ANNUAL REPORT

of

THE BIOLOGICAL LABORATORY

1954-1955

LONG ISLAND BIOLOGICAL ASSOCIATION

COLD SPRING HARBOR

LONG ISLAND, NEW YORK

LONG ISLAND BIOLOGICAL ASSOCIATION

Incorporated 1924

ANNUAL REPORT

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THE BIOLOGICAL LABORATORY

Founded 1890

Sixty-Fifth Year

1954-1955

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

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MESSAGE FROM THE PRESIDENT

This year again I wish to preface your Annual Report with a layman's interpretation of some of the activities that you will find detailed in its pages.

My present report is written shortly after the close of the twentieth Cold Spring Harbor Symposium, when the Biological Laboratory played host to about 200 of the world's outstanding biologists, more than 40 of whom came from abroad. These scientists participated in a week-long conference on "Population Genetics — the Nature and Causes of Genetic Variability in Populations."

Technical as this may sound, their researches and deliberations have very real meaning to all of us, for included in that scholarly title are such pertinent subjects as: the ultimate effects of radiation from atomic explosions on the populations of the world; the problem of increasing production and quality of food for an ever-growing world population; the resistance of insects to insecticides such as DDT, and the resistance of bacterial infections in humans to some of the new antibiotic drugs.

These are but a few of the questions that were discussed by our staff and our visitors, some of whom came from as far away as Australia, South America, Uganda, England, France, Holland, Italy, Spain, and Yugoslavia. An indication of the importance attached to this work is the fact that this year the Symposium was supported by the Atomic Energy Commission, Rockefeller Foundation, Carnegie Corporation, National Science Foundation, and the Association for the Aid of Crippled Children, which is interested in the hereditary factors involved when children are born with abnormalities.

Members of our own staff at the Laboratory played an important role in this conference, and this, of course, is a source of great pride to all of us. At the close of the Director's report is a list of those members of the Association who gave dinners for the visiting scientists. This was a generous contribution to the success of our international meeting, and I also take this occasion to thank them on behalf of the Association. The Symposium this year was dedicated to Dr. Vannevar Bush, who is retiring as President of the Carnegie Institution of Washington, and who has been a most loyal supporter of the Long Island Biological Association.

It isn't generally known that the Biological Laboratory is one of the few laboratories in this country, outside of the large universities, devoted to pure biological research. The amount of skill and patience required to conduct these studies is made evident by the fact that Dr. Bruce Wallace, our Assistant Director, has been working on one important experiment for six years. In that time, by subjecting 120 generations of fruit flies to continuous exposure to radiation, he has been able to make findings which are equivalent to a period of 3000 years in human terms. His evidence points to the

fact that down through the generations populations of these organisms are not overburdened by the deleterious effects of radiation. This experimentation is not complete, and it must of course be extended to a much broader field, but it is furnishing some of the most basic information available to us to date. It indicates that much more time exists for the study of this problem than would be implied by the reports from some sources, and that our fears, in view of the low level of radiation expected in the immediate future, have been excessive.

Other areas in which the Laboratory has been working are studies of the effect of insecticides on insect populations, under Dr. J. C. King, and of bacterial resistance to antibiotics, under Dr. Bryson. In the first of these areas, discoveries are being made which will greatly increase our knowledge as to the control of mosquitoes, house flies, body lice, scale insects, and the many other insect pests. Principles developed in the research which traces the ability of bacteria to resist antibiotics apply to such diseases as tuberculosis and other crippling afflictions of the human race.

I know that you like to be kept informed, through our Annual Report, of these doings in our neighborhood and that you share with me our great pleasure in the world-wide recognition which is afforded our work.

AMYAS AMES, *President*
Long Island Biological Association

REPORT OF THE DIRECTOR

During the past year there were several significant developments in the administration of the Laboratory which warrant mention in this report. Expansion and ramification of our activities had greatly increased the work of the Director's office, and it had been evident for some time that the administrative load was becoming too heavy to be carried by one person, who, in in addition to being Director of the Laboratory, was also directing the Department of Genetics and conducting his own research program. To remedy this situation, the Board of Directors at its January, 1954 meeting approved the action of the Executive Committee in appointing Dr. Bruce Wallace Assistant Director, to collaborate with the Director in the administration of the Laboratory. This arrangement is proving to be a satisfactory solution of the problem.

Another important development was the improvement of our facilities for summer research with microorganisms. For almost fifteen years the Laboratory has been functioning as a research center for scientists studying the genetics of microorganisms, particularly bacteria and bacterial viruses. Whereas our facilities were adequate at first, when the problems being investigated required only simple equipment, the needs for specialized apparatus had increased to such an extent that our provisions for such work were no longer satisfactory. Last year, in consultation with scientists interested in working here during the summers, a plan was worked out for improvement of the research facilities. Through the generous support of the National Science Foundation and the help of the Rockefeller Foundation, funds became available to put this plan into effect. Consequently, the John D. Jones Laboratory is now being reorganized and re-equipped for research with bacterial viruses and other microorganisms.

A third major occurrence of the past year was the receipt of financial support from the United States Public Health Service for the courses in Bacterial Genetics and Bacterial Viruses. For ten years and four years, respectively, these two courses have been carried on through the cooperative efforts of the teachers, who contributed their services, and the Laboratory, which provided a major part of the funds for running expenses and equipment. Last year, however, it became evident that some help from outside sources would have to be obtained in order to continue this work. The grant from the Public Health Service has solved our problem for one year.

Fourth, and most important, in the record of this year is the fact that 1955 marks the end of an extremely significant period in the life of the Cold Spring Harbor laboratories, since it is the last year of Dr. Bush's administration. In 1939, when Dr. Bush assumed office as President of the Carnegie Institution of Washington, the future of the Biological Laboratory was very

uncertain. It was through his efforts and those of Mr. Arthur Page, then President of the Long Island Biological Association, that the activities and programs of the Department of Genetics and the Biological Laboratory were coordinated — an event which tremendously strengthened the position of both laboratories and made possible the development of their present program. As mentioned in a later section of this report, the 1955 Symposium was dedicated to Dr. Bush, and expressions of appreciation to him opened the last session of the meetings.

Once again the Laboratory enjoyed during the past year the fine support of the members of the Long Island Biological Association and of the Wawepex Society, which made it possible to meet running expenses. Research grants were received from the Army Chemical Corps, the Atomic Energy Commission, the Office of Naval Research, the Office of the Surgeon General of the Army, and the National Tuberculosis Association; and grants in support of the Symposium were contributed by the Association for the Aid of Crippled Children, the Atomic Energy Commission, the Carnegie Corporation of New York, the National Science Foundation, and The Rockefeller Foundation. Dr. Abramson's research projects were supported by grants from the Josiah Macy, Jr. Foundation and the Geschickter Fund for Medical Research.

With regret we record the death of Mr. Henry Hicks, an old friend of the Laboratory. He was one of the incorporators of the Long Island Biological Association and a member of the Board of Directors since 1924. His interest in plants, and particularly his thorough knowledge of the local flora, helped him develop the Hicks Nurseries into a leading establishment on Long Island; and during his long connection with the Laboratory he unhesitatingly contributed his advice and supplied materials for the landscaping of our grounds.

Research

The work of Dr. Bruce Wallace and his collaborators with fruit flies (Drosophila), in addition to advancing our knowledge about the effects of radiation on genetic structure of populations, is making a significant contribution to our understanding of the role played by the hereditary mechanism in evolution. During the past year his studies have consisted primarily in an experimental demonstration that the pool of genes contained in each local population is established by virtue of selectively advantageous interaction among the individual genes. This demonstration was based on the finding that, in general, the rate of survival of larvae carrying a combination of genes acquired from several different populations is lower than that of larvae carrying gene combinations characteristic of a single population. This indicates that the genes within one population are selected on the basis of

their interactions with one another, and helps to explain the nearly normal viability of flies carrying chromosomes from irradiated populations. The particular genes found in these populations are there as a result of integrating selection.

The investigations of resistance to insecticides carried on by Dr. James C. King with Drosophila indicate that this resistance is built up by the accumulation of numerous separate genetic units, each of which contributes a small increment of resistance. The most surprising fact revealed by these experiments is that two different resistant lines, stemming from the same stock and selected in the same way, do not consolidate the same units. Instead, each develops its own system, and when different systems are crossed a decline in resistance results. This finding is of considerable significance from the point of view of population genetics and evolutionary theory. Previously, such differences in genetic systems were thought to require geological periods for their development.

Dr. Vernon Bryson and his collaborators have continued their investigations with bacteria, and by making crosses between different strains they have been able to determine the mechanisms of hereditary transmission of a number of characteristics. Crosses between strains K-12 and B of *Escherichia coli* support the view that such interstrain crosses produce bacteria which are genetically diploid with regard to small and random sections of the genome, and that diploid sections are frequently lost by segregation. Studies of twenty auxotrophs for resistance to six antibiotics revealed that auxotrophs having the same nutritional requirement may differ in drug resistance. This finding suggests a new method of screening for blocks at different levels in a biosynthetic pathway. Dr. P. D. Skaar, continuing extensive studies of the mutable K-12 strain, obtained evidence that its high mutability depends on a single gene, which can be localized by recombination.

Dr. E. Englesberg has been conducting experiments on the acquisition of apparently new biochemical functions by bacteria as a result of mutation. These experiments are of special interest, since most of the mutations studied by microbial geneticists have involved only losses, not gains, of specific chemical capacities.

Dr. H. A. Abramson and his collaborators have continued their studies of the biological effects of LSD-25 (lysergic acid diethylamide), a compound which has a very striking effect on the nervous system and which, when administered in extremely small amounts, can produce in normal people states that resemble schizophrenia. They have found two organisms that are suitable for bioassays of the drug — *Betta splendens*, the Siamese fighting fish, and *Ambularia cuprina*, the "mystery" snail. *B. splendens* reacts in a very characteristic manner to treatment with extremely small concentrations of LSD-25 (0.4 micrograms per milliliter). Studies by Dr. L. H. Geronimus

of the effect of this drug upon brain metabolism have revealed that LSD-25 is not clearly differentiated from the other ergot drugs.

During the summer of 1954, Dr. and Mrs. Reinhard W. Kaplan, of Frankfurt am Main, Germany, and Columbia University, carried on experiments to determine the time after ultraviolet irradiation when normal and mutant cells of Serratia begin to divide. They found that the long lag period before the appearance of freshly mutated cells observed by Ryan in *Escherichia coli* does not occur in the case of color mutations in Serratia.

Dr. Alan W. Bernheimer, of New York University, made a search among plants growing in the vicinity of the Laboratory for substances that would inhibit the enzymatic degradation of ribonucleic acid.

Teaching

The Nature Study Course in the summer of 1954 was again taught by Dr. Pauline James, of Pan American College, Edinburg, Texas, with the collaboration of Mr. John I. Green, of the Science Education Department of Cornell University. They were assisted by former students of the course, Donna Granick, Lee Granick, and Louise Shemin. This course is designed to stimulate interest in nature among the young people of the community, by improving their observation of the many animals and plans 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 course was given in five major sections, according to the ages of the students, and classes were subdivided for field trips and other projects. One hundred and eighteen children attended the course.

For the tenth consecutive year a three-week course was offered in techniques and problems of research with bacterial viruses. As in six previous summers, it was taught by Professor Mark Adams of New York University College of Medicine. Eighteen students were enrolled.

The course in bacterial genetics was given for the fifth year, and was conducted by M. Demerec, V. Bryson, and E. M. Witkin, in collaboration with P. FitzGerald, E. W. Glover, H. Moser, and P. D. Skaar, and with the assistance of I. Blomstrand. This course emphasizes the newer methods used in the study of heredity in bacteria, and some of the recent results in this field. There was an enrollment of sixteen students and two auditors.

Lectures

A regular schedule of scientific lectures was arranged during the summer of 1954, in cooperation with the Department of Genetics of the Carnegie

Institution. Dr. Vernon Bryson was in charge of the arrangements, and the speakers and their titles were as follows:

July 28: Robert Austrian, State University of New York. Bacterial transformation.

August 4: Alan W. Bernheimer, New York University College of Medicine. Biochemical properties of some ribonucleases.

August 11: Sydney Brenner, University of the Witwatersrand, Johannesburg, South Africa. Some studies on auxotrophic mutants.

August 18: David Shemin, College of Physicians and Surgeons. Biosynthesis of porphyrins.

September 1: Karl Maramorosch, Rockefeller Institute for Medical Research. Biological transmission of plant viruses by animal vectors.

During the year our lecture hall was used by several organizations. An illustrated lecture sponsored by the Yale Club, and three Audubon Society lectures sponsored by the parents of the Cold Spring Harbor Nursery School, were held there. The Suffolk County Medical Society used the lecture hall for one of its regular meetings, which included a program of lectures in which E. C. MacDowell and Vernon Bryson took part; and the Suffolk Section of the League of Women Voters came there for a discussion meeting.

Symposium

The twentieth Cold Spring Harbor Symposium on Quantitative Biology met from June 6 to June 13, 1955. The subject this year was "Population Genetics: the nature and causes of genetic variability." Discussions centered on the mechanism by which a group of organisms — a population — becomes adapted to the environment in which it lives by hereditary selection of the best-fitted individuals from generation to generation. A large part of the conference was devoted to considerations of the theoretical aspects of adaptation, and mathematicians took a prominent part in these discussions. Since an understanding of these mechanisms forms the basis for methods used in improving the plants and animals we use as food, scientists who study plant and animal breeding also participated in the conference. In fact, one of the primary objectives of the meeting was to bring together evolutionary geneticists and agricultural geneticists, working on theoretical and on practical problems, in an effort to establish an area of agreement on fundamental concepts common to both divisions of population genetics. Such agreement will facilitate a rapid exploitation of advances made in one division by workers in the other.

The program was organized by a committee consisting of R. E. Comstock (North Carolina State College), J. F. Crow (University of Wisconsin), Th. Dobzhansky (Columbia University), Earl L. Green (Ohio University

and United States Atomic Energy Commission), I. M. Lerner (chairman; University of California), and Bruce Wallace (Biological Laboratory).

All together, thirty-two lectures were given in nine sessions. At the first session Professor Dobzhansky presented an introductory survey of the problems facing population geneticists, and at the last session Professor Lerner summarized the high points brought out in lectures and discussions.

The registered attendance was 191. More than 40 of the participants came from foreign countries, including Great Britain, France, Germany, Sweden, Italy, Yugoslavia, Holland, Spain, Japan, Korea, Australia, Uganda, South Africa, Brazil, and Chile. The Biological Laboratory was able to finance the expenses of the conference with funds obtained from the Carnegie Corporation of New York, Atomic Energy Commission, National Science Foundation, Association for the Aid of Crippled Children, and Rockefeller Foundation.

Dedication to Dr. Bush

At the end of 1955 Dr. Vannevar Bush is retiring as President of the Carnegie Institution of Washington. In token of our appreciation for what he has done for the Cold Spring Harbor Laboratories during his administration, this year's Symposium was dedicated to him. At the concluding session, held on the evening of June 13th, the officers of the Long Island Biological Association joined with the scientific staffs of the Biological Laboratory and the Department of Genetics in expressing their gratitude. At that session Amyas Ames spoke on behalf of the Association and the community, and M. Demerec on behalf of the laboratory staffs, after whch I. M. Lerner, of the University of California presented a concluding survev of the Symposium meetings. Dr. Caryl P. Haskins, president-elect of the Carnegie Institution, was chairman of this session, which was attended by about two hundred participating scientists and members of the Association. Earlier that evening a dinner in honor of Dr. Bush was given by Mr. and Mrs. Amvas Ames, whose guests included the officers of the Long Island Biological Association, members of the scientific staff of the laboratories, the Executive Officer of the Carnegie Institution, and the speaker of the evening.

When Dr. Bush assumed office as President of the Carnegie Institution, the future of the Biological Laboratory was uncertain, and serious consideration had been given to the possibility of terminating its work. Through the efforts of Dr. Bush and Mr. Arthur Page, the activities of the Department of Genetics and the Biological Laboratory were coordinated, which strengthened the position of both laboratories and made possible the development of their present joint program. One of the important consequences of this action was an improvement of the physical facilities at the Department of Genetics and the erection of a lecture hall for the joint use of both laboratories. This new

lecture hall, by providing a more comfortable meeting place, has contributed a great deal to the success of our recent Symposia. Since, in addition, Dr. Bush's interest and influence have been important factors in insuring the continuity of these Symposia, particularly during the critical early-war and postwar periods, it was considered most appropriate to use this year's Symposium as a suitable occasion of honoring him and expressing our appreciation.

Special Events

On Sunday, September 19, 1954, 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. Dr. V. Bryson gave a brief talk on "Research for Human Well-Being," in which he discussed the importance of basic research for progress in our civilization, and pointed out the significant role played by research laboratories like ours in providing the basic information essential for the development of new means of raising our living standards. 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 1954 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.—Investigator: Dr. R. W. Kaplan, Frankfurt am Main, Germany. Students: R. E. Beardsley, Columbia University; S. W. Bowne, Jr., State College of Washington; J. W. Greenawalt, Western Reserve University; S. B. Greer, Columbia University; Dr. F. Kaudewitz, Max-Planck-Institut für Virusforschung, Tübingen, Germany; C. E. J. Kirchner, Kansas State College; J. E. Matheson, Syracuse University; and Dr. H. Moser, Zürich, Switzerland.

Dorothy Frances Rice Fund.—Student: Gertrude C. Emery, New York University.

Temple Prime Scholarships.—Investigator: Dr. E. W. Caspari, Wesleyan University. Students: L. B. Bernstein, Kansas State College; and F. Wassermann, New York University.

Buildings and Grounds

During the year major work was carried out on three of our buildings: new roofs were put on the Wawepex Laboratory and Hooper House, and extensive repairs were made to the porch of the Jones Laboratory. Work was also begun to adapt the laboratory facilities in the Jones building for research with microorganisms. In cooperation with the Department of Genetics, a black-top surface was laid on the road which leads from Blackford Hall past the Jones Laboratory and joins the Department of Genetics road at the Power House.

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 makes grateful acknowledgment of the grants given by 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 Bacterial Genetics section: the Army Chemical Corps, the Office of Naval Research, and the National Tuberculosis Association.

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 National Science Foundation, the Association for the Aid of Crippled Children, the Atomic Energy Commission, and the Rockefeller Foundation.

For the summer courses: the United States Public Health Service.

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

The Laboratory recognizes with gratitude the important service contributed by the teachers of our two research courses, Professor Mark Adams, Drs. Evelyn M. Witkin, Vernon Bryson, H. Moser, and P. D. Skaar, Miss Patricia FitzGerald, and Mr. S. W. Glover.

I wish to express thanks to Mrs. Amyas Ames for arranging the dinner in honor of Dr. Bush at the time of the Symposium, and to the following members of the Association for entertaining our symposium guests at

dinner parties: Mrs. Edward S. Blagden, Mrs. Crispin Cooke, Mrs. Duncan Cox, Mrs. Maitland A. Edey, Mrs. R. S. Emmet, Mrs. Nevil Ford, Mrs. George S. Franklin, Mrs. Grinnell Morris, Mrs. George Nichols, Mrs. William B. Nichols, Mrs. Arthur W. Page, Mrs. Walter H. Page, Mrs. Francis T. P. Plimpton, Mrs. Roland L. Redmond, Mrs. Franz Schneider, and Mrs. Alexander M. White, Jr.

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

Mrs. Van S. Merle-Smith again donated to the Laboratory several pieces of household equipment, which were gratefully received.

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

GENETIC AND BIOCHEMICAL STUDIES OF BACTERIA

V. Bryson, P. D. Skaar, H. Davidson, J. Hadden, and G. Bompiani

This report is based upon work done for the Chemical Corps, Camp Detrick, Frederick, Maryland, under Contracts No. DA-18-064-CML-2449 and No. DA-18-064-2621 with the Long Island Biological Association, Cold Spring Harbor, New York.

Relatively greater emphasis has been placed during the past year on genetic experiments, with biochemistry confined to the researches of Dr. Englesberg described elsewhere in this Report. Many of our own studies have been extensions or continuations of last year's projects, involving use of the turbidostat and analysis of bacterial recombinants for resistance to freeze-drying. In addition, cooperative investigations have been initiated with Drs. H. Fricke and H. P. Schwan on the dielectric constant of bacterial cell suspensions, and with Dr. A. Garen on tracer analysis of bacterial recombination. The comparative sensitivity of auxotrophic strains to antibiotics has been examined, with the aim of discovering if lack of specific phenotypic abilities or enzymatic capacities would confer resistance. In addition, the system of "*fer*" mutations has been studied more extensively, these mutations being detectable through the partial diploidy of interstrain crosses (B/r $\times$ K-12). Such interstrain crosses have also served to show that radiation resistance in strain B/r and in strain K-12 probably does not depend on the same genetic factors. Other experiments have shown the recombinational basis of polymyxin resistance, and the increased incidence of polymyxin-resistant mutants in a strain containing a mutator gene. In the limited space available it is possible to refer only briefly to a few of the projects now under investigation.

Antibiotic resistance of E. coli auxotrophs. Using the gradient-plate method, twenty-one auxotrophs were compared for resistance to aureomycin, terramycin, chloramphenicol, streptomycin, polymyxin B, and neomycin. Requirements in minimal media were for arginine, tryptophane, leucine, methionine, threonine, cystine, phenylalanine, lysine, and histidine. It was soon observed that independent mutants with the same deficiency did not necessarily exhibit the same degree of resistance. For example, one histidine-less strain (W74) was more resistant to aureomycin and terramycin than two others with a similar requirement. The same strain was resistant to chloramphenicol but exhibited only a background of resistant colonies when heavily streaked on polymyxin plates that permitted growth of wild type. In the course of these experiments it was found that addition of polymyxin to streptomycin would selectively prevent growth of streptomycin-dependent cells as contrasted with the streptomycin-resistant type.

Recombination of ultraviolet-resistance factors in crosses of strain B/r and K-12. Compared with *E. coli* strain B, both B/r and K-12 are comparatively resistant to ultraviolet radiation. In previous experiments we observed that if strain B is exposed to certain toxic chemicals, notably those that act as oxidizing agents or redox modifiers, the surviving cells exhibit the characteristics of strain B/r. The most conservative interpretation is that the toxic chemical agents have acted selectively to favor survival of spontaneous B/r mutants occurring in populations of strain B. This interpretation became amenable to proof with the discovery by Calef that strain B or B/r could be crossed with K-12. By means of such crosses it has been possible to establish that the chemically isolated UV-resistant mutants show the same amount of recombination for sensitivity as does strain B/r, when any of them is crossed with K-12. The emergence of radiation-sensitive recombinants from crosses of two unrelated resistant strains (K-12 $\times$ B/r) strongly implies a separate basis for resistance in the two strains. However, the peculiar segregation observed for fermentation modifiers in such crosses suggests a possible chromosomal dissimilarity.

Resistance genes that were once alleles might later be separated by major chromosomal rearrangements.

Fertility of E. coli strain B. The ability of strains B and B/r to cross with K-12, provided the latter is F+ or Hfr, has been examined, using a series of derived strains. Observable recombinant frequency appears to be largely a function of linkage between selective markers employed. However, one strain undergoes recombination with K-12 F+ with a frequency about ten times higher than would be expected from the location of the selective markers employed. This strain, IMN64, requires tryptophane, tyrosine, phenylalanine, and PABA, owing to a single genetic lesion in the vicinity of the locus for streptomycin resistance. Significantly, this enhanced fertility is not evident in crosses with K-12 Hfr; that is, frequency of recombination is high, but not higher than that in crosses of other B auxotrophs with K-12 Hfr. A few B/r auxotrophs, all characterized by subnormal growth on complete medium, fail to show any evidence of fertility.

SBA method. A convenient method for the location of new K-12 markers involves the use of a series of selected B auxotrophs (SBA), each deficient in a different segment of the genome. If the new K-12 marker is unselected, it occurs most frequently among the prototrophic recombinants arising from the cross with the B auxotroph with whose deficiency it is most closely linked.

The advantages of the method resemble those afforded by transducing systems, and stem from the low efficiency with which B is F-transduced (thus minimizing spurious reverse matings) and the limited amount of F+ gene donation.

Mechanism of recombination. In crosses between K-12 W1177 F+ (T^- L^- B_1^- *xyl*$^-$ S^r) × IMN64 (presumed shikimicless, *xyl*$^+$, S^s) the frequency of occurrence of the F alleles of *S* and *xyl* is high (in contrast to other unselected markers), but the occurrence of the two is not correlated. This suggests that *shi* lies between the two loci, *S* and *xyl*, and that, if breaks of the F chromosome explain linkage anomalies, there must be at least two of them.

In all K-12 × B crosses, a fraction of the recombinants are novel fermentative phenotypes, showing varying degrees of ability to produce acid on fermentable sugars. These characters are discontinuous, heritable, and not specific for particular sugars. The new types are presumed to have arisen as a result of segregation for fermentation modifiers (*fer* genes) for which the parental strains differ. After repeated purification many recombinants are unstable with regard to fermentation grade, although they give rise to two stable types upon subculture, suggesting segregation from a heterozygote. Such segregants, however, are generally identical with respect to other parental markers and, where different, differ for genes known to be linked to the F− selective marker employed in the cross. Thus, *fer* segregants from a K-12 W1177 F+ × IMN65 cross have been found to differ for *S*, but not for *lac*. *Fer* segregants from the cross K-12 W1177 F+ × IMN16 (pyridoxine$^-$, linked to *lac*) have been found to differ for *lac*, but not for *S*. *Fer* segregants from K-12 W1177 F+ × IMN60 (tryptophane$^-$, not linked to *S* or *lac*) have not been found to differ for either *S* or *lac*. The modalities of such systems suggest that multiple breaks occur in the F+ chromosome and that a first step in recombinant formation is the proliferation of cells heterozygous for small segments of the genome. If true, this may be a function of structural differences between K-12 and B which lead to breaks; or, alternatively, these occurrences may be a feature of all crosses (including those within K-12) and may be rendered more easily detectable here because of the existence of many *fer* differences.

Mutability. Treffers and co-workers have shown that derivatives of the K-12 strain 58-278 (phenylalanineless) mutate to high-level streptomycin resistance with about 100 times the frequency exhibited by other K-12 strains, and further that the increased mutability is independent of the phenylalanine deficiency. Recombination analyses by Skaar and Lederberg (cited in Cavilli-Sforza, J. Lederberg, and E. Lederberg, Symp. Growth Inhibition and Chemoth., Rome, 1953, p. 108) revealed that the same *S* locus was undergoing mutation in the mutable strain and that the mutability modifier (*Mut*) segregated independently of the parental deficiency and of the locus affected.

That other loci than *S* are affected by *Mut* is revealed by the heavy pappilation of many *lac*$^-$, *mal*$^-$, and *xyl*$^-$ prototrophic recombinants issuing

from the cross 58-278 (F+ *pa*$^-$ *Mut*$^+$) × W677 (F− *T*$^-$ *L*$^-$ *B*$_1$$^-$ *lac*$^-$ *mal*$^-$ *xyl*$^-$). If this cross is supplemented with B_1 most of the recombinants are B_1^- and mutable at this locus. Mutations involving different characters are not associated. If the mutations to *lac*$^+$, B_1^+, etc. represent reversions, genes representing all segments of the readily mappable genome are susceptible to the action of *Mut*. Significantly perhaps, the *pa*$^-$ gene is remarkably stable in the presence of *Mut*, and is also not clearly linked to known markers.

Over 90% of the recombinants from the 58-278 × W677 cross are mutable, suggesting strong linkage to one of the F− selective markers (*T*, *L*, or B_1). The cross singly supplemented with B_1, cited above, yields an equally high frequency of mutable recombinants, eliminating close linkage to B_1. In addition SBA crosses of 58-278 with B/r strains auxotrophic for *M*, *shi*, and *T* give frequencies of mutable recombinants of 4%, 6%, and 28%, respectively. Thus, *Mut* appears to lie in the T-L segment.

Crosses of 58-278 × W677 supplemented with threonine yield L^+T^- recombinants, 60% of which are mutable. Crosses supplemented with leucine yield L^-T^+ recombinants, only 8% of which are mutable. A close linkage of *Mut* to *L* is indicated, and examination of the rare crossovers between these two loci suggests the order: *lac*—V_1—*Mut*—*L*—*T*.

Attempts to influence mutation rate in a nonmutable strain by simply growing in the presence of a mutable one have been unsuccessful. *Mut*$^+$ *mal*$^+$ *S*^{s} cells were grown in the presence of *Mut*$^-$ *mal*$^-$ *S*^{s}, treated with streptomycin, and assayed for *mal*$^-$ *S*^{r}. The observed frequency of the latter was no higher than when the *Mut*$^-$ was grown in isolation, although the resolving power of the technique was sufficient to have detected an increase of as little as threefold.

In an attempt to influence the mutagenic action of *Mut* by environmental conditions, the following experiments were performed in collaboration with Dr. Ellis Englesberg. Relatively anaerobic conditions of growth were achieved by the use of pyrogallol–Na_2CO_3 plugs for 10-ml broth cultures in tubes. Aerobic conditions were achieved by standard culture in tubes without bubbling or agitation. Samples of grown cultures were assayed for *S*^{r} mutants, and median values were used to calculate mutation rates. In each of three experiments, mutation rate under "aerobic" conditions was normal, that is, about 4×10^{-8}. Under anaerobic conditions, however, mutation rate was diminished by a factor of 5-10. Reconstruction experiments failed to reveal a greater selective disadvantage of *S*^{r} under anaerobic conditions, and there was no clear indication of greater clumping or larger cells under aerobic conditions. It is tentatively concluded, therefore, that these are effects upon mutation rate, paralleling similar effects of oxygen tension in other systems.

Mutation to polymyxin resistance. Extensive experiments have been

conducted with the mutable strain and its nonmutable prototype to determine the influence of mutability on resistance pattern. In the nonmutable strain, resistance to polymyxin can be selected with difficulty by growth of cells on nutrient agar containing graded concentrations of the drug. The pattern does not coincide exactly with the typical survival curves for cells selected and repeatedly plated on either penicillin (multistep pattern) or streptomycin (facultative one-step pattern). Actually, the first-step mutants survive over a rather wide range (0.40 μg/ml) but the numbers encountered are relatively low and surviving colonies are sometimes found to be persisters. The latter show abundant growth when isolated in the presence of the drug, as large and distinct colonies. After subculture in the absence of the drug, however, no further evidence of resistance is encountered. It is apparently possible, as other investigators have noted, to have isolated clones exhibiting a pronounced phenotypic resistance which is not inherited after removal of cells into a new environment where the selective agent is no longer present.

Recombination studies have been performed involving crosses of polymyxin-resistant strains with strains carrying the conventional unselected markers for fermentation insufficiency. These crosses show clear segregation of sensitive prototrophs, yet at the same time virtually all possible combinations of unselected markers are found in both the drug-sensitive and the drug-resistant recombinants.

Some intermediate grades of polymyxin resistance are obtained. These experiments are now in progress and already permit the conclusion that the genetic basis of resistance involves more than one locus, and that the major locus for resistance is not closely linked to the unselected markers. In contrast with ordinary K-12 strains, polymyxin resistance can be obtained very readily in the mutable strain already observed by Treffers and co-workers to give a higher incidence of streptomycin resistance. We found that in the mutable strain the rate of mutation to polymyxin resistance was increased about 100-fold.

Clonal analysis of clustered mutations induced by manganous chloride. In the past few years we have done experiments which show that mutagen-treated *Escherichia coli* which are selected because they have a particular mutation are more likely than the rest of the cells in a treated population to have unselected mutations as well. The selected mutation in all these experiments was resistance to phage T1. The incidence and possibly the type of additional mutation varied with the mutagen used. When the same selective process was used to screen untreated sensitive populations for spontaneously arisen T1-resistant mutants, additional mutations were rare.

More recently we have repeated the experiment with $MnCl_2$ as the mutagen, in order to test whole clones of multiple mutants for homogeneity in respect to genetic structure. If the clones were found to be uniform we

would conclude that the multiple mutations arose simultaneously, as the immediate result of a particular event. If only a sector of the mutant clone contained the additional mutation we would conclude that the $MnCl_2$ had induced an unsettled genetic state, or had a residual effect in the cells.

The tabulation below shows very clearly the clustering of mutations within the treated population. Types of additional mutations serving as objects of search were auxotrophy, changes in ability to ferment lactose, and resistance to T3. No T3-resistant mutants were found. Twenty per cent of the original induced population of T1-resistant mutants were also auxotrophs,

Total Prototrophs		59
Resistant to T1 (B/1)	1	
Resistant to both T1 and T5 (B/1, B/1,5)	58	
Changed in regard to lactose fermentation	6	
Total Auxotrophs		15
Resistant to T1	2	
Resistant to both T1 and T5	13	
Changed with regard to lactose fermentation	7	
Having an additional nutritional deficiency	3	

and probably more than 20% of the auxotrophs had more than one nutritional deficiency. The double mutations to metabolic deficiencies listed last were found to be genetically separable in crosses with K-12 strains. The genes for phage resistance and fermentation also segregated independently of each other and of the deficiencies. Among the prototrophs, the number of mutants with additional mutations affecting lactose fermentation was 10%. Among the auxotrophs, or those mutants which had already been found to have one additional mutation, the proportion having a third mutation affecting lactose fermentation was 46%.

After identification of the colonies in which there were multiple mutations, these colonies were tested for homogeneity. The entire colony from the nutrient agar plate on which the $MnCl_2$-treated cells had been plated and sprayed with T1 was suspended in saline and diluted, and samples were plated on nutrient agar, on minimal medium, and on minimal medium plus the exact supplement required if this had been possible to determine. If the mutant had more than one requirement, it was plated on medium containing both supplements and also on media containing each supplement separately.

Fermentation was tested on minimal and complete media containing lactose, eosin, and methylene blue (EMS and EMB). One thousand or more cells were plated on each type of medium.

Eleven of the 13 colonies examined in this way were found to be uniform. One of the two exceptions was 99.9% auxotrophic and like B/r in lactose fermentation. One-tenth per cent was prototrophic and, unlike B/r,

developed a green sheen when grown on lactose EMB. The prototroph was stable when subcultured, but the auxotroph continued to produce a few prototrophs with the fermentation mutation for several more generations before it too became stable. The second exception was a clone of which 20% was glutamicless and serine- or glycineless and the rest only glutamicless. Both types were stable when subcultured. All the cells plated on lactose EMB had the sheen mutation. Thus it differed from the other mixed clone in two ways. The loss of one mutation was not linked to the gain of another. Since both types were present in considerable numbers and were stable when first tested, it appears likely that mutability was limited to the early divisions of the clone.

In summary, when a B/r culture was treated with $MnCl_2$, followed by the application of phage T1, many of the induced B/1 and B/1,5 mutants had additional mutations to auxotrophy or modifications in the ability to ferment lactose. This confirmed previous observations that manganous-induced mutations are frequently multiple. Among 13 T1-resistant clones involving complex phenotypes it appeared probable that most of the unselected mutations arose at approximately the same time as the phage resistance, as if they were consequences of the same inductive process. However, in two of the 13 mutant clones tested for homogeneity more than one type of individual was found, suggesting either a residual effect of the chemical mutagen or the consequences of temporary genetic instability of the bacterial genome.

GAIN MUTATION IN MICROORGANISMS

ELLIS ENGLESBERG

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Mutations from the wild type toward increased, or apparently increased, metabolic activity are found in many microorganisms, and can be classified biochemically in three groups: (1) those resulting in decreased growth-factor requirements (meiotrophic mutations); (2) those producing increased potentiality for the synthesis of cellular substances, such as capsular material, toxins, antigens, and so forth; and (3) those conferring increased potentiality for a particular catabolic activity. These mutations may actually represent gains in potentiality for the synthesis of enzymes previously absent or undetectable (gain mutations), or they may involve the loss of enzyme inhibitors.

Since it is improbable that any one primordial cell contained the complete genetic-enzymatic complex as present in the variety of life existing today, the importance of the study of gain mutations as the necessary raw material of positive evolution can readily be seen.

The immediate goal of this study of gain mutations is the selection of biological tools and methods for their analysis. The following organisms and mutations have been under investigation: (1) *Pseudomonas fluorescens*, mutation to utilization of sodium itaconate, mesaconate, and citraconate (2) Coliform organism, mutation to citrate utilization; (3) *Escherichia coli*, mutation to utilization of sodium itaconate, mesaconate, and citraconate; (2) mutation to ability to utilize D-ribose; (4) *Pasteurella pestis*, meiotrophic mutation from the requirement for cysteine, phenylalanine, methionine, valine, and isoleucine for growth; (5) *Pasteurella pestis*, mutation to rhamnose utilization. This report will summarize the studies with *Pasteurella pestis*.

Meiotrophic mutants in Pasteurella pestis. *Pasteurella pestis*, strain A1122, B1, avirulent, has been shown to require cysteine (C), phenylalanine (P), methionine (M), valine (V), and isoleucine (I) for growth at 30° C in a mineral glucose medium (Englesberg, *J. Bact.* **63**: 675-680, 1952). These amino acid requirements are not characteristic only of this particular strain, but appear to represent the norm among the various isolates of *P. pestis*, as demonstrated by a study of 25 virulent and avirulent strains by Rockenmocker. Thus, they are in no way comparable to the naturally occurring methionine dependence in strains of *E. coli* (Monod, *Ann. Inst. Pasteur* **72**: 889-890, 1940), the tryptophane requirement of some of the Salmonella, or the laboratory-produced auxotrophy in mutants of *E. coli*. In the first two

of these cases, since the majority of *E. coli* and Salmonella strains are complete autotrophs (i.e., they can utilize for their entire nitrogen and sulfur requirements inorganic salts of ammonia and sulfate), the most likely explanation of the deficiencies is that they result from loss mutation occurring in the widespread autotrophic population. One would therefore expect that change to independence in these strains would probably result from mutation of a so-called negative allele, although this is not necessarily so. In *P. pestis*, however, the situation is completely different; since most of the amino acid requirements are common to all strains, we may infer that they have evolutionary significance in the formation of this "species" and have been present for a considerable period of time.

Thus the significance of employing *P. pestis*, strain A1122, as a tool in the study of microbial evolution may be summarized as follows: (1) its amino acid requirements are representative of the entire "species," so that this organism may never have attained the state of a full autotroph; (2) if descent from an autotroph did occur, the inactive alleles previously involved in the biosynthesis of amino acids may have mutated to other vital functions during the course of evolution, and therefore by selecting meiotrophic mutants it may be possible to demonstrate the simultaneous loss of other functions and thus offer evidence of the gain of a new gene (Lederberg, *Heredity* **2**: 145-198, 1948).

An analysis of the dual sulfur requirement of *P.* pestis has previously revealed that the inability of this organism to synthesize cysteine and methionine from inorganic sulfate is the result of breaks in the conversion of sulfate to sulfite and cysteine to cystathionine, and the lack of an alternate pathway for the conversion of the sulfur of methionine to the sulfur of cysteine.

Attempts have been made recently to study the mechanism of requirements for the other amino acids, by a process of selecting meiotrophic mutants. In general the following isolation procedure was employed. A portion of a culture of *P. pestis* grown on a casein hydrolyzate (3%) mineral glucose (0.2%) medium (CH) was streaked heavily on a mineral glucose medium supplemented with the required amino acids, less the ones to which meiotrophy was desired. For example, the methionine-independent mutant was isolated by streaking the prototroph on mineral glucose agar supplemented with L-cysteine, DL-phenylalanine, DL-valine, and DL-isoleucine (40 μg/ml; L forms can be substituted for DL forms). Faint, almost imperceptible growth of the wild type was accompanied by the appearance of large secondary colonies. Mutant colonies originally present in the inoculum usually appear in 72-96 hours, but plate mutants continued to arise in this case for periods of a week or more.

Since valine and isoleucine are sometimes both required as a result of the loss of a common enzyme (transaminase), an attempt was made to select

a valine-isoleucine meiotroph in one step by streaking the prototroph onto CPM medium by a procedure similar to that employed for the isolation of M^+. The wild type failed to grow on this medium, but mutant colonies appeared after several days' incubation and were isolated in pure culture and found to be extremely stable. Thus the apparent requirement for both valine and isoleucine was lost in one step. These results seemed to indicate that it would be possible to develop a mutant strain of *P. pestis* which would grow on CP alone, by a two-step mutation and selection procedure. When the valine-isoleucine-"independent" mutant was streaked on CP, further mutation occurred leading to methionine independence, apparently confirming this hypothesis. However, an entirely different picture developed when mutants were selected in the reverse manner. When an M^+ meiotroph isolated on CPVI was streaked on CP medium, growth of the entire population occurred. Thus initial mutation to methionine independence resulted in a simultaneous loss of the "requirement" for valine and isoleucine. When the wild type was streaked directly on CP, similar M^+ mutants were isolated in one step. In both cases, the methionine, valine, and isoleucine requirement appeared to be lost as a unit.

These contradictory findings have now been shown to be the consequence of a methionine inhibition, which resulted in only an apparent requirement for valine and isoleucine. This was demonstrated in the following manner. When M^+ (which can grow on CP) was streaked on CPM, growth was completely inhibited, except for the mutant colonies that appeared. It now seems obvious from these results that methionine is required, but also inhibitory. The valine-isoleucine "requirement" is involved in the neutralization of the methionine inhibition. Thus the addition of valine and isoleucine to CPM neutralizes the methionine inhibition, allowing for the growth of the prototroph. Does methionine actually interfere with the metabolism of both valine and isoleucine? When the mutant isolated on CPM agar (now called methionine resistant, M^r) is streaked on CPMI, visible growth is completely inhibited, demonstrating an isoleucine inhibition, However, mutation to isoleucine resistance (I^r) occurs on these plates. When the M^r meiotroph is streaked on CPMV, normal growth occurs, similar to that which occurs on CPMVI. No growth of the wild type occurs on either CPMV or CPMI. These results indicate that isoleucine is required to neutralize the methionine inhibition, but is itself inhibitory, and that valine is required to neutralize the isoleucine inhibition. The isoleucine-valine relationship can also be shown by the failure of the M^+ mutant to grow on CPI whereas excellent growth occurs on CPV or CPVI. As a result of this investigation, the genotype of the *P. pestis* prototroph may be tentatively described as $C^-\ M^-\ P^-\ M^s\ I^s$.

The discovery of the unexpectedly complex relationship between methionine, valine, and isoleucine demonstrates the utility of the selection of meio-

trophic mutants for elucidating the amino acid requirements of heterotrophic microorganisms. The requirement for valine and isoleucine, as involved in the neutralization of the methionine inhibition, could not have been discovered without first isolating a methionine-independent meiotroph. Thus, it seems that this method of approach would be essential for any clear, unambiguous statement of the growth-factor requirements of other, more fastidious microorganisms, such as the Staphylococcus, Lactobacillus, Clostridia, etc.

Lag in phenotypic expression of meiotrophic mutants. Washed suspensions of the M^r mutant grown in a casein hydrolyzate mineral glucose (CH) medium exhibit a prolonged lag (24-36 hours) when inoculated into a liquid CPM mineral glucose medium. (Growth fails entirely when small inocula are employed, approximately 10,000 cells/ml.) However, the same suspensions inoculated into a complete mineral medium (CPMVI) demonstrate a lag of approximately 16 hours. Suspensions prepared from primary growth on CPMVI and on CPM behave similarly, exhibiting a typical 4-hour lag for growth in both CPMVI and CPM media. Growth on CPMVI and CPM media, therefore, adapts these cells for growth in either of these two media. The extreme lag or failure in growth shown by M^r grown on CH when inoculated into CPM is no doubt partially due to the preferential use of preformed amino acids during growth on the CH medium, which results in cells lacking essential enzymes involved in amino acid synthesis. The 16-hour lag of M^r (CH-grown cells) inoculated into CPMVI probably represents the time required for the synthesis of these enzymes. However, it does appear that for full phenotypic expression of methionine resistance (growth in CPM), growth has to occur initially under conditions in which methionine is present in at least slightly inhibitory amounts. (This appears to be the condition of growth on CPMVI agar, since the M^r mutant grows almost twice as fast as the wild type on this medium, indicating that even with the presence of valine and isoleucine, methionine is still partially inhibitory. Since growth of M^r and of the prototroph are similar in the CH medium, presumably methionine is not inhibitory in this medium.) Although the huge difference in growth in CPMVI and in CPM of inocula of M^r grown on CH might at first glance suggest that the longer lag in CPM represents the time required for the biosynthesis of enzymes involved in valine and isoleucine synthesis, this cannot be the explanation, since M^r grown on CPMVI and CPM behave similarly when subcultured into CPM. It appears that the shorter lag exhibited by CH-grown cells on CPMVI, as compared to growth on CPM, must be explained on the basis that valine and isoleucine are involved in partial neutralization of the methionine inhibition, and that increases or decreases in enzymes involved in valine and isoleucine synthesis (if this does occur) are not significant in this case. Thus, the extreme lag in CPM represents the time required for the synthesis of a methionine-

resistant pathway under conditions of methionine inhibition, as well as the time required for production of enzymes for amino acid synthesis.

As a corollary of these conclusions, it should be almost impossible to isolate M^r mutants from the wild type grown on CH by streaking on CPM agar. This appears to be the case when small, washed inocula are employed. With large unwashed inocula, however, such mutants appear, probably as a result of contaminated amino acids, valine and isoleucine being provided in the inoculum. Apparently the ability of M^r (CH-grown) to adapt in CPM (when inoculated in large amounts) can also be explained on the basis of the supply of valine and isoleucine under these conditions, perhaps as a result of partial cell lysis.

A similar example of lag in phenotypic expression has also been demonstrated with regard to growth of M^+ from a casein hydrolyzate medium in CP.

Mutation to rhamnose utilization in P. pestis. When isolated from nature and maintained on conventional media, *P. pestis* is unable to decompose rhamnose. However, when this organism is streaked onto a peptone rhamnose Endo indicator medium, growth of the wild type is followed by the appearance of a few secondary rhamnose-positive (R^+) mutant colonies. One interest in this mutation stems from the claim made by several investigators that only glycerine-positive *P. pestis* can give rise to rhamnose-positive mutants, and that mutation to rhamnose utilization converts this organism into *Pasteurella pseudotuberculosis*, an organism which differs from *Pasteurella pestis* in being motile, producing colonies on ordinary medium within 24 hours (*P. pestis* requires at least 48 hours), fermenting glycerol, and producing the enzyme urease. Isolation of rhamnose-positive mutants from glycerine-positive and -negative *P. pestis* and from both virulent and avirulent strains, and the absence of changes in glycerol fermentation and urease production, completely contradict this claim.

Additional interest stems from the fact that although fucose is present in the blood-group substances, and rhamnose itself is a component of the cell wall in many bacteria and found in many plants, very little information is available concerning the metabolism of these methyl pentoses.

A comparison of the ability of resting cells of R^+ and of the wild type to oxidize rhamnose after growth in peptone and in peptone rhamnose media demonstrated that only R^+ grown in the presence of rhamnose is able to oxidize this substrate. Adaptation of R^+ (peptone grown) to rhamnose utilization occurs in the presence of a complex nitrogen source with rhamnose as the inducer, and precedes cell division, demonstrating the adaptive nature of rhamnose oxidation by R^+. The R^+ mutant oxidizes rhamnose only partially, taking up approximately 1.3 micromoles of O_2 and liberating 1.3 micromoles of CO_2, whereas the same cells oxidizing glucose consume 3.0 micromoles of 0_2 and evolve 3.0 micromoles of CO_2. Analysis for possible

products of this apparently incomplete oxidation of rhamnose has shown the absence of any volatile or nonvolatile acids, ethanol, diacetyl, butylene glycol, or acetoin. Small amounts were found of a compound which forms an osazone with 2,4-dinitrophenylhydrazine at room temperature. The absorption spectrum of this osazone in an alcoholic NaOH solution coincides with the osazone formed from methylglyoxal. Its identity is also indicated by melting-point analysis. However, the fact that the original unknown compound is nonvolatile indicates that it is not methylglyoxal, as such, but forms an osazone which is indistinguishable from the osazone formed from methylglyoxal. Lactic aldehyde is the only compound known which fits this description. However, until an authentic supply of this compound is available for comparison and additional derivatives are prepared the unknown can be only tentatively identified as lactic aldehyde. Although quantitative recovery was not attempted, indications are that this compound can account for only a very small fraction of the rhamnose apparently not utilized. Paper chromatography analysis indicates the presence of other reducing substances which have not yet been identified.

Comparative studies of cell-free extracts of R^+ and the wild type grown in a casein hydrolyzate mineral medium with the addition of glucose or rhamnose have demonstrated that the cell extract of adapted R^+ differs from the cell extracts of the unadapted and the wild-type cells by possessing at least two "new" enzymes: (1) a rhamnose isomerase, which converts rhamnose into a new sugar, rhamulose; and (2) a kinase, which probably phosphorylates rhamulose. The question arises whether mutation to R^+ leads to the gain in potentiality for the production of two enzymes or whether this is a case of acquired ability to produce the isomerase and simultaneous adaptation of the kinase. In the latter case, the wild type has the potentiality of producing the "rhamulokinase," but since it cannot produce the inducer substrate, rhamulose, the kinase activity is never expressed. A test of this hypothesis will have to await the synthesis of sufficient amounts of pure rhamulose.

The activity of the R^+-adapted extract is not inhibited by the addition of wild-type extracts, demonstrating that mutation to R^+ is a true gain mutation. This gain mutation has no detectable effect on the existing ability of this strain to attack Kreb-cycle compounds or on its ability to utilize various hexoses and pentoses for growth. However, when rhamnose was incorporated as the sole carbohydrate in place of glucose in the casein hydrolyzate mineral medium, it was observed to "inhibit" the growth of the R^+ mutant, whereas the wild type was not so affected. (The wild type and R^+ can grow in this medium without a carbohydrate, since there is sufficient casein hydrolyzate for use as carbon, energy, and nitrogen source.) There was no similar inhibition of growth in peptone rhamnose agar.

RADIATIONS AND POPULATIONS

B. WALLACE, C. V. MADDEN, A. BARRETT, G. COSILLO, AND B. STUARD

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During the past year the work at this laboratory has dealt primarily with analyses of irradiated populations and with experiments designed to help our understanding of these populations. One of these experiments was a repetition of a study described in last year's Annual Report (1953-1954); the purpose was to demonstrate differences between gene pools of different populations of the common fruit fly.

Samples of flies from five widely separated natural populations were used in this year's experiment. Flies were obtained from Chile, Israel, California, Virginia, and New York through the courtesy of Drs. D. Brncic, E. Goldschmidt, T. Prout, Max Levitan, and J. C. King.

Individuals from these different populations were mated and remated systematically in order to obtain offspring carrying a variety of different combinations of genes from the five localities. There is no need to tabulate the 150 gene combinations included in this study; their general nature can be shown in summary form, using the numerals 1 through 5 to represent the populations:

a) 11 × 11 and all similar crosses of flies from one population with other flies from the same population.

b) 11 × 22 and all other crosses of flies from one population with flies from a different locality.

c) 11 × 12 and similar crosses of flies from one population with hybrids of that and another population.

d) 11 × 23 and similar crosses of flies from one population with hybrids of two entirely different populations.

e and f) 12 × 34 and all similar "double-cross" hybrids. There were two types of matings of this sort: in the first, special "F_3" males carrying crossover chromosomes were used; in the second, F_1 hybrid males which passed on to their offspring nonrecombinant chromosomes.

g) 12 × 12 and all similar crosses of F_1 hybrid males and females.

h) 12 × 33 and all similar crosses of F_1 hybrid males with females of an entirely different population. (This combination differed from (d) in that the hybrids here were F_1 males; in (d) the hybrids were either females or "F_3" males.)

The experiment dealt with the ability of larvae carrying these different

gene combinations to survive under semistarvation conditions. Twenty-five larvae of each genotype were transferred to 15 x 45-mm shell vials containing 2 cc of molasses-agar medium. Twelve replicate vials of each of the 150 genotypes were studied. The numbers of adults hatching in these vials were recorded.

The results of the experiment are summarized in Table 1. In this table the term "heterozygous" refers to the presence of alleles from different geographical localities at homologous loci. A "derived" chromosome is defined as one carrying genes from two different populations; by definition, this type of chromosome could not arise in a natural population. A "derived"

TABLE 1

Mean numbers of flies in the final counts of vials, grouped on the basis of the proportion of "heterozygous" loci and the degree of recombination. The figures represent the numbers of survivors in groups of 12 vials, or survivors from 300 larvae. *N* indicates "natural" haploid set; *D*, "derived" haploid set. Definitions of these and other terms are given in the text.

"Derived" chromosomes		"Heterozygous" loci: 100%		50%		0%	
0%	N	171.8	1.9	———		156.0	3.0
	D	159.1	1.1	———		———	
50%	N	165.3	1.2	161.0	1.5	———	
	D	148.4	1.1	159.0	1.9	———	
100%	N	146.7	1.6	———		———	
	D	———		———		———	

haploid set of chromosomes is the type transmitted by F_1 hybrid males; individual chromosomes carry genes from one locality only, but different chromosomes can carry genes from different populations. The results listed in the table agree very well with those of the earlier experiment reported last year. Recombination between different populations lowers the percentage survival of these flies. Flies with the highest survival rate are the F_1 hybrids (11 × 22, 11 × 33, etc.). It is especially important to notice that larvae as heterozygous as the F_1 hybrids have lower survival rates, including the lowest percentage observed.

A rather simple model of the genetic structure of a population can be

based upon the results of these two experiments. Selection within any one population establishes a pool of genes, each of which has been tested in individuals of many genotypes in previous generations. The pool existant at any one time is not a fortuitous aggregation but an integrated collection built up by the continuous coadaptation of different genes to one another through natural selection. The problem of maintaining this coadaptation through successive generations in spite of gene recombination, cross-fertilization, migration, and mutation demands a sacrifice on the part of selection. The extent of this sacrifice is indicated by the higher survival of F_1 hybrids, whose genotypes, by definition, are not coadapted. The success of coadaptation is indicated by the low percentage of survival accompanying interpopulation gene recombination.

* * * * *

During the past year there has been an enormous increase in public concern over the effects of radiations on populations. The problem is an extremely complex one that cannot be covered adequately in the short releases generally published in the daily press. Although the following account is also too short to do justice to the general problem, members of this Association may find it of some interest.

There is an unfortunate disagreement concerning the induction of gene mutations in man by irradiation. Many persons are familiar with the action of radiations on more dynamic physiological processes; with these systems there is generally a threshold dose below which irradiation has no apparent effect. In radiation genetics no comparable threshold has been observed; the frequency of genetic effects is proportional to the total amount of irradiation for all doses and all intensities. This lack of a threshold has been demonstrated in many experimental animals. It has not been demonstrated in man, since the induction of mutations themselves has not been demonstrated in human beings. This lack of evidence is not at all critical, however, because the demonstration of radiation effects in any organism has always depended upon the use of techniques and tools not available for the study of man.

Assuming that radiation does induce gene mutations in man, what will be the effect of these mutations on human populations? In answering this question it is necessary to distinguish between *individuals* and *populations*. One can predict with almost absolute certainty that the exposure of a large segment of the population of the United States to even a small dose of radiation (X-rays, atomic radiation from "fall-out," or natural background radiation) will induce at least one mutation. One can also predict that this mutation will eventually harm some individual. These predictions follow from what we know of the genetic equilibrium established within a gene

pool through the interaction of gene mutation and elimination. As a result of this knowledge, there is only one morally defensible position one can take regarding the exposure of persons to radiations: No exposure without justification. Negative arguments that irradiation has no genetic effect or that the effect consists merely of a few deleterious mutations are indefensible since they are based on a callous indifference to the health and happiness of other persons.

Related to the effect radiation-induced mutations will have on individuals is the problem of counseling exposed persons regarding possible genetic damage to their own children. At the moment this is not a serious problem; it may become serious if many persons are exposed to and survive fairly large amounts of radiation. The chief concern in this instance will be centered on dominant and semidominant genetic changes; recessive mutations accumulate to such high frequencies within populations that the slight increase within the gametes of an exposed individual can be neglected.

The fate of a population is quite distinct from the fate of individuals. During the Middle Ages, European cities were continually ravaged by the plague but continued to exist as centers of population. In our own time, the toll of automobiles can be predicted with uncanny accuracy year by year, but the continued existence of the *population* of the United States is never questioned. The introduction of mutations by irradiation presents the two correlated problems, (1) of estimating statistically the burden genetically handicapped persons will impose upon populations and (2) of predicting whether this burden will overtax the population and lead to its extinction.

These are the problems with which we have been concerned in our radiation studies with Drosophila populations, now in their sixth year. At the moment, it seems reasonable to predict that our experimental populations could withstand exposures of 2000 r per generation indefinitely; various genetic changes amenable to investigation have established equilibria in these populations so that no future generation should ever be more handicapped than the one preceding it. It now remains to place this assurance on a more quantitative basis, to improve our understanding of the genetic architecture of populations, and to identify those aspects of human populations whose analysis will facilitate the transfer of general conclusions from Drosophila to human populations. Mankind is fortunate in that the full burden imposed by radiation-induced mutations will not be felt for many generations; provided it is not wasted, there is ample time for the enumeration and experimental evaluation of the many problems with which radiations are confronting human populations.

THE GENETICS OF RESISTANCE TO INSECTICIDES

JAMES C. KING

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The two previous annual reports outlined the general nature of the project and summarized the results of the first two years of investigation. Several different lines of *Drosophila melanogaster*, stemming from two strains of different origin, have been selected for resistance to DDT by subjecting the adults to an aerosol of the insecticide and using the survivors as parents of the next generation. Different lines are subjected to different levels of selective intensity, and some lines are carried in duplicate to see whether the same strain always responds to the same type of selection in precisely the same way.

The two strains of different origin have shown distinctly different responses. Oregon-R, a standard strain kept in the laboratory for some thirty years, responds very slowly to selection. Syosset, a wild strain collected in the summer of 1952, has responded more rapidly. Different levels of selective intensity produce different rates of response. If only 50% of the flies are killed and the remaining 50% used to propagate the new generation, definite resistance develops in the Syosset lines within a dozen generations and in the Oregon-R lines in about twice as long. More intense selection, where the survivors are only 5% or 1% of the total number of flies treated, produces a slower and more erratic response.

Crosses made between resistant lines or between a resistant line and the unselected control give in the F_1 (first filial) generation an LD_{50} (the measure of resistance) intermediate between those of the two lines crossed. If the F_1 flies are inbred, there is a lower LD_{50} in the F_2. This is true even though the two lines crossed stem from the same original stock, have been selected in the same manner, and show the same degree of resistance. All the available experimental data indicate that, in the strains used, resistance is built up by combinations of numerous genetic factors and that different lines selected separately but in parallel achieve resistance by consolidating different combinations.

During the third year the program of treatment and selection has been continued and the pattern of response has continued to unfold. The two most resistant lines, SyS-1001 and Sy-S-1002, both stemming from the Syosset strain and selected at the 50% level, now show at F_{39} LD_{50}'s approxi-

mately ten times that of the control. ORS-1001 (the Oregon-R line selected at the 50% level) has an LD_{50}, at F_{41}, between five and six times that of the control. The Syosset lines carried at the higher levels of selective intensity are more resistant than the control, but none has achieved such high resistance as the lines carried at the 50% level.

Another experiment has been carried out with two lines in which the larvae were raised on medium containing DDT. Beginning with a concentration of four parts per million, the insecticide was increased to eight, then twelve, and finally sixteen parts per million. The larvae responded rapidly to selection, and after one or two generations the lines grew as well in the poisoned medium as the controls grew in unpoisoned food. Both lines were tested in every generation by exposing the adult flies to an aerosol of DDT. The adult flies of the F_1, the first generation raised in the poisoned medium, showed a slight increase in resistance as measured by the mortality produced by the aerosol. One line, SySM-2, was carried from generation to generation by using as parents the adult flies taken directly from the culture bottles. In the other, SySM-1, the flies used as parents were survivors of an aerosol treatment which had killed 50% of the flies subjected to it. Thus the line Sy-SM-2 was exposed to DDT only in the larval state. The adult flies were treated with the aerosol only to determine their resistance, and all treated survivors of the SySM-2 line were discarded. Line SySM-1, on the other hand, was selected in the adult stage at the 50% level in addition to being raised in poisoned medium.

After the initial increase in resistance of the adults, SySM-2 showed no further change in its reaction to the aerosol treatment, even though the concentration of DDT in the medium was increased fourfold. At F_{26} the adults showed no greater resistance to the aerosol than they had shown at F_1. SySM-1 roughly paralleled the behavior of SySM-2 until F_{13}, when the resistance began to show a further increase. By F_{26}, SySM-1 was showing an LD_{50} btween six and eight times that of the control.

In another project, carried out at the University of Kansas, Dr. Robert R. Sokal has been selecting *D. melanogaster* for resistance to DDT in the larval state, not only raising the larvae on poisoned medium, but measuring their resistance by placing known numbers of larvae in vials of medium containing different concentrations of the insecticide and counting the numbers of adult flies that ultimately emerged. He was able to build up a resistant line which thrived on medium containing twenty-three parts per million of DDT. He noticed that these resistant larvae pupated around the edges of the vials; the larvae of his controls pupated over the surface of the medium. By selecting pupae from the edges of the medium in the control strain, he built up a line of predominantly peripheral pupaters which had never come in contact with DDT. On testing these, he found that they were resistant to DDT, although somewhat less so than the line which had been

selected directly for resistance. Very possibly, peripheral pupation was the result of larval behavior which reduced the effectiveness of DDT in the medium as a larvicide. The two characters — resistance and peripheral pupation — were correlated, although not perfectly.

As a result of consultation with Dr. Sokal, we decided to exchange flies and test each other's lines by our own methods. When tested by the aerosol method, Dr. Sokal's resistant line showed no higher LD_{50} than his control strain and neither differed significantly from our Oregon-R or our Syosset strain. On the other hand, SyS-1002 F_{21} (which had an LD_{50} between five and six times that of its control when tested by the aerosol method) showed as great resistance in the larval state when tested by Dr. Sokal as did his own resistant strain. What is more surprising, SyS-1002 showed no tendency to peripheral pupation.

The results of the tests on these exchanged flies and our own experience with SySM-1 and SySM-2 not only bear out the conclusion that resistance in *D. melanogaster* can be the result of combinations of numerous genetic factors, but also give us some insight into the varied nature of these factors. A great deal of thinking about inherited resistance has assumed that resistance is the result of a single process, generally envisaged as physiological, each genetic factor contributing an increment to the efficacy of the process. But peripheral pupation may be correlated with resistance in some lines and not in others. It looks very much as though there is no one road to resistance, either genetically or phenotypically. The individual insect may escape DDT poisoning by behavior which keeps his exposure at a minimum, by the ability to render DDT harmless through some physiological process, or by goodness knows what other clever tricks.

It is highly probable that resistance to DDT is in no way unique in this respect. Other phenotypic characters on which selection operates are probably similar. If there is more than one way to elude the effects of a poison, it is very likely true that there is more than one way to develop a thick skin, to withstand high temperatures, or to do any of the other things necessary to survival in an inconstant and capricious environment. And the more versatile the members of a population are in meeting the requirements of the environment, the better fitted the population is to cope with environmental crises.

As was pointed out in the last annual report, the meager knowledge that we now have concerning the genetics of resistance may very well serve to increase the effectiveness of pest control. The experimental program is being continued in the hope of finding more and more practically pertinent information. In addition, it is interesting to point out that a research program which was organized primarily to seek practical knowledge has given us data of real value in increasing our understanding of the theory of population genetics and the operation of the process of evolution.

EFFECTS OF LSD-25

L. H. Geronimus, H. A. Abramson, L. J. Ingraham, and B. Sklarofsky

The work reported below was supported by a grant from the Geschickter Fund for Medical Research, Inc.

The previous annual report (1953-1954) concerned itself in part with investigations of the effects of LSD-25 and other ergot derivatives on the oxygen consumption of crude preparations of guinea pig brain. The purpose of these investigations was to determine whether there was any evidence suggesting that LSD-25 had some sort of qualitatively or quantitatively unique effect. If evidence of such an effect could be found, than a program of trying to isolate the precise systems involved could be undertaken. At the end of the period covered by the last annual report it indeed seemed that the inhibition of oxygen consumption brought about by LSD-25 when added to the two different kinds of preparation used (minced brain and brain so homogenized that the cells were disrupted) was sufficiently different from the effects of the other ergot derivatives investigated to warrant the attempt to localize its site or sites of action. For LSD-25 was more inhibitory than either *d*-lysergic acid or ergonovine with both kinds of preparation; and it entered the minces more readily than ergotamine or dihydroergotamine and was therefore, initially at least, more inhibitory than these drugs.

While preliminary experiments designed to suggest a fractionation procedure were under way, however, some compounds very closely related to LSD-25 were obtained, and it was decided to test the effects of these substances on the crude guinea pig preparations before more time was spent on what promised to be a difficult and time-consuming task. These substances were the levo isomer of LSD-25 (*l*-LSD-25), the iso isomer of LSD-25 (*d*-isoLSD-25), 2-bromoLSD-25 (BOL-148), and *d*-lysergic acid ethylamide (LAE-32). The results of Warburg experiments with the optical isomers of LSD-25 are shown in Figures 1 and 2. From these data it can be seen that there is no apparent difference in the degree of inhibition of oxygen consumption brought about by LSD-25, *l*-LSD-25, and *d*-isoLSD-25. BOL-148 is markedly more inhibitory than LSD-25; and LAE-32 is less inhibitory, but considerably more so than ergonovine.

In a study designed to compare the psychodynamic effects upon man of LSD-25 with that of other ergot drugs, Jarvik, Abramson, and Hirsch, members of the group at Mt. Sinai Hospital which is in close collaboration with the present project at the Biological Laboratory, had already found that ergonovine in large doses ($\geqq$7 micrograms per kilogram of body weight) has no psychological effects upon man whereas equally large doses of LAE-32 and BOL-148 do elicit a small number of responses of the kind brought forth by LSD-25. Since ergotamine and dihydroergotamine are used clini-

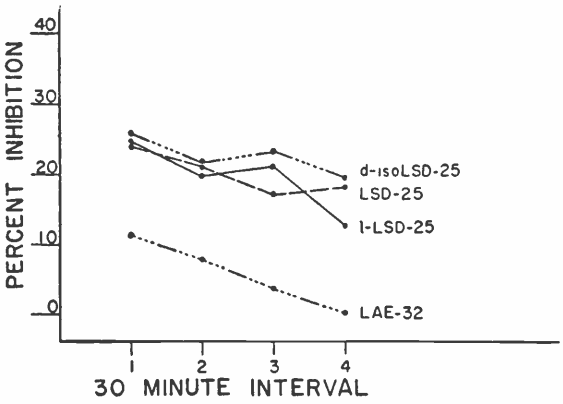

Fig. 1. The effect of 4×10^{-5} M solutions of ergot derivatives upon respiration of homogenized guinea pig brain. (187 mg tissue per vessel; Q_{O_2} of control = 3.3 for first hour, 3.0 for second hour.)

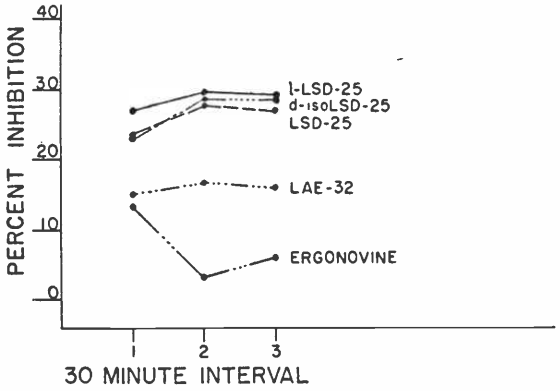

Fig. 2. The effect of 4×10^{-5} M solutions of ergot derivatives upon respiration of minced guinea pig brain. (198 mg tissue in control vessel; Q_{O_2} of control vessel = 4.9 for first hour, 4.7 for second hour.)

cally in amounts vastly larger than the dose of LSD-25 (<1 microgram per kilogram) which is sufficient to induce schizophrenia-like symptoms in man, and have never caused such symptoms, it remained necessary only to test the effects of one or both of the optical isomers of LSD-25 available to us in order to find out whether there is a correlation between effect on the oxygen consumption of guinea pig brain preparations and effect on the human

psyche. Therefore, experiments were done in which normal human volunteers, known to react strongly to as little as 25 μg of LSD-25, were given 100 μg of *l*-LSD-25. There was no reaction. After a suitable interval (one or more weeks), these subjects were given 100-μg doses of *l*-LSD-25 followed shortly by 25-μg doses of LSD-25. The resulting effect in the case of each subject was simply that of a 25-μg dose of LSD-25.

From the foregoing it is obvious that at this time it is not possible to say that there is a relationship between the metabolic effects of LSD-25 observed on the Warburg apparatus and the mode of action of this drug as a psychoticum. In fact, a cursory examination of the data gives the impression that all the evidence is against such a relationship. However, there are reasons to believe that the metabolic data gathered here at the Biological Laboratory do have some bearing on the problem in question. This can be made clear if we consider that the experiments on man indicate that at least two of the structural properties of the LSD-25 molecule are necessary for its remarkable activity. First of all, the nucleus must be *d*-lysergic acid. If one of the optical isomers of *d*-lysergic acid is substituted for it, or even if one of the hydrogens on the indole ring is replaced by a bromine atom, there is a loss of psychodynamic activity. Secondly, alteration of the diethylamide side chain brings about a loss of psychodynamic activity. Removal of one ethyl group reduces this activity drastically, and a change in the side chain completely erases it. The in vitro inhibition of brain metabolism under the conditions of our experiments is brought about equally well by lysergic acid diethylamides irrespective of whether they are the dextro, levo, or dextro-iso isomers, and regardless of whether or not the *d*-lysergic acid nucleus is substituted in the indole ring. However, within the series of derivatives of unsubstituted *d*-lysergic acid that have been used, it is clear that the nature of the side chain is important once ergotamine and dihydroergotamine are removed from consideration. This is allowable because the experiments with minces have established that these substances do not enter brain cells readily even when present in the circumambient fluid in relatively tremendous concentrations. The remaining *d*-lysergic acid derivatives are in order of decreasing inhibitory activity: LSD-25 > LAE-32 > ergonovine > *d*-lysergic acid. This is also the order of psychodynamic activity of at least the first three substances in man. Although too much weight should not be given to this relationship, because the series is short (only 6 order arrangements are possible with three objects, and only 24 if we also consider the fourth member of the metabolic series, *d*-lysergic acid), still the relationship is sufficiently suggestive to encourage further investigation.

A program was also undertaken to determine in which tissues LSD-25 localizes. This question is of importance because if LSD-25 acts directly upon the neurons of the brain, it should be found in the brains of animals

which have been injected with it. There have been several attempts, using indirect methods, to do this. One such attempt, made at the University of Rochester, consisted in administering LSD-25, labeled in the side chain, to animals and then sacrificing these animals twelve hours later and assaying various organs and tissues for radioactivity. Although the side chain of LSD-25 is undoubtedly readily removed by the transamidases and similar enzymes present in mammalian tissues, and although the animals were killed too long a time after the administration of the labeled LSD-25 (the reaction in man is virtually over within 6 to 8 hours after the drug is taken), much has been made in certain quarters of the fact that very little radioactivity was detected in the brain. In contrast, other experiments performed at the Sandoz Laboratories in Basle suggest that a considerable amount of LSD-25 penetrates the brain. Mice were sacrificed at various time intervals (10-120 minutes) after LSD-25 had been injected intravenously, and anti-serotonin activity, which indicates the presence of LSD-25 or of a derivative of LSD-25, was assayed. These experiments, although well designed and cogent, are still open to the criticism that they, too, are indirect; and the problem could be only resolved by the application of a direct test for LSD-25 activity. Such a test had been developed at the Biological Laboratory by Abramson and Evans, namely, the assay using *Betta splendens*, the Siamese fighting fish. The following type of experiment was therefore performed.

Small rats (50-100 grams) were injected intravenously with 40-50 μg of LSD-25 per gram of body weight. About an hour later the animals were sacrificed and various of their organs were homogenized. The resulting homogenates were serially diluted. Three juvenile Siamese fighting fish were placed in aliquots of each dilution. Appropriate controls, including a titration of a homogenate of normal brain to which LSD-25 had been added after homogenization and water controls containing various concentrations (including one of zero) of LSD-25, were also run. This experiment produced evidence that one hour after injection there is a concentration of LSD-25 in the brain of a rat approximately one-fourth of what could be expected if no destruction of any of the LSD-25 had occurred and if the LSD-25 had been equally distributed through the rat's tissues. This experiment was repeated, except that the homogenates were prepared in concentrated form and dialyzed against distilled water. By using the dialysate (i.e., the clear solution outside the semipermeable bag) for the titration, it was possible to rule out the complication of the fish eating bits of the tissue in the homogenate. The results were the same as with the ulfiltered homogenate. Appreciable amounts of LSD-25 do enter the brains of rats injected with large amounts of it.

BIOASSAYS FOR LSD-25

H. A. Abramson, L. T. Evans, L. H. Geronimus, A. Haggis, M. E. Jarvik, C. Kornetsky, B. Wallace, and C. W. Wickersham, III

The work reported below was supported by a grant from the Geschickter Fund for Medical Research, Inc. Begun as a summer project, this program showed so much promise that it was continued through the fall and winter by the permanent staff at the Biological Laboratory.

As the investigation of the psychodynamic effects of lysergic acid diethylamide (LSD-25) has progressed, it has become more and more evident that a specific assay for minute amounts of this drug is necessary for the exploration of a number of avenues of research which present themselves. A concentrated effort was therefore made to find a bioassay for LSD-25. The biological rather than the chemical approach was chosen not only because it seemed more promising, but also because the search for a bioassay implies an investigation of the mode of action of the drug. In fact, certain portions of the program set up were perhaps more oriented toward an investigation of the effect of LSD-25 than toward a bioassay for it. Such a primarily etiological study was the following embryological one, which was based upon the rationale that, since LSD-25, after administration to adult animals, elicits manifestations which may be referable to an effect upon the central nervous system, it could be conjectured that this compound might have some reproducible effect on the early development of the nervous system of the chick embryo. Experiments were therefore performed in which fertile hen's eggs were injected at various times of incubation with varying amounts of LSD-25, then excised after 8 days of incubation and examined grossly for visible abnormalities. Thus, distilled-water solutions containing 0.2, 0.5, 1.0, and 2.0 mg of LSD-25 were injected into groups of eggs before incubation and also 18, 24, 48, 72, and 96 hours after the beginning of incubation. Non-injected eggs, as well as eggs injected with appropriate solutions of neutral sodium tartrate (LSD-25 is actually *d*-lysergic acid diethylamide tartrate), served as controls. It was found that there were no significant differences in the frequencies or types of abnormalities observed in the embryos from non-injected, sodium tartrate injected, and LSD-25 injected eggs.

Various animals, both vertebrate and invertebrate, were treated with LSD-25 and observed for obvious vegetative, motor, or behavioral responses. The test animals included crustacea, molluscs, amphibia, turtles, insects. and fish. Definite responses to LSD-25 were elicited from *Daphnia* sp., *Drosophila melanogaster*, *Ambularia cuprina* (popularly known as the "mystery" snail), and various species of fish, including *Carassius auratus*, the common gold-fish, and *Betta splendens*, the Siamese fighting fish.

Daphnia sp., as supplied by the local pet shop, was immobilized and apparently killed by concentrations equal to or greater than 20 μg/ml of LSD-25. This is not as high an order of sensitivity as was exhibited by the other susceptible species.

Each of eight strains of *Drosophila melanogaster* showed a significant change in preferences for light of different colors after being fed LSD-25 in sugar solution. The experiments were performed in an apparatus consisting of a glass Y-tube whose walls were covered with black tape. One arm, into which the flies were introduced, was plugged and the other two arms were covered with glass light filters which transmitted different parts of the visible spectrum. Two pairs of such contrasting filters were used in each experiment. One pair consisted of a Wratten #11 filter (light green) and a Wratten #35 filter (purple); the other pair consisted of a Wratten #45 filter (blue-green) and a Wratten #47 filter (royal blue). Six flies of a given strain were fed a drop of sugar solution and were later introduced into the Y-tube, and the number of these flies choosing each filter was noted. Each group of 6 flies was tested in this manner 10 times against each pair of filters. Then the procedure was repeated with 6 new flies which had been fed sugar solution containing LSD-25. The amount of LSD-25 ingested by each fly was estimated to be of the order of magnitude of one microgram. An analysis of variance was performed with the data, and the following significant ($p < .05$) results became manifest: (1) Each strain showed a preference for one color of light over the other with each pair of filters. The preferences varied from strain to strain. (2) The pattern of color preference was altered for each strain by the ingestion of LSD-25.

The mystery snail, *Ambularia cuprina,* was found to respond to 4 hours' immersion in as little as 0.01 μg/ml of LSD-25 by opening its operculum and showing evidences of a characteristic type of disorganized movement. When a concentration of 0.1 μg/ml was used, the opening of the operculum and the extrusion of proboscis, tentacles, and gastropod occurred within 2 hours. The gastropod waved about in a disorganized manner which seemed to preclude adhesion to any surface. LAE-32 (*d*-lysergic acid ethylamide) was similar to LSD-25 in its effect upon *A. cuprina,* whereas BOL-148 (LSD-25 brominated in the indole ring) generally did not bring about an LSD-like reaction although it might do so if administered in a relatively high concentration. Since LSD-25 is a very potent antagonist of serotonin in molluscs, serotonin was added to the water containing lysergized snails. Such treatment resulted in a cessation of the waving and a closure of the operculum. This apparent reversal of the LSD-25 reaction lasted for several minutes, after which the snails reopened and continued to wave and undulate in an abnormal manner. Although *A. cuprina,* as a test animal, has the disadvantage of reacting to at least LAE-32 in the way that it reacts to LSD-25, it is suscep-

tible to lower concentrations of LSD-25 than any other intact organism thus far studied.

Betta splendens, the Siamese fighting fish, in contrast to *A. cuprina*, has been found to be an excellent test animal from the point of view of reacting to no other compound tested in precisely the same way as it reacts to LSD-25. The concentrations of LSD-25 which brought out the full-blown effect described below varied with conditions and the size and age of the fish, but it could always be elicited in juveniles 2-3 cm long by treatment with 0.5 μg/ml for one hour, and 0.1 μg/ml always brought about abnormal motor and behavioral responses although they might not with certainty be recognized as being caused by LSD-25 unless the fish were exposed for four hours.

When under the influence of LSD-25, *B. splendens* manifested the following striking aberrations from normal appearance and behavior: (1) The fish showed a stuporous or "trancelike" effect, that is, a tendency to remain motionless for minutes at a time. This "trance" could be broken by even slight stimuli, but the fish reverted to it as soon as the stimulus was removed. (2) When the fish moved in the absence of exogenously applied stimuli, they moved slowly and languidly — unlike normal fish, which tend to move swiftly and suddenly. (3) They showed a tendency to keep the head up, with the body more or less vertical and the snout usually near or at the surface of the water. (4) They had a tendency to swim backward while in the near-vertical position. This movement was apparently brought about entirely by the pectoral fins. (5) Their bodies were kinked in a spastic sort of way, so that they resembled commas when viewed laterally and letter S's when viewed from above. (6) The fish often changed their position or their direction of backward swimming by a peculiar rotation around the nearly vertical long axis of the body which is best described as a "barrel-roll." (7) From time to time the fish slowly sank to the bottom tail first in a manner reminiscent of a "cartesian diver." (8) The fish periodically displayed laterally. This display involved both the ventral and dorsal fins but not always the caudal fin. (9) Almost immediately after the administration of the drug, the fish (especially if they were juveniles) became more intensely pigmented.

Because all these attitudes and motions are part of the repertoire of normal Bettas, and since the attitudes typical of LSD-25 intoxication were not held by the fish all of the time, it was necessary to observe a treated fish in conjunction with an untreated normal fish for a period of minutes. It was soon found that the observation period could be cut down considerably when the fish were treated as populations of 3 or 4 per container. When washed and transferred to fresh spring or distilled water, the lysergized fish gradually recovered and could be reused two or three weeks afterwards. Unlike *A. cuprina*, *B. splendens* was not killed by even large concentrations of LSD-25.

Experiments were then performed to ascertain which, if any, of the deviations from normal brought about in *B. splendens* by LSD-25 were brought about only by LSD-25. The fish were treated with compounds belonging to two categories. The first category included substances active on the central nervous system such as diethyl ether, meperidine hydrochloride (Demerol), mescaline, and various barbiturates. The fish reacted to none of these in any manner suggesting a reaction to LSD-25, even when these compounds were applied in milligram rather than microgram amounts. The second category included compounds which are derivatives and isomers of LSD-25: *l*-LSD-25, *d*-isoLSD-25, LAE-32 (*d*-lysergic acid ethylamide), BOL-148 (2-bromoLSD-25), *d*-lysergic acid, ergonovine, ergotamine, and dihydroergotamine. These substances were tested over a range of concentrations from 0.2 μg/ml to 20 μg/ml. It was found that the peculiar syndrome defined by the first five criteria listed above was sufficient to differentiate LSD-25 from the other ergot drugs. In fact, LSD-25 could be differentiated from every other drug tried on the basis that it induced all three of the following reactions: (a) the trancelike state; (b) the snout-up-at-the-surface position; and (3) the kinking, including the comma-like appearance.

Despite the precise selectivity of the effects, related reactions are brought about by both LAE-32 and BOL-148. However, multiple observations over a two-hour period combined with special stress on the kinking criterion will permit even an untrained observer to differentiate a reaction to LSD-25 from reactions to LAE-32 and BOL-148.

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REPORTS OF SUMMER INVESTIGATORS

BERNHEIMER, ALAN W., College of Medicine, New York University, New York, N. Y. — A search was made among seed-bearing plants growing in the vicinity of Cold Spring Harbor for substances that would inhibit the enzymatic degradation of ribonucleic acid. Plant tissues, in most instances those of the flower or leaf, were extracted with water with the aid of a Waring Blendor. The aqueous extracts were tested for inhibition of pancreatic ribonuclease by examining their effect on the disappearance of acid-precipitable substrate (yeast ribonucleic acid). The results may be summarized in three categories. (A) Of a total of 49 extracts, 3 gave evidence of inhibiting the action of pancreatic ribonuclease. Two of these extracts, one derived from the leaves of bayberry *(Myrica carolinensis)* and the other from leaves of dogwood *(Cornus florida)*, were found to precipitate the gelatin used as a stabilizing agent in the test system, and they inhibited ribonuclease action apparently by co-precipitation of the enzyme. A third inhibitory extract was one derived from lilac *(Syringa vulgaris)* leaves. This extract was found to contain a heat-stable, dialyzable substance which inhibited the depolymerization of ribonucleic acid by pancreatic ribonuclease but not that brought about by ribonucleases derived from spleen and Neurospora. This inhibitor is under further study. (B) Forty of the 49 extracts showed little or no inhibition of pancreatic ribonuclease. (C) Six of the 49 extracts themselves depolymerized ribonucleic acid, namely, extracts derived from flowers of *Yucca filamentosa*, flowers and leaves of *Asclepias syriaca* (milkweed), flowers and seeds of Allium, flowers of Phlox, and flowers and leaves of *Lathyrus sp.* The distribution of ribonuclease in the plant kingdom has been little studied, and the plants named apparently constitute new sources of the enzyme. For further details, see an article published in the *Proceedings of the Society for Experimental Biology and Medicine* (**89**: 123, 1955).

KAPLAN, REINHARD W., AND KAPLAN, CHARLOTTE, Frankfurt am Main, Germany, and Columbia University New York, N. Y. — During the few weeks of our stay a study of the color sector *(s)* mutation process in Serratia was continued. Experiments were done to reveal the time after ultraviolet irradiation when the normal (r = red colonies) and the mutant cells (s = sectored, w = white colonies) start to divide. Suspensions of r cells were treated with ultraviolet rays, leaving 2×10^{-3} survivors, which included, besides the r type, about 16% s mutants, mainly induced, and 4% w cells, mostly of spontaneous origin. After plating on agar, the surfaces were respread after 1 to 10 hours' incubation to separate members of clones. When about 100 living cells were inoculated per plate the absolute number of colonies remained constant until the sixth hour; later respreading caused a strong increase owing to cell fission. The numbers of s and w colonies first

decreased slightly; then after 6 hours increase by cell fission began. When about 10^4 living cells were inoculated and after different periods the plates were washed with saline to determine the viable count, already after 6 hours an increase above the initial cell number was observed for all cell types. No difference in lag period was observed between the normal *r*, the "old" spontaneous *w*, and the "freshly" induced *s* mutants. Thus the long lag of freshly mutated cells in *E. coli* observed by Ryan *et al.* (1954) does not apply in the case of color mutations in this strain of Serratia.

The *w*, *r*, and *s* types increased differently, however, after this equal lag period; respectively, about 140, 30, and 6 times the initial numbers were produced by the tenth hour. The smaller increase of the *s* type and the larger increase of the *w* type may perhaps be caused not only by a slower growth rate of the *s* and a faster growth rate of the *w* cells but also by a turnover of the unstable "premutational" *s* state to the more stable *w* (and *r*) type. This is indicated by the formation of *w* and *r* sectors during the growth of the *s* colonies and by other earlier observations. It may be added that the observed divisions of the *s* cells excludes the possibility that the *s* colonies arise from mere clumps of *r* and *w* cells.

The percentages of *s* and *w* among the survivors decreased with time until respreading (*s* from 16% at 0 hours to 3% at 8 hours, *w* from 4% to 0.5% at 3¼ hours); after longer periods these percentages increased again. The total number, representing mainly the *r* type, remained constant until 6 hours. This "loss" of both *s* and *w* cells could be due to some selective killing of these cells by the respreading procedure — for example, through osmotic shock when saline was added. To test this, distilled water, nutrient solution, or a dry bent glass rod alone were used for respreading after 4 hours. Only small, insignificant losses were found with the dry rod; higher ones were found with H_2O and nutrient solution, and the highest with saline. So it seems that the *s* and *w* cells develop a sensitivity to respreading, particularly to resuspension in fluid, after ultraviolet irradiation, with maxima of damageability at the middle *(w)* or end *(s)* of the lag phase. Thus some caution is necessary in respreading. Further, the physiological change causing this sensitivity is not comparable with the one causing the delay of fresh mutants observed by Ryan *et al.*, since it applies to the "fresh" *s* mutants as well as the "old" *w* cells.

MARAMOROSCH, KARL, The Laboratories of the 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. These were (1) "Transmission of blueberry-stunt virus by *Cloanthanus magdalensis*" and (2) "Seedlings of *Solanum tuberosum* L. as indicator plants for potato leafroll virus."

SANDOW, ALEXANDER, Department of Biology, Washington Square College of Arts and Science, New York University, New York, N. Y. — This summer my work was devoted to completing a paper (with Arthur J. Kahn), "Effects of bromide, nitrate and iodide on responses of skeletal muscle." This paper was entered in the 1954 A. Cressy Morrison Prize Contest of the New York Academy of Sciences and was awarded an Honorable Mention. It is now in press with the *Annals of the New York Academy of Sciences.*

COURSE ON BACTERIAL VIRUSES

June 21–July 10, 1954

Instructor: MARK H. ADAMS, New York University.

Assistant: EVELYN WADE, New York University.

The tenth anniversary of the bacteriophage course is an appropriate occasion for a brief summary of the accomplishments of this phase of the summer activities of the Biological Laboratory. The course was founded in 1945 by M. Delbrück who was the instructor for the first two years. It was taught for seven years by M. H. Adams and for one year by A. H. Doermann. The course is designed to teach techniques and theory in the highly specialized field of bacteriophage research at a postgraduate level. The sustained interest in the course, which has resulted in capacity enrollment year after year, is full justification of the foresight and vision of Dr. Delbrück and the continued interest and support of Dr. Demerec.

During these ten years 146 students have completed the course, of whom 76 were research workers with the doctoral degree and 70 were graduate students or research technicians. Most of the graduate students have since obtained advanced degrees. At least 20 of the students have been from foreign countries, distributed as follows: Israel, 5; Denmark, 2; Iceland, 2; England, 2; Italy, 2; Germany, 2; and 1 each from Australia, Canada, France, Norway, and Switzerland. In addition, students have been attracted from most parts of the United States. Most of the phage course graduates are actively engaged in research in some branch of microbiology, many in virus research.

In the 1954 session the following 18 students were enrolled:

Robert E. Beardsley, Columbia University, New York, N.Y.
Lewis B. Bernstein, Kansas State College, Manhattan, Kan.
William A. Clark, Ph.D., American Type Culture Collection, Washington, D.C.
Robert S. Edgar, University of Rochester, Rochester, N.Y.
Gertrude C. Emery, New York University, New York, N.Y.
Maurice Green, Ph.D., Children's Hospital of Philadelphia, Philadelphia, Pa.
Sheldon B. Greer, Columbia University, New York, N.Y.
Fritz Kaudewitz, Ph.D., Max-Planck-Institut für Virusforschung, Tübingen, Germany.
Carl E. J. Kirchner, Kansas State College, Manhattan, Kan.
Aimlee D. Laderman, Columbia University, New York, N.Y.
Grace Leidy, Columbia University, New York, N.Y.
Hermann Moser, Ph.D., Zürich, Switzerland, and Carnegie Institution, Cold Spring Harbor, N.Y.

John I. Payne, University of Pennsylvania, Philadelphia, Pa.
Alexandra W. Phillips, University of Pennsylvania, Philadelphia, Pa.
Mary Louise Robbins, Ph.D., George Washington University Medical School. Washington, D.C.
Andrew J. Vargasko, Jr., George Washington University, Washington, D.C.
Elliot Volkin, Ph.D., Oak Ridge National Laboratory, Oak Ridge, Tenn.
Felix Wassermann, New York University, New York, N.Y.

In connection with the course a series of lectures was given, as follows:
G. Bertani—Lysogenicity.
M. H. Adams—Serological properties of phages.
A. D. Hershey—The properties of vegetative phages.
M. L. Robbins—Chemotherapy of viruses.
G. Leidy—Bacterial transformations.
E. Volkin—Chemical composition of phage.
S. Benzer—Studies on *R* mutations in phage T4.
M. H. Adams—The stability of phages.

It is appropriate to acknowledge the assistance which the bacteriophage course has received indirectly from the National Foundation for Infantile Paralysis. During the seven years that the course has been taught by Dr. Adams, his laboratory assistant has been paid by a National Foundation research grant, and much of the biological material used during the course has been prepared in connection with research conducted under the grant. Thus the Foundation has contributed greatly to the success of the phage course.

COURSE ON BACTERIAL GENETICS

July 14–August 3, 1954

Instructors: M. DEMEREC, V. BRYSON, and E. M. WITKIN, in collaboration with P. FITZGERALD, S. W. GLOVER, H. MOSER, and P. D. SKAAR.

Assistant: INGBRITT BLOMSTRAND.

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

Lewis B. Bernstein, Kansas State College, Manhattan, Kan.
S. W. Bowne, Jr., M.S., State College of Washington, Pullman, Wash.
Sydney Brenner, Ph.D., University of the Witwatersrand, Johannesburg, South Africa.
Robert Stuart Edgar, University of Rochester, Rochester, N.Y.
Daniel M. Eisler, Ph.D., Naval Biological Laboratory, Oakland, Calif.
Gertrude C. Emery, M.A., New York University, New York, N.Y.
Walther F. Goebel, Ph.D., The Rockefeller Institute for Medical Research, New York, N.Y.
Maurice Green, Ph.D., The Children's Hospital of Philadelphia, Philadelphia, Pa.
John W. Greenawalt, Western Reserve University, Cleveland, Ohio.
F. Kaudewitz, Ph.D., Max-Planck-Institut für Virusforschung, Tübingen, Germany.
Aimlee D. Laderman, New York, N.Y.
John Edward Matheson, Syracuse University, Syracuse, N.Y.
Clarence L. E. Monroe, Ph.D., Morgan State College, Baltimore, Md.
William N. Pearson, Ph.D., Vanderbilt University, Nashville, Tenn.
Wilfred Yaphe, Ph.D., Martime Regional Laboratory, National Research Council, Halifax, Nova Scotia, Canada.
Leonard N. Zimmerman, Ph.D., The Pennsylvania State University, State College, Pa.

Auditors:

Henrietta G. Kalicki, Manhattanville College of the Sacred Heart, New York, N.Y.
Alexandra Wharton Phillips, University of Pennsylvania, Philadelphia, Pa.

The following lectures and seminars were given in connection with the course by instructors, students, and research workers at the laboratories:

V. Bryson—Origin of resistance in microorganisms.
J. Gots—Systematic analysis of auxotrophy.
E. M. Witkin—Delayed appearance of mutations.
F. Kaudewitz—Lethals induced in *Amoeba proteus* by P^{32}.
P. D. Skaar—Genetic recombination in *Escherichia coli*.
S. Brenner—Theories of Thinshelwood on inheritance.
H. Moser—Kinetic studies of selection in the chemostat.

NATURE STUDY COURSE

June 28–July 30, 1954

Instructors: PAULINE JAMES, Department of Biology, Pan American College, Edinburg, Texas.
JOHN I. GREEN, Department of Conservation, Cornell University, Ithaca, New York.

Assistant: DONNA GRANICK, New York, New York.

Junior Assistants: LEE GRANICK, New York, New York.
LOUISE SHEMIN, New York, New York.

The five-week Nature Study Course was in general conducted in a manner similar to that of previous years although the greatly increased enrollment made certain changes inevitable. Emphasis remained on field natural history, and every effort was made to acquaint all students with as many varied forms of nature as possible and to create in each student a basic understanding and appreciation of man's relation to his environment and his complete dependence upon it.

The students were divided into five major age groups as usual, but with two instructors it was then possible to subdivide each class into smaller groups for field trips and project work. This was especially useful in dealing with the Senior and Advanced students, who were allowed to work in Wawepex Laboratory between 8:00 a.m. and 12:00 m. on Fridays as well as at their regular Monday and Wednesday classes.

In addition to field trips to the many varied habitats within easy access from the headquarters at Wawepex Laboratory, trips were taken to the New York State Fish Hatchery at Cold Spring Harbor, the Roosevelt Bird Sanctuary at Oyster Bay, and the Tackapausha Nature Preserve near the south shore of Long Island. The nesting colonies of shore birds at Jones Beach were also visited, and large numbers of nesting common terns *(Sterna hirundo)* and black skimmers *(Rynchops nigra)* were observed. On days when weather conditions were unsuitable for field trips, special nature films and slides were shown and laboratory projects were continued.

There was an usually large number of students in the Senior and Advanced classes this year. Some of them worked together on group projects but many chose to work on individual problems. Two collected and observed caddis fly larvae, taking particular note of preferences exhibited in the choice of materials used in building cases. One group made a study of the identification and occurrence of trees in the area, while others worked on soil analysis and the study of locally collected fossils. One group of the Seniors collected specimens and set up a fresh-water aquarium of local plants and animals. One project of special interest to some of the older boys was that

of learning to prepare museum study skins of birds and mammals. Without previous experience, the boys in this group did unusually good work and had an excellent exhibit at the Open House. It should be noted, however, that all the birds so prepared were provided from highway casualties, and so forth — particularly from Jones Beach, where major highways bisect the nesting areas. None were "collected" for preservation purposes. Likewise, many of the mammals were provided from discarded Biological Laboratory experimental specimens.

The large and always enthusiastic Intermediate class participated in many activities. One of special note was the collection of a number of red-backed salamanders *(Plethodon cinereus)* and two-lined salamanders *(Eurycea bislineata)*, which were established in terraria and observed for the duration of the course, then released in their native habitat.

Younger members of the course took part in various field activities, taking particular interest in marine life and insect life. Some were able to collect and study certain vertebrates, including one young cottontail *(Sylvilagus floridanus mallurus)*, which was cared for and observed until the end of the course when it was released on the Demerecs' lawn.

The Nature Study Course closed on July 30, 1954, with an open house in Wawepex Laboratory for the parents and friends of the students. Visitors were shown various exhibits and demonstrations prepared by members of the course during the five-week period. The group then adjourned to the lawn, where refreshments were served.

The following students were enrolled in the course:

Aellen, Rosemarie
Ames, Robert Charles
Benzer, Barbara
Bernheimer, Alan
Bolger, Jeffrey
Bonime, Karen
Brooks, Gregory
Bruen, Marian
Bruen, Nicky
Brunauer, Gail
Bunce, John Allan
Bunker, Sheffield
Burnett, Jean P.
Burtch, Florence
Cardile, Francis
Carhart, Susan E.
Cherici, Pier R.
Cleaveland, Ed
Colyer, Barry
Dewey, John
Dewey, Michael
Elder, Robert
Elder, Sarah
Emmet, Robert
Flagg, Mrs. W. Allston
Fochtman, Grace
Fraser, Barbara
Galehouse, Shelley
Gerbino, Charis
Gerbino, Jason
Gibney, Allan
Gibson, William
Gottlieb, Gail
Graesser, Gerald M.

Granick, Donna
Granick, Lee
Grieser, Katherine
Guille, Jane
Guille, Jimmy
Harris, Jean
Henning, Frances
Henning, Helene
Hoguet, Constance
Hoguet, George R.
Howe, Jonathan
Irick, John
Jayne, Richard
Jazombek, Bonnie
Johnson, David Starr
Kelly, John Linsey
Kernan, Anita
Koenig, Harold Otto
Koenig, Julian
Lane, Susan
Leonardi, Carl
Ley, Richard
Lutjen, Ann Louise
Lutjen, George P.
MacCoun, James
MacKay, Robert
Maramorosch, Lydia
McClintock, Cynthia
Meirs, John
Melzig, Perry
Melzig, Ricky
Merton, Andy
Minarik, Brooke Ellen
Montgomery, David
Mulligan, William
Niven, Billy
Norins, Wendy
Norton, Billy
Noyes, Susan
O'Connor, David
Olds, Robly
Olmsted, Robert
Pierce, Josiah
Pivnick, Carol
Pratt, David
Prytz, John McDonald
Regan, Gordon B.
Rehwinkel, Charles D.
Reisch, Linda Carol
Riccardi, Barbara
Richmond, Dean
Ridley, John
Rippere, Kenneth
Roberts, Gordon
Robertson, Anne
Robertson, John
Ross, Charles
Ross, Joe
Rusch, Cecile
Samoiloff, Martin
Sandow, Gregory
Sandow, Lisa
Schlaikjer, Dana
Schneider, Ann
Schneider, Franz, Jr.
Scrivener, Rodney
Seem, Jonathan
Shemin, Louise
Smith, Randall D.
Smoot, Sarah
Stroupe, Susan
Takami, Jonathan
Titus, Jonathan
Tucker, Louise
Tucker, Thomas
Turner, Mary
Walker, Elaine
Walker, Louise
Watt, Heather
Webber, Kathy
Wells, Julia
Wheeler, David
Wheeler, Sally
Ziegler, 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 Applications of Tracer Elements, xii + 222 pp.
Vol. XIV (1949) Amino Acids and Proteins, xii + 217 pp.
Vol. XV (1950) Origin and Evolution of Man, xii + 425 pp.
Vol. XVI (1951) Genes and Mutations, xvi + 521 pp.
Vol. XVII (1952) The Neuron, xiv + 323 pp.
Vol. XVIII (1953) Viruses, xvi + 301 pp.
Vol. XIX (1954) The Mammalian Fetus: physiological aspects of development, xii + 225 pp.

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*Out of print.

LABORATORY PERSONNEL

* Abramson, Alexandra—Technical Assistant
Abramson, Harold A.—Research Psychiatrist
* Abramson, Harold A., Jr.—Technical Assistant
* Adams, Mark H.—Bacteriologist, Instructor
† Auriana, Louis—Research Assistant
Barrett, Audrey E.—Research Assistant
Bennett, Esther F.—Technical Assistant
* Bernheimer, Alan W.—Summer Guest
Bompiani, Gioietta—Research Assistant
* Bruening, Betty—Research Assistant
* Bryson, Constance—Technical Assistant
Bryson, Vernon—Geneticist
Burtch, Ethel—Stenographer
* Caspari, E. W.—Summer Guest
* Connell, Charles—Typist
Cosillo, Gloria—Research Assistant
* Cutalo, Beatrice—Clerical Assistant
Demerec, M.—Director
Demerec, Zlata—Research Assistant
Dyson-Hudson, Vera Rada—Research Associate
† Elliott, Dorothy W.—Technical Assistant
Englesberg, Ellis—Bacteriologist
* Evans, L. Thomas—Naturalist
Farrington, Margaret—Technical Assistant
Franzese, Eleanor—Business Manager
Fricke, Hugo—Associate
Furguiele, Anthony—Maintenance Man
Gardner, Henry—Technical Assistant
Geronimus, Lippman H.—Bacterial Physiologist
* Granick, S.—Summer Guest
* Green, John—I. Nature Study Course Instructor
Hadden, Joanna—Research Assistant
* Haggis, Alex J.—Biologist
† Haworth, Barbara J.—Research Assistant
† Heplar, Joseph—Bacteriologist
Hershey, Harriet D.—Research Assistant
† Ingraham, Laura—Research Assistant
Israel, Robert—Research Assistant
* James, Ina Pauline—Nature Study Course Instructor
* Kaplan, Charlotte—Summer Guest
* Kaplan, Reinhard W.—Summer Guest
Kennard, John F.—Gardener
King, James C.—Geneticist
Kornetsky, Conan—Psychologist
* Lo Monaco, Mario—Research Assistant

† McCann, John—Maintenance Man
Maciura, Stephen—Carpenter
Madden, Carol V.—Research Assistant
* Maramorosch, Karl—Summer Guest
Matson, Joan A.—Stenographer
† Merlino, Aldo—Maintenance Man
† Merlino, Joseph—Maintenance Man
† Murdock, Rosamond—Research Assistant
† Ness, Charlotte—Stenographer
Neviackas, Gwendolyn—Stenographer
Orszag, Myrna—Research Assistant
Puglisi, Vincent A.—Research Assistant
* Ray, Eva—Research Assistant
Reddy, William—Maintenance Man
* Sandow, Alexander—Summer Guest
† Scheffler, Gudrun—Business Manager
† Schnur, Gloria L.—Stenographer
* Shemin, David—Summer Guest
Skaar, Palmer D.—Geneticist
Sklarofsky, Bernard—Psychobiologist
* Stuard, Barbara—Research Assistant
Thurston, Robert K.—Superintendent of Buildings and Grounds
Treanor, Ellen T.—Maid
† Tuttle, Susan—Research Assistant
† Vander Schalie, Margie—Research Assistant
† Van Houten, Ralph—Technical Helper
Wallace, Bruce—Geneticist; Assistant Director
Warren, Katherine Brehme—Executive Editor of Symposia

* *Summer or temporary.*

† *Resigned during the year.*

REPORT OF THE SECRETARY

The 31st Annual Meeting of the Association was held in the Lecture Hall at Cold Spring Harbor on June 22, 1954, with nineteen members present and President Ames presiding. The Secretary reviewed the chief acts of the Association during the year, and his report was voted approved. The report of the Treasurer also was approved. A resolution was presented and unanimously voted to amend the By-Laws with respect to the time of meetings of the Board of Directors. It was previously specified that these meetings be held in the months of January and July; the new amendment specifies only that they be held twice in each year. The following members were named by the Nominating Committee and elected or re-elected to the Board of Directors to serve until 1958: Amyas Ames, George W. Corner, Th. Dobzhansky, Mrs. Maitland A. Edey, Rollin D. Hotchkiss, Ernst Mayr, and Walter H. Page. Dr. Demerec, speaking as Director of the Laboratory, reported on the Symposium on "The Mammalian Fetus" held in June, the summer courses on bacterial viruses and bacterial genetics, the summer research program, and the nature study course for children. He discussed chiefly the year-round research program of the Laboratory, and explained the need for an expendable research fund to assure the salaries of key scientists for a period of years. Dr. Demerec referred with pleasure to the fact that during the past year the income of the Association had exceeded expenditures, as shown by the Treasurer's report — a fact that made it possible to increase the reserve fund for research. He concluded by expressing appreciation and gratitude to the two lecturers who delivered talks of general interest during the year, Dr. Corner and Dr. Murphy; to the teachers who contributed their services in the summer courses, Drs. Adams, Bryson, and Witkin; to the foundations and agencies that made grants in support of research and of the Symposium; to the many friends who during the year contributed as members of the Association; and to the Wawepex Society for its generous gift.

The 69th meeting of the Board of Directors was held after the Annual Meeting on June 22, 1954, in the Lecture Hall at Cold Spring Harbor. The Executive Committee was re-elected to serve for the following year, the members being: Amyas Ames, Mrs. George S. Franklin, E. C. MacDowell, Grinnell Morris, William B. Nichols, Arthur W. Page, and Mrs. Walter H. Page. Transactions of the Finance Committee, consisting of the sale and purchase of stocks, were reported and approved. Possible ways and means were discussed of building up an expendable research fund.

A meeting of the Executive Committee was held at the home of President Amyas Ames on November 14, 1954. By unanimous vote, the office of Assistant Director of the Laboratory was created, and Dr. Bruce Wallace was appointed to this position, beginning December 1, 1954. By unanimous vote

the Treasurer was empowered to open a Laboratory account in the First Suffolk National Bank of Huntington.

The 70th meeting of the Board of Directors was held January 30, 1955 in the Lecture Hall at Cold Spring Harbor. The minutes of the last meeting of the Board and the November meeting of the Executive Committee were reviewed and approved. In a report concerning the work of the Laboratory, Dr. Demerec discussed the significance of Dr. Bruce Wallace's research with irradiated Drosophila as it relates to the timely and controversial question of the effect of atomic radiation on human populations. He then reported briefly on the progress of other research groups at the Laboratory, on plans for the 1955 Symposium, and on the proposed modernization and improvement of facilities for summer research with bacteria and bacterial viruses. In connection with the summer program, he pointed out the need for a larger scholarship fund. Dr. Demerec presented the budget for the coming year, which was voted approved. President Ames reported plans for the use of the Lecture Hall, and urged that the need for a large sustaining fund be kept constantly in mind by members of the Board.

E. C. MacDowell, *Secretary*
Long Island Biological Association

REPORT OF THE TREASURER

AUDIT CERTIFICATE

April 30, 1955

MAIN AND COMPANY

CERTIFIED PUBLIC ACCOUNTANTS

NEW YORK, N. Y.

Long Island Biological Association,
Colp 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, 1955. 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 Bioligical Association at April 30, 1955 and the results of its operations for the year ended on that date in conformity with generally accepted accounting principles applied on a basis consistent with that of the preceding year.

MAIN AND COMPANY

New York, N. Y.
June 22, 1955

BALANCE SHEET

April 30, 1955

ASSETS

General and Endowment Fund				
Cash:				
In banks		$39,277.62		
On hand		100.00	$39,377.62	
Investments (market value $28,809.76)			26,980.14	
Accounts receivable:				
On special grants and contracts		$26,647.78		
Other	$5,325.91			
Less: Reserve for uncollectible accounts	985.00	4,340.91	30,988.69	
Inventory of books, at cost		$38,985.17		
Less: Reserve for obsolescence		5,854.00	33,131.17	
Deferred expenses			5,707.70	
Land, buildings and equipment			296,676.54	$432,861.86
Special Funds				
Cash in bank			$ 1,143.82	
Investments (market value $15,728.86)			15,710.00	16,853.82
Total				$449,715.68

NOTE: Subject to comments of report.

LIABILITIES AND NET WORTH

General and Endowment Fund			
Liabilities:			
Accounts payable		$ 13,790.92	
Accrued payroll		758.96	
Special grants and contracts		2,000.64	
Total liabilities		$ 16,550.52	
Deferred income		16,000.00	
Reserve for Scientific Research		17,405.27	
Endowment Fund:			
Dr. William J. Matheson Bequest		20,000.00	
Net worth		362,906.07	$432,861.86
Special Funds			
Blackford Memorial Fund: Principal		$ 5,000.00	
Charles Benedict Davenport Memorial Fund:			
Principal	$4,934.75		
Unexpended income	908.99	5,843.74	
Charles Benedict Davenport, Junior, Fund:			
Principal		1,037.12	
Temple Prime Scholarship Fund:			
Principal	$2,500.00		
Unexpended income	65.10	2,565.10	
Dorothy Frances Rice Fund:			
Principal	$2,284.60		
Unexpended income	123.26	2,407.86	16,853.82
Total			$449,715.68

LAND, BUILDINGS AND EQUIPMENT

April 30, 1955

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. deForest 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	101,265.00
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
Total			$296,676.54

* Built on land leased from Wawepex Society.

STATEMENT OF NET WORTH
For the Year Ended April 30, 1955

Balance, May 1, 1954	$355,215.70
Add:	
Adjustment made as at May 1, 1954 to reflect travel expenses paid in advance to participants for June 1954 symposium	3,500.93
Adjustment to record estimated Accounts Receivable at May 1, 1954 from symposia book sales	2,400.00
Excess of income over expense for the year ended April 30, 1955	1,789.44
Balance, April 30, 1955	$362,906.07

STATEMENT OF INCOME AND EXPENSE
For the Year Ended April 30, 1955

Income:			
Contributions:			
Dues and contributions		$ 8,292.83	
Grants for Annual Symposia:			
Association for Aid to Crippled Children	$5,000.00		
Carnegie Corporation	6,000.00		
National Science Foundation	6,500.00	17,500.00	
Wawepex Society		1,900.00	
John D. Jones Scholarship		600.00	$28,292.83
Symposia:			
Book sales		$14,062.83	
Registration fees		122.00	14,184.83
Dining hall			6,569.14
Rooms and apartments			12,200.25
Research fees			18,167.89
Interest and dividends on investments			962.29
Summer course tuition			2.272.03
Nature study course			2,337.52
Beach permits			925.00
Annual distribution from Walter B. James Fund			165.00
Miscellaneous income			236.00
Total income			$86,312.78

Expense:		
Symposia:		
Cost of books sold and publication expense	$ 8,885.97	
Expense of participants and lecturers (Note "A")	10,175.17	$19,061.14
Dining hall		8,938.38
Rooms and apartments		4,167.62
Research expenses		2,160.44
Summer course expense		1,228.20
Nature study course expense		1,424.09
Expense of patroling beach		732.50
Distribution of John D. Jones Scholarship		755.00
Buildings and grounds maintenance:		
Salaries	$11,019.16	
Materials and supplies	6,220.13	
Heat, light and water	3,632.55	20,871.84
General and administrative:		
Salaries	$ 5,792.21	
Equipment	4,620.00	
Insurance	906.99	
Printing and stationery	1,220.71	
Telephone, telegraph and postage	291.18	
Other	1,114.04	13,945.13
Provision for reserve for scientific research		8,000.00
Provision for obsolescence of inventory of books		2,254.00
Provision for uncollectible accounts receivable		985.00
Total expense		$84,523.34
Excess of income over expense		$1,789.44

NOTE "A": Above amount does not include portion of dining hall and rooms and apartment expense applicable to invited participants nor does it include the applicable portion of other expenses.

STATEMENT OF GRANTS AND CONTRACTS FOR SPECIAL RESEARCH
For the Year Ended April 30, 1955

From Whom Received	BALANCE, MAY 1, 1954		TRANSACTIONS MAY 1, 1954 TO APRIL 30, 1955			BALANCE, APRIL 30, 1955	
	Due to Association (Accounts Receivable)	Unexpended Balance of Grant	Amounts Received	Expenditures Charged Against Grant or Contract	Income to Association Charged Against Grant or Contract	Due to Association (Accounts Receivable)	Unexpended Balance of Grant
The Jane Coffin Childs Memorial Fund for Medical Research		$ 405.27			$ 405.27*		
Geschickter Fund		1,672.51	$ 22,925.00	$ 20,679.01	3,556.84		$ 361.66
Josiah Macy, Jr. Foundation	$ 83.72		880.00	31.03	180.00		585.25
National Tuberculosis Association		1,303.39	5,314.63	5,152.12	412.17		1,053.73
Rockefeller Foundation			5,000.00	5,121.30		$ 121.30	
United States Atomic Energy Commission		14,743.36	16,049.00	30,440.31	3,278.99	2,926.94	
United States Department of the Army:							
Chemical Corps:							
Studies on Bacteria	8,463.18		21,437.52	21,808.45	2,901.76	11,735.87	
Studies on Mouse Leukemia	63.22		63.22				
Office of Surgeon General	12,412.89		26,502.05	14,645.55	6,362.93	6,919.32	
United States Department of the Navy:							
Office of Naval Research	3,249.63		8,243.53	8,674.17	900.00	4,580.27	
United States Public Health Service				364.08		364.08	
	$24,272.64	$18,124.53	$106,414.95	$106,916.02	$17,997.96	$26,647.78	$2,000.64

* Transfer of unexpended balance of grant to Reserve for Scientific Research.

NOTE: Subject to comments of report.

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Laboratory Director: M. DEMEREC
Assistant Director: BRUCE WALLACE

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To serve until 1958

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ERNST MAYR	Harvard University
MRS. WALTER H. PAGE	Cold Spring Harbor, N. Y.

To serve until 1957

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M. DEMEREC	Carnegie Institution
NEVIL FORD	Huntington, N. Y.
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ROBERT CUSHMAN MURPHY	Setauket, N. Y.
JOHN K. ROOSEVELT	Oyster Bay, N. Y.

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WILLIAM B. NICHOLS	Syosset, N. Y.
MRS. ALEXANDER M. WHITE, JR.	Oyster Bay, N. Y.
B. H. WILLIER	Johns Hopkins University

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Ross G. HARRISON ..... Yale University

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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
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 1890-17
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
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Mrs. Elisha Walker, Jr.
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William J. Wardall
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Ethelbert Warfield
Bradford Warner
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Armitage Watkins
Gordon J. Watt
Wawepex Society
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Mrs. Percy S. Weeks
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Alexander M. White
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Mrs. John C. White, Jr.
C. W. Wickersham, Jr.
Douglas Williams
Mrs. Douglas Williams
B. H. Willier
William W. Willock, Jr.
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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.