuals whose basic needs are similar, estimates of relative fitnesses can be obtained. The information published in the last several annual reports has been collected largely through the use of a single technique: the comparison of the frequencies of flies heterozygous for random pairs of second chromosomes from the tested populations developing in the presence of mutant competitors in test cultures. The average frequencies of flies carrying second chromosomes from the tested populations are relatively constant for each population and, hence, they offer a basis for comparing these populations. Two new techniques have now been added to our routine tests.

The first of the new methods involves an extremely simple modification of the original technique. Without going into numerous details, the new method consists of establishing test cultures in which four types of flies—CyL/Pm, CyL, Pm, and wild-type—should occur in equal proportions. The CyL/Pm flies do not carry second chromosomes from the experimental populations and therefore they serve as an independent standard against which to compare the frequencies of the other three classes. By dividing the number of flies in each class by the number of CyL/Pm flies (actually, by one more than this number) one gets an estimate of the relative viabilities of these classes. The average viability of the wild-type flies of different populations can then be compared in order to estimate the relative fitnesses of these populations.

The relative adaptive values of seven experimental populations obtained by this new CyL/Pm technique are listed in table 1. The results shown in this table are based on relatively few tests and so they are subject to change as additional information is obtained. According to the evidence available, however, flies carrying random combinations of chromosomes from a population irradiated continuously for about 150 generations (population No. 6) are nearly as viable as those carrying similar chromosomes from an un-irradiated, control population (No. 3). Another population (No. 19), started with flies removed from population No. 6 during the 126th generation showed little change in the results obtained by this type of analysis as a result of its maintenance without irradiation.

A small irradiated population (No. 5) shows a considerable effect of irradiation (estimated adaptive value equals .90) and a partial recovery after some 40 generations in the absence of irradiation (No. 17 and No. 18). Finally a population that receives a low dose of gamma-irradiation (No. 7) appears in these tests to be affected more than population No. 6 which receives a considerably larger amount of irradiation; this is an unexpected result which needs additional verification before it can be considered seriously.

Table 1

Preliminary estimates of adaptive values of experimental populations of D. melanogaster obtained by the new "CyL/Pm" technique.

Population	Number	Adaptive Value
3	275	1
5	367	.90
6	292	.99
7	367	.94
17	447	.96
18	534	.95
19	454	.98

The same test of second chromosomes can be used to determine whether lethals and semilethals have average semidominant deleterious effects. Every chromosome studied in the heterozygous condition (random pairs) is studied in homozygous individuals as well. Therefore, the random pairs of chromosomes can be classified as those in which both, one, or neither is lethal when homozygous. (This classification is not possible in the case of chromosomes from population No. 3 where only heterozygous combinations are studied.) The relative viabilities of flies carrying these combinations of chromosomes from different populations are shown in table 2. It is worth noting in this table that evidence for semidominance of lethals and semilethals is scant; this is emphasized in the bottom row in which the results of all tests are averaged. There is an indication, though, that the situation varies from population to population.

Table 2

Estimation of the semidominance of lethal and semilethal second chromosomes obtained by comparing the adaptive values of individuals carrying two, one, or no lethal second chromosomes. Only heterozygous combinations not lethal or semilethal in themselves are included in this table. N=normal chromosomes; L=lethal or semilethal. Adaptive value of N/N defined as 1.00 for every population.

Population	N/N	N/L	L/L
5	1	1.06	1.01
6	1	.98	.93
7	1	.95	.98
17	1	.96	.97
18	1	1.00	.98
19	1	1.06	1.07
Average	1	1.00	.99

The second technique for estimating the relative fitness of populations consists of determining the total number of offspring produced in vials by 50 single-pair matings of flies from each population. Two sets of fifty-pair matings representing two different populations are set up in each experiment; eventually all possible combinations of populations will be represented in these tests. From each set of fifty vials, fifty males and fifty virgin females are collected and these fifty males and females are mated at random in vials once more. Each set of fifty vials, then, is continued through ten generations by these random, single-pair matings of flies collected from the preceding set of vials. Theoretically we expect three types of information from this type of analysis: from the consolidation of all first-generation matings of each population we should get an estimate of the original productivity of random pairs of matings. This can be regarded as our best estimate of that population's fitness—an estimate based on the entire genotype of each original parent and upon many components of fitness. This type of estimate, by including more chromosomal material and more stages of the fly's life cycle, appears to be preferable to earlier estimates that were limited to second chromosomes and to larval survival alone. Second, we might expect that during the course of ten generations in non-irradiated vials an improvement in fitness resulting from the elimination of deleterious mutations will occur. This improvement would be related to the intensity of selection against these mutations. Finally, the change in population size from about 10,000 individuals in population cages to the vial "populations" of 100 flies or less may alter the role of certain genes. Genes that were retained in the original populations because of their effect on heterozygous individuals may, by becoming homozygous more frequently in the smaller populations, lower the fitness of these latter populations. In the case of material taken directly from irradiated populations these last two effects will oppose one another and, in the absence of any technique for separating them, only the net effect will be obvious.

The results of the first eight generations of the vial tests for the populations studied so far are listed in table 3. Data from the first two generations in vials indicate that only populations No. 5 and No. 6 differ substantially in fitness from the control (No. 3). Single pair matings of

flies from population No. 6 are, on the average, only about 75% as productive as those of the control population while similar matings of flies from population No. 5 are some 89% as productive. Population No. 1 (exposed to X-rays in the first generation but un-irradiated since that time), population No. 7 (exposed continuously to about 300r of gamma-radiation per generation), and populations No. 17, No. 18 and No. 19 (daughter populations of No. 5 and No. 6 that have been removed from irradiation for about 20 generations) are all essentially equal in fitness to the control population according to this test.

Table 3

Estimations of the adaptive values of populations based upon the average number of offspring produced by single-pair matings of flies from the different populations. Only the results of the first generation or two should be used to compare the original populations; later generations in vials reflect the elimination of deleterious mutations from irradiated material as well as the effects of curtailing population size sharply in this type of experiment. The adaptive value of population 3 in every generation has been defined as 1.00.

Population	Generations in vials							
	1	2	3	4	5	6	7	8
1	1.03	1.00	.80	.94	.95	1.06	.85	.80
3	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
5	.87	.91	.92	.95	1.02	.94	.91	.85
6	.71	.79	.82	.90	1.04	.98	1.00	.89
7	.99	1.09	1.13	1.27	1.26	1.16	1.15	1.12
17	1.05	1.11	1.14	.....	.....	.....	.....	.....
18	.96	1.01	1.02	1.11	1.22	.97	1.14	1.06
19	.98	.95	1.01	.99	1.18	1.01	.95	.85

The relatively meagre information in table 3 indicates that populations recover from irradiation rather rapidly; that is, that a great deal of the effects of new mutations are expressed in heterozygous individuals. The results obtained from No. 17, No. 18, and No. 19 indicate that these populations are substantially improved in fitness over populations No. 5 and No. 6 that have continued to be exposed to irradiation. Furthermore, with the exception of the eighth generation the average fitness estimated for populations No. 5 and No. 6 combined increased regularly until it was virtually equal to that of the control population. Although part of this increase may be relative only, part of it undoubtedly reflects a real improvement of the flies from the irradiated populations.

The two new techniques described here have been used on a sufficiently large scale to make some conclusions worthwhile. First, contrary to the genetic tests of second chromosomes, the "vial populations" indicate that

the average productivity of single pairs of flies from population No. 7, a continuously irradiated population, is greater than that for any other experimental population. Second, the same studies indicate that the irradiated populations recover fitness within relatively few generations after the cessation of irradiation. Third, the changes in relative fitnesses occurring during successive generations of the "vial populations" indicate that the recovery of fitness after irrradiation takes place through the utilization of new gene mutations, quite possibly induced mutations. Finally, with the exception of the data on population No. 7, the new genetic test gives results similar to those obtained in the earlier test; substitution of CyL/Pm flies as the standard of comparison for the CyL flies previously used for this purpose does not seriously alter the results. The main difference between the early generations of the "vial populations" and the genetic test is the exaggeration of the difference between the continuously irradiated populations and the others.

The work reported here was done under contract No. AT-(30-1)-557, U. S. Atomic Energy Commission.

THE GENETICS OF RESISTANCE TO INSECTICIDES

James C. King

The three preceding annual reports of the Biological Laboratory have contained progress reports on the work concerning the inheritance of resistance to DDT in Drosophila melanogaster. The essential findings can be briefly summarized. Every attempt to produce a line of resistant flies by treating adult insects with DDT and breeding from the survivors has been successful. But the level of resistance achieved within a given number of generations has varied with the strain used and the details of the techniques employed. A strain labeled Syosset, descended from about two dozen flies collected in a grocery store in July 1952, has responded more rapidly to selection than the standard Oregon-R strain which has been kept in the laboratory for some thirty years. The most effective method of selection has been to expose flies of successive generations to a dose of DDT which kills about half the total and use the survivors as parents. More intensive selection effected by a heavier dose of DDT has been less consistent in producing resistance although one very resistant line has been built up in this way. Resistance always develops slowly. Ten or twelve generations of selection have been necessary to produce clearly demonstrable results. By raising larvae in medium containing DDT, lines showing resistance in the larval state have been obtained. But when such larvae become adult, they show only a slightly greater resistance than the untreated controls. On the other hand, whenever the larval offspring of a line of resistant adults have been tested on poisoned medium, they have shown definitely higher resistance.

If one wishes to investigate the mode of inheritance of any character, the most obvious method is to make crosses between individuals which differ with respect to the character and then observe the presence or absence of the character in the first filial (F_1) and subsequent generations. Consequently, as soon as lines with demonstrable resistance had been built up, a series of crosses was begun to study the pattern by which resistance was inherited. Unfortunately, it is not practical to measure the resistance of an individual Drosophila. Individuals vary greatly in their ability to withstand the insecticide and the only practical measure of resistance is the LD_{50}—the dose which will kill half of a given group of flies. This figure is obtained by subjecting several batches of flies of common origin to different doses, observing the mortality resulting from each dose and then calculating by a statistical procedure the dose that will effect a mortality of 50%. This means that crosses cannot be made using single pairs of flies. Instead, mass matings must be made and the LD_{50}'s of the flies of the two parent generations compared with those of the offspring in the F_1 and succeeding generations. Fortunately, when selection is stopped, resistance does not decline perceptibly for at least four generations; so it is possible to observe the effects of crossing through four generations without subjecting any of the hybrids to further selection.

In previous years some data on the results of crosses had been obtained. During the past year we have concentrated on making and studying crosses and we now have a somewhat better picture of the mode of inheritance. So far as the F_1 is concerned, we can generalize by saying that whenever crosses have been made between lines of flies differing in resistance, the offspring have shown an LD_{50} intermediate between those of the two parental lines. Furthermore, we can say that LD_{50} of the offspring will be nearer to that of the less resistant parental line. We can be even more precise and say that the LD_{50} of the offspring will approximate the geometric mean of the two parental lines. The geometric mean of two values is the square root of their product and thus is nearer to the smaller. The arithmetic mean is one half the sum of two values and hence lies half way between them. For example, the arithmetic mean of 27 and 3 is 15 (½ of 30); the geometric mean is 9 ($\sqrt{81}$). If we cross two lines, the LD_{50}'s of which are in the ratio of 27 to 3, the LD_{50} of the F_1 offspring will be approximately 9 and not 15.

This apparently occult phenomenon becomes somewhat less mysterious when we point out that the relationship of dosage to per cent mortality is a logarithmic one. If we plot per cent mortality against dosage, we will get the smooth sigmoid curve of the cumulative normal distribution only if we use a logarithmic scale for dosage, that is, a scale where the distance between 2 and 4 is the same as the distance between 4 and 8 and not half as great—in other words where the distances between values are equal to the ratios between them and not to the differences. To get a mean on such a scale, one must average logarithms, not numbers, and this is exactly the same as taking the square root of their product.

To translate all this into genetic terms, we can say that resistance in all our lines of Drosophila is neither dominant nor recessive and that the F_1 of a cross shows a resistance which is the average on a logarithmic scale between the two parental lines. When two resistant lines are crossed, the same rule applies. If they are equal in resistance, the F_1 will be the same as the parents.

If the F_1 flies of a cross are inbred to produce an F_2, the situation is more complicated. In the early crosses, both between a resistant line and the control and between different resistant lines, it was found repeatedly that the LD_{50} of the F_2 generation was lower than that of the F_1. This phenomenon has been studied most intensively in a series of seven crosses made between two resistant lines at intervals while both lines were being selected for resistance. The seven crosses were made at generations 18, 23, 29, 37, 46, 49 and 54 of the selected lines. In the first four crosses there was a significant drop in the LD_{50} between F_1 and F_2. In the last three crosses there has been no significant drop. In the first four crosses the amount of the decrease declined from cross 1 to cross 4 so that we appear to have been observing a patterned change. The drop in LD_{50} from F_1 to F_2 seems to be characteristic of crosses between lines in the early stages of selection for resistance and disappears after selection has proceeded for

RELATIONSHIP BETWEEN MUTATION AND BIOCHEMICAL FUNCTION IN BACTERIA

Ellis Englesberg, Laura Ingraham, and Joanna Hadden

During the past year we have continued our study of gain mutations in Pasteurella pestis, involving the following two aspects: (1) mutation to rhamnose utilization; and (2) meitrophic mutants. In addition, a third project was initiated, consisting of a combined enzymatic and genetic study of certain mutants of Salmonella typhimurium.

Mutation to Rhamnose Utilization in Pasteurella pestis. It has been previously reported that all strains of Pasteurella pestis, both virulent and avirulent, are unable to use L-rhamnose as a carbon or energy source, but give rise to apparently rare rhamnose utilizing mutants (R+). Enzymatic analysis indicated that R+ differs from R– in possessing the ability to produce two adaptive enzymes, which R– apparently is not able to produce: (1) an L-rhamnose isomerase, which converts rhamnose into a new sugar, rhamnulose (6-deoxy L-fructose); and (2) a kinase, which phosphorylates rhamnulose probably to rhamnulose-1-phosphate. During the past year, considerable effort has been made to further characterize the enzymes involved and to attempt to explain the mechanism that resulted in the gain in ability to produce these two enzymes.

Cell-free extracts were prepared by the alumina grinding technique of McIlwain, using cells grown in casein hydrolyzate mineral glucose (CHMG) or rhamnose (CHMR) media. Extracts prepared from R+ grown on CHMR will be referred to as R+ adapted extracts or R+ (CHMR), while extracts of R+ grown on CHMG will be called R+ unadapted extracts or R+ (CHMG). Similarly extracts of R– will be referred to as R– (CHMR) or R– (CHMG), respectively. Rhamnose isomerase activity was determined by measuring the production of the keto sugar employing the sensitive cysteine, sulphuric acid, carbazole tests of Dische and Borenfreund. The kinase activity was determined either manometrically (Colliwick) and Kalkar) or by measuring rhamnose or rhamnulose disappearance in a zinc sulfate, barium hydroxide supernatant (Somogyi). All enzyme reactions were carried out at 30°C.

Under the optimum conditions established for the activity of the isomerase (200 micromoles of rhamnose, 100 micromoles of $MgCl_2$, pH 7.5 tris (hydroxymethyl) amino methane buffer (90 micromoles), in a total volume of 2.1 ml/5 mg dry weight of extract protein), 7.8 micromoles of rhamnulose were produced per hr/mg of extract protein by the R+ adapted extract, while no activity was detected in R+ unadapted extracts or with either of the R– extracts. Under conditions of lower rhamnose concentration, which gives increased sensitivity of the assay for rhamnulose, the R+ unadapted extracts and the R– extracts were found to have less than 1/300 of the activity of the R+ adapted extract.

Under optimum conditions for kinase activity (adenosine triphosphate (ATP) 10 micromoles, rhamnulose 5 micromoles, pH 7.0 tris buffer, (20 micromoles of $MgCl_2$ were added although no absolute requirement for Mg^{++} was demonstrated in these undialyzed preparations) in a total volume of 2.1 ml per 5mg dry weight of extract protein), the R^+ adapted extract was found to phosphorylate 0.86 micromoles of rhamnulose per hr per mg of protein. Under these conditions there was no detectable activity with the other three extracts. The sensitivity of the test is such that it is possible to say that the R^+ adapted extract had at least 100 times the activity of the other three extracts. Analysis for products of this reaction with rhamnose and rhamnulose as substrates has demonstrated the presence of an acid labile phosphate ester of rhamnulose which is probably rhamnulose-1-phosphate. No other phosphate ester was detected. These results therefore, indicate the presence in the R^+ adapted extract of a rhamnulokinase and the absence of any rhamnokinase or phosphorhamnomutase.

Since previous work relating mutation to enzyme activity has shown that a single mutational event directly affects the synthesis of only one enzmye ("one gene, one enzyme" hypothesis), our findings that mutation to R^+ resulted in the apparent gain in ability to synthesize two enzymes required careful scrutiny.

One possible explanation of our results is that mutation results in the gain in ability to produce the isomerase and simultaneous adaptation of the kinase. In this case, the wild type would have the potentiality for producing the rhamnulokinase, but since it cannot produce the inducer rhamnulose from the substrate rhamnose, the kinase activity is never expressed. To test this possibility, the wild type and the R^+ mutant were grown in a 0.2% rhamnulose peptone medium for 24 hours. The medium was assayed for residual rhamnulose and the cells were harvested and assayed for ability to oxidize rhamnulose and rhamnose. There was no rhamnulose utilization in the medium in which the wild type cells were grown, and the wild type failed to oxidize either rhamnose or rhamnulose. On the other hand, the medium in which R^+ was grown was completely devoid of any residual rhamnulose, and manometric experiments demonstrated that R^+ was adapted to both rhamnose and rhamnulose oxidation. Thus, it appears that the wild type lacks the ability to produce both the isomerase and the kinase, and that mutation results in a gain in ability to produce both enzymes.

Another question to be dealt with was whether this mutation leading to the gain in two enzymes was really a single mutational event. The possibility existed that on rhamnose Endo agar, which was employed in selecting R^+, mutation first occurred leading to the gain in ability to produce one of the enzymes and that then a subsequent mutation occurred leading to the production of the second enzyme. Since no selection can be postulated for either supposed mutation occurring alone, the double mutation could only occur if the mutation rate of each event was fairly high. The mutation rate determined from R^- to R^+ was found to be quite low (2.5 x

10^{-11}), so that this possibility seemed plausible. Therefore, attempts were made to select for mutants on both rhamnose as well as rhamnulose agar. If two mutational events occurred, it might be possible to demonstrate a large increase in frequency of mutation on rhamnulose agar and to pick mutant colonies on this medium which would only be able to oxidize rhamnulose, or in the event of further mutation, colonies containing a mixture of cells, some of which could use only rhamnulose while others could use both rhamnose and rhamnulose. Results, however, demonstrated that the frequency of occurrence of mutation on these two types of media were not noticeably different, and entire mutant colonies isolated on rhamnulose and rhamnose agar used both rhamnose and rhamnulose. Therefore, there can be little doubt that the gain in ability to produce both enzymes occurs simultaneously as a single mutational event.

Meiotrophic Mutants of Pasteurella pestis. By successive selection steps on defined agar media, we have succeeded in isolating meitrophic mutants of Pasteurella pestis A1122 that are able to grow in a mineral glucose medium with ammonium chloride as the sole source of nitrogen, and sulfite or thiosulfate as the sole sulfur source. The mutation rates toward autotrophy are high, so that the process of selection can easily be repeated. It will be recalled that the prototrophic amino acid requirements (wild type requirements) of this organism at 30°C are: cysteine (C), methionine (M), phenylalanine (P), valine (V), and isoleucine (I). In the last report we descibed the isolation of a methionine independent, methionine resistant mutant (M^+M^rP-) (which grows in a medium with phenylalanine as the sole amino acid), and the apparent biochemical significance of the methionine, valine, and isoleucine requirement. By streaking M^+M^rP- (which we shall simply refer to as P–) onto a cystine or thiosulfate mineral glucose medium, we were able to select a mutant (P^+1) which requires six days to produce visible colonies on this medium and which gives maximum and rapid growth in liquid medium with the addition of only 0.1 gamma per ml of phenylalanine. When streaked onto a phenylalanine free medium, P^+1 gives rise to two additional mutants: P^+2 and P^+3. Both P^+2 and P^+3 yield visible colonies in three days on this medium. P^+3, however, is an excellent feeder of P–, while P^+2 does not feed at all. Indications are that the P^+ mutants involve adjustments of a single functional deficiency in phenylalanine biosynthesis. Feeding experiments between Pasteurella pestis and a series of phenylalanine auxotrophs of Escherichia coli and Aerobacter aerogenes (supplied by Dr. B. Davis) indicate that the phenylalanine requirement of Pasteurella pestis A1122 (prototroph) is the result of a break between prephenic acid and some compound "X" which is apparently past the common precursor for tryptohane. It is interesting to note that prephenic acid, which has been postulated as an intermediate in phenylalanine and tyrosine biosynthesis in Escherichia coli, is not utilizable by the intact cell. The fact that Pasteurella pestis is able to utilize this compound is the first demonstration, we believe, of its actual biological significance. Since Pasteurella pestis, with a deficiency before prephenic acid, requires only phenylalanine and not

tyrosine, and since experiments have also shown that tyrosine in relatively large concentrations can substitute for phenylalanine, it appears that prephenic acid is not a common branching-off point in tyrosine and phenylalanine biosynthesis (as has been demonstrated in Escherichia coli and Aerobacter aerogenes by B. Davis and coworkers), and that we must assume a direct interconversion of phenylalanine and tyrosine which is independent of prephenic acid.

The following diagram summarizes what we know concerning the natural deficiencies in amino acid biosynthesis in Pasteurella pestis A1122:

sulfate—/—sulfite——thiosulfate——cysteine—/—cystathionine——homocysteine——methionine

shikimic————————X—/—prephenic——phenylpyruvic——phenylalanine—?—tyrosine

The breaks in all cases are several steps removed from the final step in the synthesis of the amino acids concerned. Apparently the ability to convert cystathionine to homoysteine to methionine, sulfite to thiosulfate to cysteine, prephenic to phenylpyruvic to phenylalanine, and the peculiar breaks descibed must have some selective value to the bacterium. The fact that the synthesis of only three amino acids has been affected and with these the entire pathways have not been "lost" during the evolution of this organism in its living hosts (as had been postulated in general in such circumstances by many investigators) is an indication of our gross simplification of the possible selection factors involved. Because of the position of the breaks it is interesting to speculate on how they may have originated. To do this, let us review our present concept of the evolution of biosynthetic pathways.

According to the Haldane-Oparin-Horowitz hypothesis of evolution, life originated as a self-duplicating unit, dependent upon the surrounding sea of organic matter for growth. With the depletion of specific compounds of this organic sea, selection occurred in a step-wise fashion for those forms which evolved various synthetic abilities. This continuous process eventually led to the development of the complex autotroph, an organism which could synthesize all its requirements from inorganic compounds. Thus, a cycle in nature was completed and the earth was again abundantly supplied with organic substances, but this time the organic matter was the product of living organisms and not the result of chemical evolution.

Lwoff, in a study of present day colorless and plastidless protozoa, has advanced the thesis that organisms existing today with growth factor requirements have evolved by loss mutation from such a primitive autotroph. He states: "In whatever fashion the evolutionary development of living substances occurred in earlier times, one fact remains without doubt: in the free living or parasitic organisms which we know, the requirement for growth factors is the consequence of losses of function".

A corollary to Lwoff's conclusions is that all descent must of necessity trace back through completely autotrophic ancestors, and this on a priori grounds is unjustified. A more plausible hypothesis is that the event of autotrophy made possible the existence of life that had not reached that state of evolution, and that various types of dependent relationships therefore arose. Thus, aside from evoking the concept of loss mutation, there is the possibility that Pasteurella pestis may have evolved the initial steps to phenylalanine and cysteine and methionine synthesis in a step-wise fashion, but this process did not go to completion because of the production of these compounds or their immediate precursors in sufficient amounts by other organisms which had evolved faster in their synthetic abilities. Similarly, mutation to ability to utilize various carbon sources for growth and energy (e.g., rhamnose, in the case of Pasteurella pestis) may also have originated by gain of a completely new ability or regain of a lost one.

Studies with Salmonella typhimurium. During the past year we have initiated a project involving a combined enzymatic and genetic study of mutants of Salmonella typhimurium, deficient in ability to utilize a series of metabolically related compounds as sole sources of carbon and energy, in an attempt to determine the relationship between intergenic and intragenic organization to permeability (active transport) processes and enzyme synthesis, organization, and function.

In this study it was decided to concentrate upon mutants deficient in ability to utilize pyruvate, acetate, and Krebs cycle compounds because: (1) the enzymes involved are present in relatively large amounts as compared to enzymes functioning in biosynthetic pathways; (2) we possess a fund of knowledge concerning the enzymes involved in the oxidation of these compounds; and (3) these compounds have been previously involved in permeability effects.

By incorporating two major changes, we were able to use the penicillin technique to isolate such mutants: (1) a 48-hour period of starvation of thoroughly washed cells before the addition of penicillin and glucose or other carbon source; and (2) the plating of the cells after the selection step onto a highly enriched glucose medium. With these modifications, we have succeeded in shifting the selection from 100% typical auxotrophs to approximately 48% "permeability" and dissimilatory mutants. An analysis of the growth responses of the mutants isolated indicates that they fall into two major groups: (1) "permeability" mutants, i.e., mutants which are unable to utilize various carbon sources although they apparently have the enzymes present for their degradation; and (2) dissimilatory deficient mutants, i.e., mutants apparently lacking the enzyme for the initial degradation of the compounds in question. Both groups are further classified into 13 subgroups (A-N) according to carbon sources not utilized. Transduction studies with phage produced from the wild type cells have demonstrated that both "permeability" and dissimilatory deficient markers are genetically controlled.

It is interesting to note that although 29 out of the 32 mutants isolated were selected on the basis of being unable to use glucose (as the carbon source in the presence of penicillin), most of the mutants are able to utilize glucose slowly. Also, 27 out of the 32 mutants described are unable to utilize acetate, while the remaining 5 grow extremely poorly on this substrate. It is also obvious from our results that growth responses on the various media employed are not sufficient to allow for a definite characterization of all mutants as to their specific deficiency. A few of the major groups will be described.

Members of groups A, B, C, and D, share in common the inability to utilize acetate as a carbon source, but differ among themselves in the time required to show visible growth on mineral agar medium supplemented with glucose, glycerol, pyruvate, citrate, succinate, fumarate, malate, malate+pyruvate, glycerol+glutamate, and aspartate.

Mutant 1-7-1 (group E) is characterized by the inability to utilize acetate, succinate, fumarate, or malate as carbon sources. It gives rise to a series of "back" mutants when streaked onto agar medium containing any of the above four substrates. Mutants isolated on acetate are able to utilize all four compounds; however, those isolated on malate and fumarate are still not able to utilize acetate or succinate, while mutants isolated on succinate either utilize all four compounds as well as the wild type or utilize acetate slowly and the rest at a normal rate. Since growth on glucose, glycerol, pyruvate, and citrate is only slightly affected in 1-7-2, it seems improbable that the inability to grow on acetate, succinate, fumarate, and malate could be the result of an enzyme deficiency in the Krebs cycle. More likely this mutant is an example of a "permeability" mutant, and this may indicate that in the prototroph these four compounds may have one site that they share in common. The "back" mutants may represent partial modifications of this site or perhaps production of other sites or mechanisms for the transport of these compounds.

The two mutants of group H are unable to utilize pyruvate, acetate, citrate, succinate, fumarate, malate, and pyruvate+malate for growth. One uses glucose and glycerol as well as the wild type, while the other shows only slow growth with both these substrates. It should be pointed out that the mutants of all groups (except perhaps 1-7-2, which was present in large numbers in the wild type population) probably arose by single mutations. We are inclined to view these two mutants of group H as "permeability" mutants because of glucose utilization in the absence of glutamate and aspartate.

Group I is characterized by inability to utilize acetate and glucose and by slow growth on the other compounds tested. All give rise at fairly rapid rates to "back" mutants on glucose agar.

Growth responses of group K may be the result of a deficiency in ability to oxidize alpha-ketoglutarate or succinate.

Growth responses of mutants in group N appear to be localized in the initial step in pyruvate oxidation. These mutants are unable to utilize glucose, glycerol, or pyruvate for growth; acetate is used poorly, while all members of the Krebs cycle are utilized as the wild type. This group of mutants may be extremely valuable in elucidating the steps in the pyruvate oxidation system. They should also provide a very useful tool in the study of "permeability" responses. Although the addition of growth factor quantities of glutamate and aspartate permits these mutants to grow with glycerol as the carbon source, the substitution of glucose for glycerol inhibits growth. Similarly, excellent growth occurs in a mineral agar medium with 0.2% citrate as sole carbon source; the addition of glucose, however, completely inhibits this growth. We have recently isolated glucose resistant (G^r) mutants on glucose citrate medium and on glucose medium supplemented with glutamate and aspartate. These G^r mutants are still unable to grow with glucose, glycerol, or pyruvate as sole carbon sources. G^r isolated on glucose+glutamate+aspartate are still inhibited by glucose on the glucose citrate medium, whereas some G^r mutants isolated on glucose+citrate grow equally well on glucose+glutamate+aspartate, while others grow poorly. Apparently the glucose inhibition is fairly non-specific and is involved in preventing citrate, glutamate, aspartate, and probably other compounds from getting into a position where they can be utilized by the cell. Mutation to glucose resistance appears to be a partial or complete modification of one or more of such sites or perhaps the development of additional sites. Cross transduction experiments with the three mutants of group N resulted in a low frequency of wild type being produced, indicating the close linkage of the alleles involved.

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GENETIC STUDIES OF ESCHERICHIA COLI

P. D. Skaar and H. Davidson

Bacteria are known to accomplish genetic recombination by several methods. One of these, conjugation, involves the cell to cell transfer of hereditary materials during the physical contact of two sexually differentiated parents (Lederberg). Another, transduction, does not require the contact of parental cells; hereditary material is transferred through the medium by a bacterial virus (Zinder). The work of the past year falls into two general categories. One line of research has been a continuation of the work of previous years and has sought to unravel the details of the conjugation process. The other has utilized transductions to examine the hypothesis that genes with related functions tend to be in close physical proximity.

Studies on the Conjugation Process. All of the new experiments reported here were done in collaboration with Dr. Alan Garen. They involve the use of Hfr (high frequency of recombination) strains. Strains of the Hfr mating type when mated with F– mating type strains yield conjugating pairs with a frequency exceeding 40%. The bacteria remain paired for a time and then separate. Genetically recombinant progeny arise from these exconjugants by cell division. However, they only arise from the F– exconjugant, suggesting that transfer of genetic material had been unilateral from Hfr to F– (Lederberg, Hayes).

A first approach to the chemistry of the mating process was made using radioactive tracers. These experiments will be reported in greater detail by Dr. Garen in the Carnegie Institution Year Book. In brief, they reveal that the transfer of phosphorus between bacteria parallels the transfer of genetic material. Further, the experiments were performed in such a way that it is likely that most of the transfer observed was that of DNA phosphorus.

One of the prominent features of recombinants arising by the conjugation process is the predominance of genes contributed by the F– (receptor) parent, with the exception, of course, of the donor genes necessary to detect a recombinant. This bias is most simply explained by saying that discontinuities arise in the donor genome at some step before the appearance of recombinants. Early explanations (Lederberg, Watson and Hayes) called for a few, specific, points at which these discontinuities appeared. Clowes and Rowley pointed out that certain linkage anomalies might disappear if it be assumed that discontinuities are multiple and non-specific. At the same time, we were obtaining data from K-12 x B/r crosses which seemed to be explicable only on this type of interpretation (see last year's report).

Recent experiments by Wollman and Jacob have strengthened this argument in a most striking manner. They found that, when mating

Hfr:F– pairs were subjected to forces which might be expected to separate them (in a Waring blendor), the genic constitution of certain selected recombinants varied in a systematic manner. The frequency of appearance of unselected Hfr (donor) markers increased in proportion to the duration of conjugation before artificial separation. These results mean that all Hfr genes are not transferred simultaneously and that discontinuities arise, or are induced, at varying sites in the Hfr genome.

By virtue of techniques developed for the tracer experiments, we were in a position to confirm these results immediately and to extend them. Hfr cells were allowed to conjugate with F– cells for varying lengths of time and then the Hfr cells were killed by a bacteriophage (T6) to which the F– parent was resistant. The remaining F– cells were then plated on non-selective medium to give isolated colonies, and these were analyzed by replica plating. Recombinant colonies were examined which contained one or more of the Hfr genes: Lac^+, V_1^r, or L^+. Those obtained after a short period of conjugation tended to contain Lac^+ only; at later times, those containing both Lac^+ and V_1^r appeared; still later those containing all three genes predominated. Other types of recombinant colonies were rare at all times. This confirms the conclusion that genes are not transferred simultaneously, and (since a population of unselected recombinants was examined) indicates further that transfer is an oriented process.

Surprisingly, the sequence of transfer observed in our experiments was precisely opposite the one reported by Wollman and Jacob. Appropriate experiments have shown that this is due to some difference between the two Hfr strains employed, possibly structural.

As indicated above, these results provide strong evidence that varying points of discontinuity either arise or are induced (by phage or the blendor) in the Hfr genome. In order to assess the relative importance of these two sources of discontinuity, advantage was taken of the fact that the gene Mal^+ (maltose fermentation) appears among recombinants with a negligible frequency in these crosses. Hfr Mal^+ were mated with F– Mal– and plated directly on non-selective maltose indicator medium. Maltose fermenting colonies represent plated Hfr cells; colonies containing both fermenters and non-fermenters represent plated Hfr:F– pairs; maltose non-fermenting colonies represent plated F– cells. The F– may or may not have experienced a mating. Replica platings showed that, with increased duration of mixed growth, an increasing number of these F– colonies contained one or more of the Hfr genes: Lac^+, V_1^r, and L^+. These spontaneous exconjugants were subjected to the same kind of analysis described above for the T6 screened recombinants and among them a virtually identical trend in relative gene frequencies could be demonstrated. Thus, varying points of discontinuity arise normally in the Hfr genome. Indeed, the inevitable contribution of spontaneous exconjugants to trends observed following phage exposure or blending is of such a magnitude as to leave the possible role of these treatments in inducing discontinuities open to question.

The Linkage of Tryptophan Loci. Mutants of E. coli, B/r, which are resistant to bacteriophage T1, but not to T5, are usually tryptophan requirers (Anderson). They respond only to tryptophan, not to either of the precursors indole or anthranilic acid. In an earlier report from this laboratory it was observed that these two associated changes are pleiotropic effects of a single genetic event. The mutation is not closely linked to any of the well-known markers in K-12, including the gene conferring resistance to both T1 and T5. Nor is it closely linked to the polyaromatic deficiency of the B/r strain, IMN64.

The ease with which this type of tryptophan auxotroph may be obtained suggested that it might be a convenient tool in the study of linkage relationships between other tryptophan mutations in E. coli. Accordingly, nine independent tryptophan requiring B/r strains were obtained from Dr. Demerec and from Miriam Schwartz and analyzed for nutritional properties. Two were satisfied by anthranilic acid or indole (tryA), three were satisfied by indole, not anthranilic, and accumulated anthranilic (tryB), one was satisfied by indole, not anthranilic, and did not accumulate anthranilic (tryC), and three were satisfied only by tryptophan (tryD). One of each of the first three (tryA-2, try B-1, and tryC-4) was subjected to T1 and a tryptophan requiring T1-resistant mutant isolated.

Each of these three double mutants, which may be symbolized as tryAD, tryBD, and, tryCD were crossed with K-12 strain, W1895 which does not require tryptophan, on minimal medium supplemented with indole. All of the recombinants from each cross were wild-type, providing presumptive evidence that tryptophan mutations are clustered in E. coli as they are in Salmonella (Demerec) and prompting further study by means of cross transductions.

The temperate phage, Plkc (provided by Dr. E. S. Lennox), was grown serially on B/r and a variant isolated (Plb) which produced more distinct plaques on B/r and from which higher titer lysates could be prepared. The methods employed in obtaining lysates and effecting transductions differ only slightly from those described by Lennox for the Plkc-K-12 system. In general, the lysates contained only about 10^9 plaque-forming units/ml, whereas the transduction frequency was satisfactory (about 10^{-6} per phage).

To test for the existence of a gene sequence (ABCD) corresponding to the synthetic sequence, as in Salmonella (Demerec), three-point tests were attempted using single mutants (e.g. tryA) as donors and double mutants (e.g. tryBD) as receptors. These were unsuccessful for reasons discussed later, so that recourse was had to two-point tests. All nine independent mutations were found to be closely linked. Four of these, representing one example of each block, have been studied in detail: tryA-2, tryB-1, tryC-4 and tryD-9. These are genetically separable and appear to lie in the order of ABCD.

The failure of the three-point tests referred to above was due to the absence of any transductant type other than that resembling the donor, regardless of parental coupling. Thus, for example, if tryBD were exposed to phage grown on tryA bacteria and plated on minimal medium plus indole, only tryA colonies appeared while wild-type were absent. Where phage had been grown on tryD bacteria, no transductants appeared, although both components could be shown to be competent by other criteria. In view of the demonstrable separability of tryA, tryB, tryC and tryD loci in two-point tests, the possibility arose that the tryD mutation associated with T1-resistance consists of a deletion encompassing much of the region concerned with tryptophan synthesis. Several types of transductions are consistent with this interpretation; the simple experiment involving transduction of a common T1-resistant (tryD-8) on minimal plus indole by phage grown on tryA-2, tryB-1, and tryC-4 is particularly clear. None of these transductions yielded wild-type colonies.

The inference that T1-resistant tryptophan requiring mutants arise by a sizable deletion is supported by other evidence. They do not revert and grow poorly even in minimal plus tryptophan. They fail to accumulate a known tryptophan precursor and this inability is imposed upon initially tryB and tryD mutants when they are made T1-resistant (Gots).

Identical conclusions have been reached in an independent study by Yanofsky and Lennox (personal communication).

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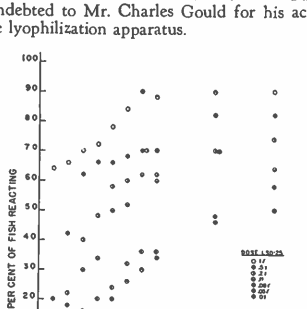
PSYCHOBIOLOGY SECTION

H. A. Abramson, N. Fremont-Smith, A. Goldfarb, M. P. Hewitt,
M. E. Jarvik, G. J. Neviakas, M. I. Orszag, B. Sklarofsky,
J. Stache

The systematic method of approach to study the nature of psychotic processes in man has continued. During the past year the research has been supported by grants from the Josiah Macy, Jr. Foundation of New York City, and by other sources.

Although LSD-25 continued to be the chemical which serves as the originator of the model psychosis in man, liaison with the State Hospital at Central Islip enables us to extend what we have learned in the past year on lower animals and non-psychotic individuals to the study of psychotics. At present there is also an active liaison with Brookhaven National Laboratory in connection with the development of freeze-drying techniques. At Central Islip, liaison in the study of the schizophrenic process in man has been accomplished with Dr. S. Merlis and Dr. W. Turner. At Brookhaven, we are indebted to Mr. Charles Gould for his active participation in improving the lyophilization apparatus.



Percentage of fish showing nose up—tail down position plotted against time for various concentrations of LSD-25 per cc.

Bioassay of LSD-25 with Betta splendens. As demonstrated by previous work at this Laboratory, the Siamese fighting fish is a satisfactory test animal to determine the presence of LSD-25. Figure 1 illustrates observations made on 400 fish. Although four criteria were used during

the period of observation (nose up—tail down, kink in body, proximity to water surface, proximity to bottom of container), the first of these was sufficiently unambiguous to give the results shown in Figure 1, where the percentage of fish showing nose up—tail down position is plotted against time for various concentrations of LSD-25 per cc. These curves at present serve as part of our technique for the quantitative bioassay of LSD-25.

Biosassay of LSD-25 with Ampullaria cuprina. The "mystery snail" is an even more sensitive indicator of LSD-25 than the Siamese fighting fish. Previous observations have indicated that it takes approximately four hours for the snail to respond with wild disorganization of the gastropod musculature at a concentration of 0.01 gamma per cc. We are presently engaged in obtaining the same type of dose-time characteristics for Ampullaria cuprina in solutions of LSD-25. The details of this technique will be published subsequently.

Reaction of LSD-25 with Serum.

(a) Electrophoretic studies: Mixtures of serum and LSD-25 were studied electrophoretically, using a modification of the Kolin apparatus. The pH of the solution was 7.2 to 7.35. When LSD-25 was run alone, no line (LSD-25 concentrated) formed in 20 minutes, and the LSD-25 did not seem to migrate. With serum, a band formed rapidly (observed by fluorescence). This indicated that the LSD had combined with one component of the serum.

The Kolin apparatus was modified to enable fractionation at the mobility zone. From the collected data it appeared that the LSD-25 migrated with a globulin in the lipoprotein region.

(b) Dialysis equilibrium studies: Preliminary investigations concerning the binding of LSD-25 by serum proteins using the dialysis equilibrium technique indicated (1) that for 0.2 my serum in 2 ml buffer (pH=7.2, c=0.01 M phosphate) dialyzed against a solution of 100 ml of the same buffer containing 2.0 mg LSD-25, binding occurs; (2) that under the same conditions, using higher concentrations of LSD-25 (10 and 20 mg LSD-25 per 100 ml), a saturation phenomenon occurs in which the per cent of bound LSD-25 is so small compared to the total LSD-25 present that the experimental error makes calculation impossible.

Liaison with Central Islip. One of the main differences between the psychotic and normal human being is the pattern of communication that he utilizes. We have inaugurated a series of experiments at Central Islip State Hospital, with the cooperation of their Research Department, to determine just what the differences are between normal and schizophrenic communication. In line with this endeavor, attempts will be made to influence the quality of verbal communication by means of drugs that are known to increase or decrease psychotic symptoms.

Thus far, an intensive series of preliminary experiments has been conducted with one psychotic and one nonpsychotic subject and a variety of experimenters. All the verbal communication at these weekly sessions is

recorded on tape and is then typed. One of the aims is to determine whether psychotics are affected to the same extent as normals by different drugs.

An attempt is being made to see whether communication can be enhanced by use of "stablemates"; that is, we are trying to test the hypothesis that schizophrenics will communicate more easily with people with whom they feel on equal terms than with medical supervisors.

Content Analysis. We have available at this laboratory a vast amount of verbal material collected from persons under the influence of LSD-25 and other drugs. Both normal and psychotic subjects have been used in these experiments. Most of these data have been unanalyzed to date with the exception of a few sessions that are reported by Lennard, Jarvik, and Abramson in "Lysergic Acid Diethylamide (LSD-25): XII. A preliminary statement of its effects upon interpersonal communication," The Journal of Psychology 41: 185-198, 1956.

It is planned to enlist the help of Dr. A. Bernstein to content-analyze some of this other material. In addition, certain clinical material resulting from psychotherapy in private practice has been and will be subjected to the same sort of analysis. Material collected at Central Islip State Hospital will also be analyzed in the same way. Table 1 shows a content analysis of the verbal production of one patient over a period of several years. This is an example of the type of data which can be derived from verbal records.

Table 1
Content Analysis of Verbatim Recordings

Date	Number patient lines	Total somatic lines	% somatic lines	Total skin lines	% skin lines	Number recorded sessions
To Summer 1949	8,635.5	356.6	4.1	221.3	2.5	55
Fall 1949 to Summer 1950	11,039.1	510.5	4.6	347.5	3.1	88
Fall 1950 to Summer 1951	8,827.8	333.5	3.7	215.0	2.4	67
Fall 1951 to Summer 1952	6,190.4	67.4	1.0	55.4	0.89	59
Fall 1952 to Summer 1953	5,101.0	81.0	1.5	43.0	0.84	34
Fall of 1953	1,695.0	10.6	0.62	9.6	0.56	11
						314

Blocking Substances. Chlorpromazine, as well as certain hormones, has been studied to evaluate the blocking process reported. Our results indicate that for chlorpromazine 50 mg by mouth may either decrease or increase the effects of LSD, depending upon the time of administration.

Freeze-drying of Urine. As part of our plan to search for substances to block or to produce psychoses, urine extraction is planned. Technical problems connected with freeze-drying large volumes of urine are being solved.

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REPORTS OF SUMMER INVESTIGATORS

Bernheimer, Alan W. and Bernheimer, Harriet P., New York University College of Medicine and State University of New York College of Medicine—It was planned to study induced enzyme formation in the large American silkworm, Platysamia cecropia, but because the larvae became diseased during attempts to rear them, it was not possible to carry out these studies. Much of the summer was spent in lyophilizing a large number of stocks of pneumococcus, streptococcus, Salmonella and Escherichia coli, in demonstrating the operation of the lyophile apparatus to others, and in library work.

Boicourt, Rose Mary Hammer, Department of Bacteriology, University of Illinois, Urbana—Attempts to Demonstrate Chemical Mutagenesis in Phage T4. We attempted to induce mutation in phage T4r140 (Benzer) to r^+. The average reversion frequency in this mutant is 5×10^{-4} as measured by plaques produced on K-12 (lamba). E. coli B was grown and infected in synthetic medium with glucose or lactate, supplemented with 20 mg/l L-tryptophan and 0.5% vitamin-free casamino acids. Caffeine, theophylline and theobromine (150 mg/l) and proflavine (4 to 15 mg/l) were tested as mutagens. The frequency of mutants was determined both in one step experiments and in single bursts. No significant differences were found either in the total number of r^+ mutants or in the frequency of mutant clones.

Since caffeine mutagenesis is readily demonstrable in the chemostat we tried using bacteria grown in the chemostat (kindly made available by Dr. Moser). Bacteria were grown in lactate medium +0.1% casamino acids, with tryptophan as controlling factor at 0.5 mg/l, at a generation time of about 6 hours. Caffeine was added (150 mg/l) and a rise in the rate of bacterial mutation to T5-resistance was observed. Then, bacteria were taken from the chemostat and immediately supplied with excess tryptophan and infected with T4r140. No significant increase was observed over controls either in total mutant frequency or in frequency of mutant clones.

Doermann, A. H., and Chase, Martha C., Biological Laboratories, University of Rochester, Rochester, New York—Benzer recently discovered that rII mutants of bacteriophages T2 or T4 fail to grow in the bacterial strain K/12 (lambda), in contrast to the wild types which grow normally. This observation permits selective scoring of r^+ recombinants in $rII_1 \times rII_2$ crosses and allows examination of the fine genetic structure in this region of the genome (Benzer, Proc. Nat'l. Acad. Sci. 41:344). One feature of the fine-structure study requires testing the linearity of genetic marker arrangement within such short regions. For this purpose several double rII phage strains had been isolated from recombination experiments. Use

of these in three-factor crosses uncovered an unusually high degree of negative interference over short regions of the rII region. Since the new phenomenon seemed to be of particular interest, and since many other multiple rII strains were needed to investigate it more fully, a new procedure was devised for obtaining such phages. The basic difficulty in isolating the multiple rII strains lies in the fact that they are not phenotypically distinguishable from the single rII phages, which, being the parental types, are present in great excess.

A replicating method was gradually evolved and found to be satisfactory for distinguishing phages containing individual r11 mutants and combinations of several of them. Platings are made by the usual agar layer technique. A "parent" plate is seeded with strain B and approximately 30 phage particles from a mixture which contains r_1, r_2, r_1 r_2, and wild type (the progeny from a cross r_1 X r_2). This parent plate is incubated for 18-24 hours at 37 C. For each parent plate to be tested, two "replica" plates are prepared. Both are seeded with 3 drops of a 1:50 mixture of B/s and K12 (lamba)s, and to both are added 2 drops of a solution (5 mg/ml) of dihydrostreptomycin sulfate (to prevent growth of B which will later be transferred to the plate). In addition, one of the replica plates is seeded with 10^8 r_1 particles and the other with 10^8 r_2 particles. All plates are refrigerated for about 2 hours (the replica plates are not preincubated) to harden the top layer and prevent peeling during the replicating process. After the plates are cold, a sterile silk velvet is stamped lightly onto the parent plate and transferred to one replica plate. A second piece of velvet is used to transfer from the parent plated to the second replica plate. The replica plates are incubated overnight and examined for clear areas, or spots. Phages from an r_1 plaque, when transferred to the r_1 replica plate, cannot form any r^+ recombinants; but on the r_2 plate they can, because they infect bacteria already infected with r_2. Thus a spot will be formed on the latter, but not on the former replica plate. The converse is true for an r_2 plaque which is replicated similarly. A plaque of r_1r_2 cannot form a spot on either replica plate, while r^+ will develop a spot on both. Thus all genotypes can be distinguished. The method was tested quantitatively with an artificial mixture of three genotypes, r_{205}, r_{271}, and $r_{205}r_{271}$. The results were found to be in statistical agreement with the numbers of each type expected.

Edgar, Robert S., Biological Labs., University of Rochester, Rochester, N. Y.—Preliminary work was initiated which would permit a detailed examination of the negative interference observed in the rII region of the genome of the bacteriophage T4B (Edgar, Chase and Doermann, PIS No. 9; Benzer, Proc. Nat. Acad. Sci. 41:344). Since suitable markers linked to the rII region were not available in T4B, but were available in T4D (Doermann and Hill, Gen. 38:79), crosses were performed between these two strains. Novel phenotypes occurred in the progeny of these crosses and subsequent backcrosses of selected progeny to the T4D parent which indicated that the two strains differed genetically in at least three loci. Thus

this approach was abandoned and eight rII mutants were isolated in T4D and their relative locations determined by two-factor crosses.

An host-range mutant of T4D, selected on K-12/4 was isolated. Crosses of this mutant have shown that it is located in the third linkage group.

Fraser, Dorothy K., University of Illinois, Urbana, Illinois—Comparison of Mutability of a T4 Stock with That of T3. Experiments were carried out to determine whether plaque-type mutants of a T4 strain appeared preferentially in the yield of infected bacteria failing to lyse at the end of the usual latent period, as do host-range mutants of T3 (Ann. Report, Carnegie Inst. of Washington, 53, 214, 1954).

T4 r48 tu 42g tu 45a was obtained from Dr. A. H. Doermann. Old plaques of this phage contain a high proportion of plaque-type mutants, while young plaques, or stocks resulting from the lysis of young bacteria in dilute suspensions, are genetically fairly pure. This resembles the situation observed with T3, and could be due to selection or to actual production of mutants in late-lysing bacteria.

When E. coli B cells are infected with T4 rtutu and allowed to lyse in anti-T4 serum, so that the yield from the cells that lyse promptly is destroyed, some infective centers are found to survive for several hours. The proportion of the survivors is higher the higher the concentration at which the infected bacteria are kept. However, these infective centers, when diluated and incubated in broth to permit lysis, gave almost no increase in titer. They may represent phage protected from anti-serum, possibly by reversible attachment to unlysed bacteria. These infective centers did not contain signficantly more mutants than the progeny of early-lysing cells.

Reconstruction experiments were carried out to determine whether selection could account for the appearance of large numbers of mutants in old liquid cultures. Known mixtures of parent and mutant strains were used to infect bacteria of different physiological ages, and the proportions of types were determined in the progeny. It was found that selection pressures change markedly with increasing age of the bacterial culture, favoring mutant types in old cultures. Thus it was felt that probably the mutants from T4 rtutu were not actually produced in late-lysing bacteria, as occurs with T3, but were selected for by growth in aging bacteria. It was also found that the number and type of plaque-type mutants of T4 rtutu stocks depended upon the strain of E. coli used for propagation. However, this result may also be due to the evidently complex process of selection.

Granick, S., Rockefeller Institute for Medical Research, New York, N.Y.—Chloroplast Fixation for Electron Microscopy. One of the important problems in photosynthesis is the organization of chlorophyll molecules in the chloroplast. With the electron microscope fine sections of chloroplasts, prepared from tissues fixed in buffered osmic acid, have been examined

and much detail has been discovered. However, we have found that buffered osmic acid alone does not preserve cholorphyll in the species of plants we have used. Chlorophyll was found to be leached out of the chloroplasts during the later stages of dehydration. If chlorophyll is leached out, then under the same conditions, it is probable that other fatty materials may also be leached out. Because of this finding, it may be necessary to modify some of the current concepts of chloroplast structure based on studies of chloroplasts that have been fixed in buffered osmic acid.

Our studies of osmic fixatives indicate that chlorophyll, and perhaps other lipids, are not leached out from chloroplasts of Spirogyra under the following conditions: Fix Spyrogyra filaments for 15 minutes in a mixture of 2 parts of 2% osmic acid plus 1 part of 2.5% potassium dichromate. Then add to the mixture 4 parts of 2% chromic acid. After 15 minutes the filaments are washed with water and dehydrated in the cutomary manner. This method of fixation however failed to prevent carotenoids from being leached out of carrot chromoplasts during dehydration.

Lennox, E. S., Department of Bacteriology, University of Illinois, Urbana, Illinois—Studies of Genetic Transduction with the Bacteriophage Pl.

A. Transduction of characters controlling beta-galactosidase formation from K-12 strain into Shigella dysenteriae strain Sh. This strain of Shigella is lactose negative absolute, i.e., it forms no detectable beta-galactosidase even in the presence of inducer. It had been reported (Cohen-Bazire and Jolit, Ann. Inst. Pasteur 84, 937, 1953) from crosses in K-12 that the genetic determinant controlling such phenotype is allelic to the one determining the galactosidase-constitutive character. This was found not to be the case with Sh. In transduction experiment an adaptive donor gave only adaptive Shigella. A constitutive donor only in rare cases (about 1 in 100) gave any but adaptive Lac^+ recipients. In later crosses of K-12 Lac^+ constitutive x Sh, 12 out of 15 purified isolates were constitutive, the remainder being adaptive.

B. Transduction of lambda production into Shigella—The coli phage lambda does not form plaques on Sh. No host range mutant for Sh has been found. We tested whether lambda could multiply in Sh if its determinant were introduced by Pl transduction. The result was positive. A Pl lysate prepared on K-12 lysogenic for lambda contains some lambda plaque-formers. These can be assayed on E. coli C, which plates both Pl as tiny turbid plaques and lambda as large clearer plaques. In such a lysate there were found particles which adsorb to Sh, sedimented with it and produced lambda plaques on C/Pl. This fraction was 1/10 of the total lambda plaque-formers on C. Control lamba phage derived from K-12 (lambda) by U. V. induction did not sediment with Sh.

Levine, Myron, Department of Bacteriology, University of Illinois, Urbana, Illinois. Lysogenization with Virulent Mutants of Salmonella Phage—Mixed infection of Salmonella typhimurium (strain LT 2) by viru-

lent (nonlysogenizing) mutants CI and CII of bacteriophange P22 results in some bacteria becoming lysogenic for the "virulent" phage CI. The hypothesis has been formulated that a number of reaction steps are necessary for the establishment of lysogeny and that the mutants are blocked at different points in the reaction sequence. In mixed infection the CI and CII phages can supplement one another giving lysogeny.

During the summer, host-induced-modified stocks of CI and CII phages were prepared by growing the phages on Salmonella gallinarum. The development of each of these modified phages in S. typhimurium is prevented. However, mixed infection of S. typhimurium by modified CI and normal CII phage gives some lysogeny with prophage carrying the CI locus. The reverse experiment, mixed infection of normal CI and modified CII phage, results in no lysogeny. If the modified phages are blocked at the same point in development, these findings suggest that the reactions(s) which phage CI can contribute toward the establishment of lysogeny precede the block induced by the S. gallinarum host, whereas the reaction(s) blocked in phage CI but performed by phage CII follow the host induced block.

The CI prophages derived from these crosses are generally recombinants for other markers present in the infecting phage. Analysis of the assortment of these markers makes it possible to localize the region of the phage chromosome controlling the host-induced-modification character.

Luria, S. E., University of Illinois, Urbana, Illinois—Serological Study of Hybrids between Shigella and Escherichia. Hybrids between Shigella flexneri (fertility F–) and E. coli K-12 derivatives (fertility F^+ or Hfr) were isolated and tested for serological cross-reactions with the parent strains. The purpose of this study was to investigate the possible role of hybridization in the serological relations between naturally occurring strains of Shigella and Escherichia, in the serological variation in Shigellas and in the origin of the natural "types" of Shigella.

Hybrids were selected as (S^R $Sugar^+$) colonies from crosses (S^R $Sugar^-$) x (S^S $Sugar^+$). They were isolated in pure cultures and tested for agglutination with sera against the parents. All hybrids tested reacted with Shigella parent antisera and not with E. coli parent antisera. One major new class was discovered among the Lac^+ hybrids in crosses Shigella flexneri 2a x E. coli. Over 95% of the Lac^+ hybrids agglutinate poorly with serum against parent Shigella flexneri 2a (less than 1:500 instead of 1:10000). They agglutinate to a high titer with homologous antiserum. This in turn agglutinates the parent strain 2a only slightly, but agglutinates several other Shigella types. These hybrids were tentatively identified as similar to the naturally occurring strains Shigella flexneri gamma (group specific). Hybridization caused a loss of specific type antigen. Whether a new type antigen is present in these hybrids is not yet clear. The new serological property appears to be controlled by

a determinant closely linked to the Lac locus, but distinct from the Lac+ allele itself.

Other hybrids reveal less drastic changes in antigenic make up, which are to be investigated further.

Maramorosch, Karl. Rockefeller Institute for Medical Research, New York, N. Y.—Part of the summer was spent in preparing for publication two papers representing experimental work carried out at the Rockefeller Institute. They were: (1) Studies on multiplication of aster-yellows virus in insect tissue cultures; (2) An automatic microinjector for insect inoculations.

Miller, Dwight D., Department of Zoology, University of Nebraska, Lincoln, Nebraska.—The object of this work was to collect and culture Drosophila athabasca from Cold Spring Harbor to use in a study of reproductive isolation between different geographical strains of this species. From August 13 through September 2, 1955, collecting was done at several places in the woods near the laboratories. Cultures of D. athabasca were established, and most of them were successfully taken or sent to the University of Nebraska. (Thanks are due Mrs. Guinevere C. Smith, who kept the cultures in the Carnegie Institution of Washington Drosophila stock room until cool weather made it possible for her to send them to Nebraska). Although research on inter-strain isolation in D athabasca is still in progress, the Cold Spring Harbor strains have already given interesting results; they cross quite readily with strains of this species from Michigan and Wyoming, between which a high degree of sexual isolation had previously been observed.

The Drosophila collections at Cold Spring Harbor yielded altogether 13,361 specimens, belonging to at least 21 species: affinis, algonquin, athabasca, busckii, colorata, duncani, funebris, immigrans, melanica, melanogaster, melanura, nigromelanica, pseudoobscura, putrida, quinaria, repleta (group), robusta, simulans, testacea, transversa, and tripunctata. Somewhat out of the ordinary was a rather high percentage of mutant D. melanogaster (more than 2% of this species), presumably due to escaped individuals from the laboratories. A dark-eyed type alone constituted about 1.9% of melanogaster (a single culture of this kind was found to contain Plum when tested by Mr. Stewart Ensign of the University of Nebraska). Very surprising was the presence of five individuals of D. pseudoobscura. According to Dr. Bruce Wallace, a large number of flies of this species were released in the area in 1948, so it seems likely that the specimens collected were descendants of these.

Wassermann, Felix E., Department of Microbiology, College of Medicine, New York University, N.Y., N.Y.—During the summer experiments were carried out to determine the kinetics of release of bacteriophage from infected populations of bacteria. Changes in the environment and in the

physiological state of bacteria prior to and subsequent to infection were investigated as to their effect on the release of phage progeny. The results of this work, done under the guidance of Dr. Mark H. Adams, were published in Virology, 2, 96-108 (1956).

Other work done was preliminary study on the efficiency of plating and plaque characteristics of several T5 hybrids in view of a planned investigation of the genetic control of their antigenic specificity.

COURSE ON BACTERIAL VIRUSES

June 20-July 9, 1955

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

Assistant: Richard H. Epstein, University of Rochester.

The eleventh session of the Cold Spring Harbor phage course was held this year. As in previous years, the course has dealt with techniques and current research problems in the field of bacterial viruses. This year the laboratory manual was extensively modified to include more of the recent developments in phage research. More specifically, three additions were made; experiments were included to emphasize the genetic approach; a new section (by S. E. Luria) was devoted to procedures with temperate phages; and a tracer experiment using phosphorus-32 and sulfur-35 was introduced (by A. Garen and A. D. Hershey). The revised laboratory manual required a stepped-up laboratory schedule. Five laboratory periods and five periods for analysis of data (rather than three, as in previous years) were held each week. In addition, the following seminars were given by leading investigators in the field:

M. H. Adams, New York University College of Medicine—Phage taxonomy.
S. Benzer, Purdue University—Genetics of r mutants in T4.
G. Bertani, California Institute of Technology—Lysogenesis.
M. Delbruck, California Institute of Technology—Ancient History.
A. D. Hershey, Carnegie Institution—Phage chemistry.
E. S. Lennox, University of Illinois—Transductions in Escherichia coli.
S. E. Luria, University of Illinois—Fundamentals of lysogenesis.
F. Wassermann, New York University College of Medicine—The mean latent period.

The eighteen students and four auditors who attended the course are below:

Robert F. Acker, Ph.D., Iowa State College, Ames, Iowa.
R. C. Clowes, Ph.D., The Wright-Fleming Institute of Microbiology, St. Mary's Hospital Medical School, London, England.
Mortimer M. Elkind, Ph.D., National Cancer Institute, Bethesda, Md.
H. W. Felsenfeld, M.S., Columbia University, New York, N.Y.
Sheldon S. Goldberg, Ph.D., Camp Detrick, Frederick, Md.
Walter Harm, PhD., Institut fur Genetik der Freien Universitat Berlin, Germany.
E. Virgil Howell, M.A., University of Utah, Salt Lake City, Utah.
D. Kanazir, M.D., Ph.D., Institute Boris Kidric, Vinca, Belgrade, Yugoslavia.

Martha Jean Paton, M.S., Amherst College, Amherst, Mass.
Herman Prell, Ph.D., University of Marburg, Germany.
John D. Ross, M.S., University of Minnesota, Minneapolis, Minn.
Richard W. Sames, M.S., Indiana University, Bloomington, Ind.
Ralph A. Slepecky, Ph.D., National Microbiological Institute, National Institutes of Health, Bethesda, Md.
Stephen R. Taub, B.A., University of Rochester, Rochester, N.Y.
Constance T. Thomas, B.A., Carnegie Institution, Cold Spring Harbor, N.Y.
Walter Vielmetter, Grad. Stud., Max-Planck-Institut fur Virusforschung, Tubingen, Germany.
Alfred M. Wallbank, Grad. Stud., Michigan State College, East Lansing, Mich.

Auditors:

Gioietta, S. Bompiani, Rome, Italy.
Helga Harm, PhD., Institut fur Genetik der Freien Universitat, Berlin, Germany.
Albert S. Kaplan, Ph.D., Yale University School of Medicine, New Haven, Conn.
Myra Weisberg, B.A., Tufts University, Medford, Mass.

COURSE ON BACTERIAL GENETICS

July 13-August 2, 1955

Instructors: M. Demerec, V. Bryson, E. M. Witkin, P. E. Hartman, and P. D. Skaar, in collaboration with P. FitzGerald, S. W. Glover, and H. Moser.

The course on selected methods in bacterial genetics, first given in 1950, was offered for the sixth time to advanced graduate and postgraduate students. The course emphasized current methods used in the study of bacterial heredity, and some of the important results of recent work in this field. The following students were enrolled:

Arthur Wesley Andrews, Jr., B.A., Camp Detrick, Frederick, Md.
Helen R. Bowser, A.B., Harvard Medical School, Boston, Mass.
Irving Davis (Capt. USAF), M.S., USAF School of Aviation Medicine, Randolph Air Force Base, Texas.
Claire Deutscher, B.S., Brooklyn College, Brooklyn, N.Y.
Harold R. Garner, Ph.D., Purdue University, Lafayette, Ind.
P. Arne Hansen, Ph.D., University of Maryland, College Park, Md.
Walter Harm, Ph.D., Institut fur Genetik der Freien Universitat Berlin, Germany.
Koyoshi Higuchi, Ph.D., Camp Detrick, Frederick, Md.
E. Virgil Howell, M.A., University of Utah, Salt Lake City, Utah.
D. Kanazir, M.D., Ph.D., Institute Boris Kidric, Vinca, Belgrade, Yugoslavia.
Charles P. Miles, M.D., University of California Hospital, San Francisco, Calif.
Hermann Prell, PhD., University of Marburg, Germany.
Stephan R. Taub, B.A., University of Rochester, Rochester, N.Y.
Charles A. Thomas, Jr., Ph.D., 6200 Delong Road, Indianapolis, Ind.
Walter Vielmetter, Grad. Stud., Max-Planck-Institut fur Virusforschung, Tubingen, Germany.
Myra Weisberg, B.A., Tufts University, Medford, Mass.
Mary G. West, M.S., Camp Detrick, Frederick, Md.

Auditors

R. C. Clowes, Ph.D., The Wright-Fleming Institute of Microbiology, St. Mary's Hospital Medical School, London, England.
Frank Lanni, Ph.D., Emory University, Emory University, Ga.
Yvonne Thery Lanni, Emory University, Emory University, Ga.

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

R. W. Barratt, Dartmouth College—Advances in linkage studies in Neurospora.
V. Bryson, Biological Laboratory—Induced multiple mutations in bacteria.

M. Demerec, Carnegie Institution—Transduction studies with tryptophan mutants in Salmonella.
R. S. Edgar, University of Rochester—Phage genetics.
D. Fluke, Brookhaven National Laboratory—Ionizing radiation studies of the Pneumococcus transforming principle.
N. H. Giles, Yale University—Recent studies with mutations involving purple adenine mutants in Neurospora.
J. S. Gots, University of Pennsylvania—Analysis of auxotrophic mutants.
P. E. Hartman, Carnegie Institution—Histidine mutants of Salmonella.
R. D. Hotchkiss, Rockefeller Institute—Transformation of genetic characters in Pneumococcus.
E. S. Lennox, University of Illinois—Linked transductions in Escherichia coli.
S. E. Luria, University of Illinois—Bacterial genetics: past, present and future.
P. D. Skaar, Biological Laboratory—Recombination in Escherichia coli.
E. Witkin, Carnegie Institution—Induced mutations in bacteria.
N. D. Zinder, Rockefeller Institute—Principles of bacterial transductions.

NATURE STUDY COURSE

June 27-July 29, 1955.

Instructors: John A. Gustafson, Department of Science, State University Teachers College, Cortland, N.Y.
Marvin Rosenberg, Biology Instructor, North Syracuse Central High School, North Syracuse, N.Y.
Jill A. Lamoureux, Port Washington, N.Y.

Assistants: Shayna Rosenberg, North Syracuse, N. Y.
Donna Granick, New York, N.Y.

As in previous years, the Nature Study Course ran for five weeks, and was attended by 112 students. The children were separated into eight groups according to age, and classes ranged in size from 8 to 20 students. Each child came to class twice a week for two hours each period. Children aged 6 to 7 were in the Beginners groups, those aged 8 and 9 in the Junior groups, and those aged 10 and 11 in the Intermediate groups. Twelve-year-olds and a few children thirteen years old were in the Senior and Advanced group, which carried on activity of a project nature under the supervision of Dr. Gustafson and Mr. Rosenberg. In addition to the senior and advanced students, Mr. Rosenberg taught the seven- and nine-year-olds, and Dr. Gustafson taught the eight- and ten-year-olds. Mrs. Lamoureux taught students six, nine, and eleven years old. Classes were held each day, Monday through Thursday, from 9 to 11 a.m. and from 2 to 4 p.m.

Wawepex Laboratory was the headquarters for the course. The building proved adequate, since the several small classes each had a room in which to meet, and in which to store and display their trophies collected on field trips. As always, field work was emphasized, but in order to correlate the information gathered in the field, and to study more closely the material collected, each instructor spent some time with each class indoors. The staff formulated the philosophy that, even though the students are young and expect to have a good time on field trips, the course is designed to teach them some of the elements of biological and geological science, and every effort was made to attain that end. Although individual instructors occasionally felt discouraged about the amount of knowledge they were putting across, it was generally agreed at the end of the course that most of the students went away with new information and ideas, and a better appreciation of the natural world of which they are a living part.

During the course several long trips by automobile were made to Jones Beach, Tackapausha Preserve, Oyster Bay Beach, and Mill Neck. Some of these were all-day trips during the last week of the course. Each child brought a picnic lunch, and parents of some of them volunteered to drive. These all-day trips were markedly successful.

This year two evenings were devoted to showing of films in the auditorium of the Lecture Hall. These programs were open to the public, and were well attended by students, their parents and friends, and persons associated with the laboratories. Some of the films were shown on the following day to younger students who could come to the evening showing. The success of this venture indicates that the practice should be continued.

On Friday, July 29, an open house was held at the Laboratory from 2 to 4 p.m. On display were the collections and projects worked on by the students. Refreshmenets were served on the adjacent lawn.

The following students were enrolled in the course:

Ames, Robert
Baldwin, Barbara
Bernheimer, Alan W., Jr.
Berry Lucinda
Billman, Christopher
Brower, Prentice W., Jr.
Bruen, Marian
Bruen, Nicholas
Buckley, Lawrence
Bunce, John Allan
Burns, Claudia
Citarella, Carol
Colyer, Barry
Cooke, George C.
Currie, Jane
Deegan, James Edward
Dewey, Janet Ellen
Dewey, John Bradford
Dowd, H. Robert
Dowd, J. Patrick
Edwards, Charles
Edwards, Sally
Elder, Sarah
Elvin, George
Fessenden, Edward E.
Fleischman, Susan
Francis, Julia Ann
Galehouse, Shelley
Gartland, Thomas
Gottlieb, Gail
Graesser, Gerry
Granick, Joel Lee
Guille, Jim
Hansel, Stephen A.
Harris, Jean
Hollander, Jane
Horwill, Sally
Hotchkiss, Paul
Ingersoll, Judy
Ingraham, Jack
Ingraham, Sandy
Ingraham, Steve
Johnson, Hugh G., Jr.
Jonason, Alan Scott
Kerr, Bradley
Kerr, Cynthia
Knight, Jesse Winthrop
Lally, P. Jeffrey
Lawton, Mary L.
Leach, Charles H.
Lee, Marion R.
Lewis, James
Litchford, Jane
Little, Sally
Macy, Florence
Maloney, Sharon Ann
Maloney, Sheila Mary
Markowitz, Jan
Marston, Mary Lee
Millett, Fred
Moore, Michele
Mulligan, Billy
Muma, Dee
Nardiello, William
Nathan, Laura
Norins, Wendy
Pierce, Josiah
Pittis, Milicent
Pivnick, Carol
Pluess, Linda Marie
Powell, James
Powell, John

Powell, Robin
Powers, Diana C.
Pratt, Hillary W.
Purdon, Nancy
Purdon, Roger
Reif, Sharon
Roosevelt, Anna
Scheffy, Bracky
Schuman, Mickey
Simon, Margo
Skojgard, Ross
Smith, Eldon
Snedeker, James
Solomon, Janet
Squair, John
Storrs, Virginia
Sungaard, Jeremy
Thomson, Paul
Thomson, Sydney
Titus, Peter
Towers, John
Townsend, Charles
Townsend, Robert T. III
Truslow, Sophia
Tucker, Benedict
Tucker, Mary L.
Tucker Thomas
Wallace, Bruce
Wallace, Peter
Warden, John S.
Warren, Constance
Warren, Margaret
Warren, Virginia
Weissman, Jo
Werkley, Christopher
Weterrings, Lea
Wickersham, Barbara
Witkin, Joseph
Witzenburg, James
Zeigler, Carol Ann

SYMPOSIA PUBLICATIONS

*Vol. I (1933) Surface Phenomena, xii + 239 pp.
*Vol. II (1934) Growth, xii + 284 pp.
*Vol. III (1935) Photochemical Reactions, xvi + 359 pp.
*Vol. IV (1936) Excitations, xii + 375 pp.
*Vol. V (1937) Internal Secretions, xvi + 433 pp.
*Vol. VI (1938) Protein Chemistry, xiv + 395 pp.
*Vol. VII (1939) Biological Oxidations, xiv + 463 pp.
*Vol. VIII (1940) Permeability and the Nature of Cell Membranes, xii + 284 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 Application of Tracer Elements, xii + 222 pp.
Vol. XIV (1949) Amino Acids and Proteins, xii + 217 pp.
Vol. XV (1950) Origin and Evolution of Man, xii + 425 pp.
Vol. XVI (1951) Genes and Mutations, xvi + 521 pp.
Vol. XVII (1952) The Neuron, xiv + 323 pp.
Vol. XVIII (1953) Viruses, xvi + 301 pp.
Vol. XIX (1954) The Mammalian Fetus: physiological aspects of development, xii + 225 pp.
Vol. XX (1955) Population Genetics: the nature and causes of genetic variability in populations.

*Out of print.

SUMMER RESEARCH INVESTIGATORS

Adams, Mark H.—New York University College of Medicine, New York, N.Y.

Bernheimer, Alan W.—New York University College of Medicine, New York, N.Y.

Bernheimer, Harriet P.—New York University College of Medicine, New York, N.Y.

Boicourt, Rose Mary Hammer—University of Illinois, Urbana, Illinois.

Doermann, A. H.—University of Rochester, Rochester, N. Y.

Edgar, Robert S.—University of Rochester, Rochester, N.Y.

Fraser, Dorothy K.—University of Illinois, Urbana, Illinois.

Goldfarb, A. R.—The Chicago Medical School, Chicago, Illinois.

Granick, S.—Rockefeller Institute for Medical Research, New York, N.Y.

Hotchkiss, Rollin D.—Rockefeller Institute for Medical Research, New York, N.Y.

Lennox, Edwin S.—University of Illinois, Urbana, Illinois

Lennox, Helen C.—University of Illinois, Urbana, Illinois

Levine, Myron—University of Illinois, Urbana, Illinois

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

Maramorosch, Karl—Rockefeller Institute for Medical Research, New York, N.Y.

Miller, Dwight D.—University of Nebraska, Lincoln, Nebraska

Novick, Aaron—University of Chicago, Chicago, Illinois

Wassermann, Felix E.—New York University College of Medicine, New York, N.Y.

LABORATORY PERSONNEL

Abramson, Harold A.—Research Psychiatrist
*Abramson, Harold A. Jr.—Technical Assistant
*Bassler, Barbara T.——Stenographer
*Beaubian, Wendell—Dining Hall Manager
Bell, Marshall L.—Maintenance Man
†Bompiani, Gioietta—Research Assistant
Branton, Geneva—Technical Assistant
*Bryson, David L.—Technical Assistant
†Bryson, Vernon—Geneticist
*Burtch, Ethel—Typist
†Carlson, Blair—Research Assistant
Cosillo, Gloria—Research Assistant
Demerec, M—Director
†Demerec, Zlata—Research Assistant
*Doermann, August H.—Instructor, Bacterial Viruses Course
Englesberg, Ellis—Bacteriologist.
†Farrington, Margaret S.—Technical Assistant
Franzese, Eleanor—Business Manager
*Fremont-Smith, Nicholas—Research Assistant
†Furguiele, Anthony—Maintenance Man
Gardner, Henry—Technical Assistant
†Geronimus, Lippman H.—Bacterial Physiologist
*Glass, Lois A.——Technical Assistant
*Gustafson, John—Nature Study Course Instructor
†Hadden, Joanna—Research Assistant
Hershey, Harriet D.—Research Assistant
Hyde, Olive—Administrative Assistant
Ingraham, Laura—Research Assistant
Isenberg, Alice—Technical Assistant
†Israel, Robert A.—Research Assistant
Jarvik, Murray E.—Psychologist
Kalish, Judith—Research Assistant
Kennard, John F.—Gardener
King, James C.—Geneticist
*Lamoureux, Jill—Nature Study Course Instructor
*Luria, S. E.—Collaborator, Bacterial Viruses Course
McMullen, Ellen—Research Assistant
†Maciura, Stephen—Carpenter
Madden, Carol V.—Research Assistant
Matson, Joan A.—Stenographer
†Matson, Marjorie G.—Technical Assistant
*Mayr, Susanne—Technical Assistant
Milton, Ulysses—Maintenance Man
Myers, Patricia D.—Research Assistant

Neviackas, Gwendolyn—Stenographer
Orszag, Myrna—Research Assistant
Reddy, William F.—Maintenance Man
*Rosenberg, Marvin—Nature Study Course Instructor
Schilling, Harold C.—Carpenter
Skaar, Palmer D.—Geneticist
Sklarofsky, Bernard—Psychobiologist
Sokoloff, Alexander—Guest Investigator
*Stuard, Barbara—Research Assistant
Thurston, Robert K.—Superintendent of Buildings and Grounds
†Treanor, Ellen T.—Maid
Wallace, Bruce—Geneticist; Assistant Director
Wallace, Miriam—Research Assistant
Warren, Katherine Brehme—Executive Editor of Symposia.

*Summer or temporary
†Resigned during the year.

REPORT OF THE SECRETARY

The 32nd Annual Meeting of the Long Island Biological Association was held in the Lecture Hall at Cold Spring Harbor on July 26, 1955, with President Amyas Ames presiding and twenty-one members present. The chief acts of the Association during the previous year were reviewed by the Secretary. The following members, proposed by the Nominating Committee, were elected or re-elected to the Board of Directors to serve until 1959: Dr. Lloyd V. Berkner, Dr. Howard J. Curtis, Dr. B. P. Kaufmann, Mr. Jesse Knight, Jr., Mr. Grinnell Morris, Mr. Arthur W. Page, and Mr. Franz Schneider. The report of the Treasurer was summarized by Dr. Demerec, who pointed out that funds received during the year as contributions and as grants for the Symposium considerably exceeded previous records, and that grants for research totaled over $100,000. Dr. Demerec presented his report as Director of the Laboratory, which has appeared in printed form in the Annual Report for 1954-1955.

A meeting of the Executive Committee was held in Dr. Demerec's office in Cold Spring Harbor on September 18, 1955, with all members present. Points under discussion were the following: formalization of the relationship between the Biological Laboratory and the Department of Genetics; the critical necessity of sufficient funds to guarantee tenure for chief investigators on the Laboratory staff; and the desirability of constructing a ten-room, motel-type residence to help accommodate increasing numbers of summer guests. The committee approved the building of such a motel, with the restriction that the total outlay should not exceed $15,000.

The 71st meeting of the Board of Directors was held on September 25, 1955, in the Lecture Hall at Cold Spring Harbor, with eighteen members present. The action of the Executive Committee in authorizing the construction of a motel for summer residents was ratified. The Treasurer reported that finances were in good condition. The major part of the Director's report dealt with an analysis of the financial position of the Laboratory. He called attention to a significant rise in membership and in membership contributions; to the increased contribution of the Wawepex Society for John D. Jones Scholarships; and to the fact that the reorganized dining hall service had shown a small profit. The last fiscal year ended with a favorable balance, which was put into the reserve fund for research. It is evident, however, that it is impossible to accumulate sufficient reserves in this way, and the Director stressed again the necessity for raising such a fund. He then reported on the re-equipment of summer laboratories for research in quantitative microbiology, the summer courses for research workers, the Nature Study course, and the topic for the 1956 Symposium. On motion duly seconded and voted, the Executive Committee as constituted for the year past was re-elected to serve for the coming year, as follows: Amyas Ames (chairman), Mrs. George

S. Franklin, E. C. MacDowell, Grinnell Morris, William B. Nichols, Arthur W. Page, and Mrs. Walter H. Page.

A meeting of the Executive Committee was held at the home of President Ames on February 16, 1956. The budget for the coming year was discussed. Dr. Demerec reported that the motel was nearing completion, and that the cost would approximate the authorized $15,000. After discussion, it was voted to authorize the purchase of first-class furnishings for the motel at an estimated cost of $4,000.

The 72nd meeting of the Board of Directors was held on March 4, 1956, in the Lecture Hall at Cold Spring Harbor, with fourteen members present. The minutes of the last meeting of the Board and the meeting of the Executive Committee were read and approved. Dr. Demerec reported on the organization of research at the Laboratory, and on plans for summer activities. The budget for the fiscal year beginning May 1, 1956 was presented, discussed, and approved. President Ames reported a conference with Dr. Caryl P. Haskins, new president of the Carnegie Institution of Washington, in which the continued cooperation of the Carnegie Institution was assured.

E. C. MacDowell, Secretary
Long Island Biological Association

REPORT OF THE TREASURER

AUDIT CERTIFICATE

April 30, 1956

MAIN AND COMPANY

Certified Public Accountants

New York, N. Y.

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

We have made an examination of the accounts of the Long Island Biological Association for the year ended April 30, 1956. Our examination was made in accordance with generally accepted auditing standards, and accordingly included such tests of the accounting records and such other auditing procedures as we considered necessary in the circumstances.

In our opinion, the accompanying balance sheet and statements of income and expense and net worth, and supporting schedules present fairly the position of the Long Island Biological Association at April 30, 1956 and the results of its operations for the year ended on that date in conformity with generally accepted accounting principles applied on a basis consistent with that of the preceding year, except that, effective with the year ended April 30, 1956, the equipment is being depreciated over a five year period, as a result of which, operations for the year have been charged with $11,588.06.

Main and Company
Certified Public Accountants

New York, N.Y.
June 15, 1956.

BALANCE SHEET

April 30, 1956

ASSETS

General and Endowment Fund				
Cash:				
In banks		$49,394.35		
On hand		100.00	$ 49,494.35	
Investments (market value $45,290.74)			42,024.15	
Accounts receivable:				
On grants and contracts for special research		$31,126.77		
Other	$4,423.46			
Less: Reserve for uncollectible accounts	956.00	3,467.46	34,594.23	
Inventory of books, at cost		$37,236.70		
Less: Reserve for obsolescence		13,606.00	23,630.70	
Deferred expenses			2,189.40	
Land, buildings and equipment			303,275.03	$455,207.86
Special Funds				
Cash in bank			$ 1,342.22	
Investments (market value $15,574.86)			15,710.00	17,052 22
Total				$472,260.08

LIABILITIES AND NET WORTH

General and Endowment Fund

Liabilities:

Accounts payable		$ 4,241.79	
Accrued payroll		1,330.64	
Grants and contracts for special research		54,718.97	
Total liabilities		$ 60,291.40	
Reserve for Scientific Research		20,000.00	
Endowment Fund: Dr. William J. Matheson Bequest		20,000.00	
Net worth		354,916.46	$455,207.86

Special Funds

Blackford Memorial Fund: Principal		$ 5,000.00	
Charles Benedict Davenport Memorial Fund:			
Principal	$4,934.75		
Unexpended income	1,043.97	5,978.72	
Charles Benedict Davenport, Junior, Fund: Principal		1,037.12	
Temple Prime Scholarship Fund:			
Principal	$2,500.00		
Unexpended income	102.80	2,602.80	
Dorothy Frances Rice Fund:			
Principal	$2,294.72		
Unexpended income	138.86	2,433.58	17,052.22
Total			$472,260.08

LAND, BUILDINGS AND EQUIPMENT
April 30, 1956

Land:			
Purchased with funds raised through public subscription		$52,198.22	
Land purchased from Estate of Mary E. Jones		15,674.99	
Henry W. deForest land		12,000.00	
Airslie land		5,000.00	$ 84,873.21
Improvements to land:			
Pipe line		$ 1,860.39	
Road		746.64	
Light and telephone poles		290.98	2,898.01
Buildings:			
Airslie building		$ 5,000.00	
Blackford Hall*		19,000.00	
Cole Cottage		2,105.00	
Davenport Laboratory		8,500.00	
Henry W. de Forest building		15,000.00	
Reginald G. Harris House		8,500.00	
Dr. Walter B. James Laboratory		13,500.00	
George L. Nichols Memorial Laboratory		13,700.00	
Williams House		11,300.00	
Urey Cottage		2,660.00	
Machine shop and garage		2,000.00	
New building		18,186.55	119,451.55
Land and buildings leased from Wawepex Society under lease expiring in 1979:			
Land		$13,500.00	
Buildings:			
Hooper House	$13,200.00		
Jones Laboratory	10,000.00		
Osterhout Cottage	5,500.00		
Wawepex Laboratory	7,500.00	36,200.00	49,700.00
Equipment:			
General		$38,577.27	
Biophysics		16,849.90	
Physiology		2,513.15	
		$57,940.32	
Less: Reserve for depreciation of equipment		11,588.06	46,352.26
Total			$303,275.03

* Built on land leased from Wawepex Society

STATEMENT OF NET WORTH
For the Year Ended April 30, 1956

Balance, May 1, 1955		$362,906.07
Less:		
Grant for annual symposium received during period ended April 30, 1955 deferred to current year	$6,000.00	
Excess of expense over income for year ended April 30, 1956	1,989.61	7,989.61
Balance, April 30, 1956		$354,916.46

STATEMENT OF INCOME AND EXPENSE
For the Year Ended April 30, 1956

Income:		
Grants, contracts and research fees		$160,585.02
Contributions:		
Dues	$ 9,216.66	
Wawepex Society	1,900.00	
John D. Jones Scholarship	700.00	
Walter B. James Fund	175.00	11,991.66
Book sales—Symposium of Quantitative Biology		8,223.18
Dining hall		16,275.94
Rooms and apartments		16,414.80
Summer course tuition		2,573.50
Nature study course		2,857.50
Beach permits		1,085.00
Registration fees for annual symposium		188.00
Interest and dividends on investments		1,172.21
Total income		$221,366.81

Expense:		
Expenditures directly chargeable against grants and contracts for special research:		
Salaries	$83,655.89	
Supplies	14,726.15	
Expense of participants and lecturers for annual symposion	31,570.64	
Other expenses	8,404.16	$138,356.54
Cost of books sold and publication expense – Symposium of Quantitative Biology		3,172.21
Dining hall		13,905.93
Rooms and apartments		6,071.45
Research expense		1,846.98
Summer course		775.84
Nature study course		2,072.49
Expense of patrolling beach		756.41
Distribution of John D. Jones Scholarship		752.50
Buildings and grounds maintenance:		
Salaries	$11,745.45	
Materials and supplies	5,336.10	
Heat, light and water	3,259.91	20,341.46
General and administrative		
Salaries	$ 6,571.21	
Insurance	2,128.11	
Printing and stationery	1,891.13	
Telephone, telegraph and postage	747.82	
Equipment	256.10	
Other	1,765.90	13,360.27
Loss on sale of investments		9.25
Provision for reserve for scientific research		2,594.73
Provision for obsolescence of inventory of books		7,752.00
Depreciation of equipment		11,588.06
Total expense		$223,356.42
Excess of expense over income		$ 1,989.61

STATEMENT OF UNEXPENDED GRANTS AND CONTRACTS FOR SPECIAL RESEARCH
April 30, 1956

	Authorized Amount	Amount Expended to April 30, 1956 (Including fees to Association)	Unexpended Balance April 30, 1956
Josiah Macy, Jr. Foundation Three year grant expiring July 1, 1958	$ 33,000.00	$ 1,000.00	$ 32,000.00
Josiah Macy, Jr. Foundation Three year grant expiring September 1955	2,090.00	704.75	1,385.25
National Science Foundation Grant for a period of two years expiring September 1957	19,800.00	2,539.30	17,260.70
National Tuberculosis Association One year grant expiring June 30, 1956	5,762.00	4,242.62	1,519.38
Special summer research grant from National Science Foundation Grant for three years expiring June 30, 1958	15,000.00	7,028.07	7,971.93
Grants for twenty-first annual symposium to be held June 1956:			
Association for the Aid of Crippled Children	3,000.00		3,000.00
Carnegie Corporation	6,000.00		6,000.00
National Science Foundation	6,500.00		6,500.00
Alfred P. Sloan Foundation	3,000.00		3,000.00
United States Public Health Service	4,000.00		4,000.00
United States Atomic Energy Commission One year contract expiring February 28, 1957	37,417.00	4,079.79	33,337.21
United States Department of the Army, Office of the Surgeon General One year contract expiring May 31, 1956	24,475.00	21,710.02	2,764.98
United States Department of the Navy, Office of Naval Research Three year contract expiring March 31, 1958	30,360.00	9,194.62	21,165.38
United States Public Health Service One year grant expiring March 31, 1957	4,600.00	318.29	4,281.71
	$195,004.00	$50,817.46	$144,186.54

ORGANIZATION OF THE ASSOCIATION

OFFICERS

President
Amyas Ames

Vice-President
Mrs. Walter H. Page

Secretary
E. C. MacDowell

Vice-President & Treasurer
Grinnell Morris

Assistant Secretary
B. P. Kaufmann

Laboratory Director: M. Demerec
Assistant Director: Bruce Wallace

BOARD OF DIRECTORS

To serve until 1960

Mark H. Adams ..... New York University
Crispin Cooke ..... Huntington, N.Y.
Mrs. George S. Franklin ..... Cold Spring Harbor, N.Y.
E. C. MacDowell ..... Cold Spring Harbor, N.Y.
William B. Nichols ..... Syosset, N.Y.
Mrs. Alexander M. White, Jr. ..... Oyster Bay, N.Y.
B. H. Willier ..... Johns Hopkins University

To serve until 1959

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

To serve until 1958

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

To serve until 1957

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

Members Emeriti

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

EXECUTIVE COMMITTEE

Amyas Ames

Mrs. G. S. Franklin
E. C. MacDowell
Grinnell Morris
William B. Nichols
Arthur W. Page
Mrs. Walter H. Page

WOMEN'S COMMITTEE

Chairman—Mrs. Alexander M. White, Jr.
Vice-Chairman—Mrs. George S. Franklin
Secretary—Mrs. Edward S. Blagden
Treasurer—Mrs. Walter H. Page

FINANCE COMMITTEE

Grinnell Morris
William B. Nichols
Amyas Ames

BUILDINGS AND GROUNDS

Mrs. George S. Franklin, Chairman

Mrs. Percy Jennings
William B. Nichols
B. P. Kaufmann

SCIENTIFIC ADVISORY COMMITTEE

George W. Corner, Chairman

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

FORMER PRESIDENTS, LABORATORY DIRECTORS, AND BOARD MEMBERS

Presidents

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

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
Atkins, C. D. 1915-23
Ayer, J. C. 1930-33
Ayres, H. M. 1892-1900
Backus, T. J. 1890-1901
Blackford, Eugene 1890-1904
Blackford, Mrs. Eugene 1906-17
Bleecker, C. M. 1926-45
Bleecker, T. B. 1946-51
Blum, E. C. 1923
Boody, D. A. 1890-1917
Brackett, G. C. 1904-08
Brower, G. V. 1899-1917
Brown, Addison 1890-1913
Brown, J. S. 1908-17
Bumpus, H. C. 1903-12; 1927-30
Butler, N. M. 1903-17
Chambers, Robert 1932-54
Cochran, D. H. 1890-1902
Cole, K. S. 1940-43
Cole, W. H. 1934-52
Coombs, W. J. 1890-1910
Crittenden, W. H. 1922-23
Crozier, W. J. 1928-44
Davenport, C. B. 1903-44
Davenport, W. B. 1916-17
de Forest, H. W. 1912-17; 1924-25
de Forest, R. W. 1902-17
Denbigh, J. H. 1923
Detwiler, S. R. 1928-42
Doubleday, F. N. 1908-11
Draper, George 1924-32
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
Mayer, A. G. 1903-17
Merle-Smith, Mrs. Van S. 1931-50
Mickleborough, John 1890-1917
Mills, D. H. 1946-52
Montant, A. P. 1902-09
Morgan, T. H. 1924-28
Newberry, J. S. 1890-93
Nichols, Acosta 1927-45
Nichols, J. W. T. 1910-17
Noyes, H. F. 1902-21
Osterhout, W. J. V. 1927-41
Overton, Frank 1924
Palmer, L. M. 1899-1913
Parshley, H. M. 1924-33
Peabody, Julian 1911-17
Perkins, A. C. 1890-92
Ponder, Eric 1937-41
Pratt, H. I. 1929-30
Prime, Cornelia 1909-17
Raymond, J. H. 1890-1900
Roosevelt, John K. 1927-1956
Rumsey, Mary H. 1924
Schiff, J. M. 1931-50
Schiff, M. L. 1924-31
Scott, Donald 1911-17
Seamans, C. W. 1906-15
Shapley, Harlow 1943-51
Stimson, H. L. 1925-36
Smith, H. C. 1913-17
Stewart, J. H. J. 1893-1917; 1924-26
Stockard, C. R. 1924-39
Stoddard, Howland B. 1951-55
Stratford, William 1890-1917
Straubenmuller, Gustav 1911-17
Strauss, Albert 1914-17
Stutzer, Herman 1911-23
Swingle, W. W. 1924-44
Taylor, H. C. 1926-42
Thompson, Edward 1903-17
Tiffany, L. C. 1892-1917
Urey, H. C. 1934-49
Vanderbilt, W. K. 1924-43
Walter, H. E. 1924-43
Webb, Alexander 1890-1902
Weld, F. M. 1914-17
Wetmore, C. W. 1902-07
White, S. V. 1890-1905
Williams, T. S. 1910-30
Wilson, E. B. 1903-17
Wood, Willis D. 1926-52
Woodbridge, C. L. 1894-1901
Woodward, J. B. 1890-96
Woodward, R. B. 1890-1914

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Dennistoun M. Bell
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Lloyd V. Berkner
Frederick Bernheim
Alan Bernheimer
Sydney Bevin
Nicholas Biddle
Mrs. Nicholas Biddle
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Mrs. Edward S. Blagden
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Mrs. Trowbridge Callaway
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Mrs. Paul Cushman
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Mrs. Henry P. Davison, Jr.
Raymond de Clairville
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Mrs. Henry W. de Forest
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Max Delbruck
M. Demerec
Mrs. M. Demerec
Mrs. William Denby

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Mrs. Richard Derby
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Mrs. Alvin Devereux
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Mrs. Caryl P. Haskins
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Ashton Hawkins
Mrs. Ashton Hawkins
R. W. Hawkins
Healy & Gekoski
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Mrs. R. Graham Heiner
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Davenport Hooker
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James C. King
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Ernst Mayr
R. B. McAdoo
Mrs. Harvey C. McClintock
Ross McFarland
Mrs. Ross McFarland
Mrs. Van S. Merle-Smith
Leo M. Meyer
William H. Miller
Alfred E. Mirsky
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Walter V. Moffitt
Frank Moore
Louis de B. Moore
Mrs. Louis de B. Moore
Grinnell Morris
Mrs. Grinnell Morris
Mrs. Ray Morris
Stuart Mudd
Robert C. Mulhauser
Mrs. Robert C. Mulhauser
John R. Muma
Mrs. John R. Muma
Mrs. Alfred E. Munier
Robert Cushman Murphy
Vincent A. Nardiello, Jr.
James V. Neel
Winthrop Neilson
Mrs. Winthrop Neilson
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William B. Nichols
John W. Nields
Mrs. John W. Nields
Juliet Nourse
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Mrs. Charles P. Noyes
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Mrs. D. Grinnell Noyes
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Mrs. Frank Powell
Francis C. Powers
Mrs. Francis C. Powers
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Mrs. Frederic R. Pratt
H. Irving Pratt
Mrs. H. Irving Pratt
Richardson Pratt
Theodore H. Price
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Mrs. G. Hale Pulsifer
E. Racker
Roland L. Redmond

Mrs. Roland L. Redmond
Mrs. Lansing P. Reed
Mrs. Gordon Rentschler
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Bernard J. Ridder
Mrs. Bernard J. Ridder
R. Oliver Rippere
Harry C. Robb
Charles S. Robertson
Mrs. Charles S. Robertson
Archibald B. Roosevelt
Mrs. Archibald B. Roosevelt
George Emlen Roosevelt
John K. Roosevelt
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William W. Willock, Jr.
Henry S. Wingate
Keyes Winter
W. Wilton Wood, Inc.
Willis D. Wood
Mrs. Willis D. Wood
Sewall Wright
Herbert H. Zeese
† Deceased

A BEQUEST FOR THE BIOLOGICAL LABORATORY

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

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

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

FORM OF BEQUEST

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

SUSAN COOPER