ANNUAL REPORT

of

THE BIOLOGICAL LABORATORY

1953-1954

LONG ISLAND BIOLOGICAL ASSOCIATION

COLD SPRING HARBOR

LONG ISLAND, NEW YORK

LONG ISLAND BIOLOGICAL ASSOCIATION

Incorporated 1924

ANNUAL REPORT

of

THE BIOLOGICAL LABORATORY

Founded 1890

Sixty-Fourth Year

1953 - 1954

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

TABLE OF CONTENTS

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JAMES, W. B. 1902-17; 1924-27
JENNINGS, H. S. 1924-27
JENNINGS, WALTER 1906-17; 1924-33
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JONES, J. D. 1890-95
JONES, O. L. 1890-1913
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OSTERHOUT, W. J. V. 1927-41
OVERTON, FRANK 1924
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PARSHLEY, H. M. 1924-33
PEABODY, JULIAN 1911-17
PERKINS, A. C. 1890-92
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PRATT, H. I. 1929-30
PRIME, CORNELIA 1909-17
RAYMOND, J. H. 1890-1900
RUMSEY, MARY H. 1924
SWINGLE, W. W. 1924-44
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
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STOCKARD, C. R. 1924-39
STRATFORD, WILLIAM 1890-17
STRAUBENMULLER, GUSTAV 1911-17
STRAUSS, ALBERT 1914-17
STUTZER, HERMAN 1911-23
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
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OUR LABORATORY IN THE WORLD TODAY

The present research program at our Laboratory might aptly be described by the title "Experimental Evolution in Its Impact on Modern Society." Although the work is scientifically technical and is in the field of pure research, the discoveries made by members of the staff have a considerable bearing on the problems that face our generation.

One group of our scientists, working for the Atomic Energy Commission, is investigating radiation—similar to that from atomic explosions—in terms of its effect on the offspring of exposed individuals. Using fruit flies, they have been able to observe precisely what changes take place in the hereditary characteristics and the vigor of populations that are continuously exposed to different intensities of radiation for about a hundred and fifty generations. These studies have indicated that, although harmful effects show up in certain individuals, a population of several thousand may become adapted within a number of generations to the presence of radiation in its environment, and be on a par, as far as vigor is concerned, with a population that has not been exposed to radiation.

A second group of our scientists is studying the question of how insect populations acquire resistance to insecticides. Why do malaria-transmitting mosquitoes, for instance, or the houseflies that are such a nuisance and health menace, or the scale insects that damage citrus fruit trees, become resistant to DDT or to some other insecticide which at first was effective in combating them?

Still a third group is concerned with the changes in bacterial populations that make them able to resist various antibiotics, or survive at abnormal temperatures, or withstand exposure to X-rays or ultraviolet rays.

It is beginning to appear that there is a very close relationship between these factors observed in our laboratories, which enable populations of insects or bacteria to adjust to unfavorable environmental conditions, and the factors that have brought about the evolution of life from the lower to the higher organisms. The further insight resulting from this research should make us better prepared to understand some of the problems that arise in our present-day world.

Another activity of our Laboratory, the international conference known as the Cold Spring Harbor Symposia on Quantitative Biology, also has far-reaching implications for the understanding and solution of some modern problems. At this year's Symposium, held June 7th to 14th, specialists in anatomy, physiology, genetics, and clinical medicine came together to discuss the results of their research on the development of the mammalian—and particularly the human—embryo. Such a conference inevitably led to greater knowledge of human physical deformities, ranging from the trivial to the serious.

Since about one or two per cent of live-born babies have serious congenital abnormalities, the Association for the Aid of Crippled Children was interested in this conference, and provided support in the form of a grant. Additional funds were also contributed by the Carnegie Corporation and by the National Science Foundation, making it possible, in all, to cover the travel expenses of twenty leading specialists from European countries, who participated in discussions with about a hundred scientists from the United States and Canada at our Symposium.

So it is that our Laboratory has become famous among scientists the world over. Out of such meetings between intelligent men, new thought, new ideas, and new inspiration are generated. Certainly our Laboratory could not have a more vital function!

AMYAS AMES, *President*
Long Island Biological Association

REPORT OF THE DIRECTOR

This year's main developments were not so spectacular as those reported last year, when our new lecture hall became available for use and the purchase of property along Bungtown Road was consummated. Significant achievements were scored, however, on all fronts of our activities. We had good success in our research program, the Symposium maintained the high standard of previous conferences, the summer activities were expanded, and our contacts with members of the Association and neighbors were strengthened. I shall briefly review here these various facets of the Laboratory's affairs.

Research

In the classical concept of genetics, the genes were regarded as independent entities, which in the processes of evolution were selected or eliminated on their own merits. We now realize that the heredity of an individual represents an integrated system, and that in order to understand evolution we must study the structure, function, and origin of this integration in various organisms. Dr. Wallace's research on the effects of radiation on the genetic structure of populations of the common fruit fly (*Drosophila melanogaster*) has opened up an approach for such studies. The data he has obtained during the past several years have made it increasingly obvious that the simple models previously used by population geneticists are inadequate to explain the experimental results obtained in irradiation of populations. This inadequacy is most apparent in the lack of correlation between frequencies of demonstrably "deleterious" genes found within populations and the effects these genes have in individuals carrying them in random combinations. The situation has called for an investigation of a more general problem—the organization of the gene pools of populations.

Wallace's most recent study of gene-pool structure was based on the interactions of genes of several gene pools. The results show quite clearly that developmental rate—the character studied—is affected to a much greater extent by disruption of the intrachromosomal, functional organization of chromosomes than by the increased concealment of recessive genes afforded by interpopulation matings.

Studying the origin of resistance to insecticides, Dr. King has found during the past two years that two lines of *Drosophila melanogaster*, treated for twenty-three generations with a dose of DDT sufficient to kill fifty per cent of the population and then propagated by breeding the survivors, responded by developing a tolerance for the insecticide. It now takes between three and four times as much poison to kill fifty per cent of the resistant flies as it takes to kill fifty per cent of the control flies. Other lines from the same stock, subjected to doses killing between ninety and ninety-nine per cent of the flies treated, have not developed so great a tolerance. Thus the fifty per cent level of selective intensity produces resistance more effectively than higher levels. Still other lines, stemming from a different stock, have devel-

oped no measurable resistance—showing that different stocks respond differently to the same type of selection. Crosses between the most resistant strains, and between them and the control, indicate that resistance is a character produced by the accumulation of many interacting genetic factors—a polygenic system. No single genes with large effects toward resistance have been found. Information of this sort may well be of practical value in solving the problem of how to control insect pests and at the same time minimize the likelihood of producing high resistance in wild populations.

The group using bacteria in their studies included Drs. Bryson, Szybalski, Skaar, and Heplar, and, until October 1953, Dr. Geronimus. They have been trying to learn more about the origin of new types of microorganisms, and what factors influence the growth and establishment of new strains in comparison with parental types. Their work has been centered in problems of microbial evolution, with the emphasis on mutation and selection.

For several years Dr. Abramson has been carrying on an extensive research program at the Mount Sinai Hospital in New York City, in which he is studying the effects of LSD25 (lysergic acid diethylamide) on human behavior. This compound has a very striking effect on the nervous system, and when administered in extremely small amounts can produce in normal people states that resemble schizophrenia. Last year Abramson was in a position to expand this program, and since October 1953 he has been conducting collateral studies of the biological effects of LSD25 on living cells at our Laboratory, with the collaboration of Dr. Geronimus, Dr. Kornetsky, and Mrs. L. J. Ingraham.

A major portion of the summer research in 1953 dealt with studies of microorganisms. S. E. Luria and E. S. Lennox of the University of Illinois, and J. D. Mandell of the California Institute of Technology, worked with bacterial viruses. Luria carried out a chromatographic analysis of the deoxyribonucleic acid synthesized by *Escherichia coli* after infection with bacteriophage T2 in the presence of proflavine. Lennox made a preliminary study of a small bacteriophage which grows on several strains of *E. coli* and on *Shigella dysenteriae*, and also continued with investigations on the protection of *E. coli* by visible light from subsequent ultraviolet irradation. Mandel investigated the inactivation of bacteriophage T4*r* by specific serum in distilled water. Max Delbrück and A. Keynan, of the California Institute of Technology, studied phototropic responses in the unicellular, multinucleate sporangiophores of a mold belonging to a Phycomyces species.

Studies in the field and in the laboratory were carried on by Mary B. Delbrück of Pasadena, California, and by A. R. Messina of the American Museum of Natural History. Mrs. Delbrück collected, classified, installed in artificial nests, and studied the habits of ten species of ants; and Miss Messina investigated the bottom sediments in Cold Spring Harbor, Oyster Bay, and Long Island Sound for a study of the living foraminifera that grow there.

Continuing work begun several years ago, A. R. Goldfarb of the Chicago Medical School and H. A. Abramson of New York City studied by means

of paper chromatography a special fraction of ragweed pollen which is potent in producing hay fever and asthma. Abramson also, in cooperation with M. E. Jarvik, carried on an analysis of material accumulated in studies of LSD made at the Mount Sinai Hospital in New York.

A number of other investigators spent their time analyzing data accumulated during the winter months and preparing manuscripts for publication.

Symposia

Because the period covered by the Annual Report has been changed from the calendar year to the year beginning July 1, it is necessary this year to report on two Symposia, one held in June 1953 and the other in June 1954.

The eighteenth Cold Spring Harbor Symposium met from June 5th to 11th, 1953. For the first time the new Lecture Hall, erected with funds provided by the Carnegie Corporation, was used for the meeting. It accommodates about 250 persons without excessive crowding. The fine acoustics, pleasant atmosphere even in hot weather, and ample space gave the participants a degree of comfort never before available at our Symposia. This was fully appreciated by those attending the meeting, and many favorable comments were made about the new building.

Two hundred and seventy-two scientists from many parts of the United States and several countries overseas participated in discussions on the subject of "Viruses." Biologists, biophysicists, biochemists, and pathologists reported and discussed the results of their experiments with bacterial, plant, and animal viruses. For more than ten years scientists had been working out techniques for studying the multiplication, heredity, and chemistry of the viruses that attack bacteria. And in the two previous years a good start had been made on adapting similar methods to investigation of the viruses that attack animal cells and cause many diseases still uncontrolled by medical science, such as poliomyelitis, influenza, and the common cold. Much of this pioneer work was reviewed at the meetings. Although by far the greatest portion of the conference was devoted to the basic discoveries of research scientists, since these are the foundation for applied and clinical research, the questions of transmission, virulence, immunity, and control—all important in the medical field—were treated often in the discussions.

Cooperating with the Laboratory in the organization of this meeting was the National Foundation for Infantile Paralysis, which supports an extensive virus research program in the effort to find a means of controlling polio. Since the Foundation invited a large number of key participants, it was possible for a good proportion of foreign visitors to be present. Participants from abroad totaled seventeen, including four from Canada, six from England and Scotland, three from France, two from Germany, one from Australia, and one from Switzerland. The grant of the Carnegie Corporation was used, with their approval, to speed up publication of the Symposium volume.

The program of the "Viruses" Symposium was organized by Dr. Max

Delbrück of the California Institute of Technology, in consultation with Dr. F. L. Horsfall, Jr., of the Rockefeller Institute for Medical Research, and Dr. H. M. Weaver of the National Foundation for Infantile Paralysis. Full credit for an unusually successful program goes to that group, and particularly to Dr. Delbrück, who devoted a great deal of thought and effort to its planning.

The nineteenth Cold Spring Harbor Symposium was held from June 7th to 14th, 1954. The primary aim of this conference was to evaluate the present knowledge about the morphological, physiological, and biochemical factors that affect the development of the embryo, especially the human embryo. The meeting began with a consideration of early embryonic development, then took up a variety of integrated physiological activities necessary for the development of the fetus, and finally discussed the metabolic characteristics of the fetus as a whole and of its different parts. All together, twenty lectures were given in thirteen sessions. The last session was devoted to summaries and evaluations, presented from the viewpoints of a biochemist, a physiologist, and a clinician.

Because Europe is the research center for problems of the kind discussed at the meeting, it was important to have a good proportion of European scientists in attendance. This was made possible because the Laboratory received, in addition to the long-term grant from the Carnegie Corporation of New York, special grants for the expenses of this Symposium from the National Science Foundation and from the Association for the Aid of Crippled Children. Twenty specialists from Belgium, Finland, France, Great Britain, Ireland, Norway, and Sweden came to this country to take part in the meeting, together with more than a hundred participants from the United States and Canada.

The program of the 1954 Symposium was organized by a committee consisting of Drs. D. H. Barron and J. S. Nicholas of Yale University, Dr. L. B. Flexner of the University of Pennsylvania, Dr. C. A. Smith of Harvard University, and Dr. S. R. M. Reynolds of the Carnegie Institution, who acted as chairman and whose energy and foresight were primarily responsible for the fine success of the meeting.

Teaching

The Nature Study Course was again given by Dr. Pauline James of Pan American College, Edinburg, Texas. She was assisted by Mrs. Melvin R. Wood, of Kingsville, Texas, and Mr. Bradford Warner, Jr., of New York. This course is designed to stimulate interest in nature among the young people of the community, by quickening their observation of the many plants and animals around them, by teaching them how to find the answers to questions raised by their observations, and by helping them realize that careful and accurate study of the smaller incidents we can all observe contributes greatly toward expanding our knowledge of natural phenomena. The course, divided into five sections, was attended by 102 young people.

For the ninth consecutive year a three-week course was offered in techniques and problems of research with bacterial viruses. As in four of the

previous summers, it was taught by Professor Mark Adams of New York University College of Medicine. Fourteen students were enrolled.

The course in bacterial genetics was given for the fourth year, and was conducted by Evelyn Witkin, V. Bryson, and M. Demerec in collaboration with Jean Hemmerly, E. L. Labrum, W. Szybalski, and N. Zinder. This course emphasizes the newer methods used in the study of heredity in bacteria, and some of the recent studies in this field. There was an enrollment of eleven students.

Scholarships

The John D. Jones Scholarship was divided among the following summer investigators and students: Howard Ko, Purdue University; David R. Krieg, University of Rochester; Dr. Dan L. Lindsley, Princeton University; Robert Rowen, New York University; and Murray Seldeen, New York University.

Lectures

A regular schedule of scientific lectures was arranged during the summer of 1953, in cooperation with the Department of Genetics of the Carnegie Institution. The speakers were summer visitors to the Laboratory; and Dr. Maurice Bernstein was in charge of the arrangements. The speakers and titles were as follows:

July 1: Max Delbrück, California Institute of Technology. Intracellular amplifying mechanisms.

July 8: Frank Fenner, Australian National University. Experimental epidemiology.

July 15: Ernst Caspari, Wesleyan University. Genes and mitochondria.

July 29: Norton D. Zinder, Rockefeller Institute. Principles of bacterial transduction.

August 5: David Shemin, College of Physicians and Surgeons. The succinate-glycine cycle; the biosynthesis of porphyrins and purines.

In the spring two lectures of general interest were organized by the Laboratory, one given on May 19, 1954, by Dr. George W. Corner of the Department of Embryology of the Carnegie Institution in Baltimore, on "Twins, Triplets, Quads, and Quins," and the other on June 2 by Dr. Robert Cushman Murphy of the Museum of Natural History entitled "On the Payroll of the Birds." Dr. Corner discussed the development of the human embryo, with particular emphasis on multiple births, and Dr. Murphy gave an account of his most recent visit to the Peruvian guano islands.

Buildings and Grounds

During the year major work was done on three of our buildings. The Wawapex Laboratory building was repainted on the outside, a new roof was put on Airslie, and in Urey Cottage the rooms were painted, the living-room floor was replaced, and new linoleum was laid in the kitchen. On the

grounds, a small parking field was built to the north of Nichols Building, a new surface was put on the road leading from Bungtown Road to Urey Cottage, and—in cooperation with our neighbors who use De Forest Drive—that road also was resurfaced.

In order to replace the fund used for the purchase of property from the Estate of Mary E. Jones the previous year, eight acres of land located on top of the hill along Moore's Hill Road were sold.

Finances

The expenses of full-time research and of the Symposia are being met by grants received from the organizations mentioned in the following section. During the year, Dr. Abramson began studies of the biological and biochemical effects of LSD25, supported by a grant from the Geschickter Fund.

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

Acknowledgments

Miss Dorothy Klem, who had been the secretary of the Laboratory since 1935, resigned in November, 1953, to take a position in the business of a member of her family. I wish to acknowledge here the very efficient service Miss Klem gave to the Laboratory and to express the regret we all feel at her leaving.

I would like to make grateful acknowledgment of the important service to the Laboratory contributed by this year's lecturers, Dr. Corner and Dr. Murphy, and by the teachers of our two research courses, Professor Adams and Drs. Witkin and Bryson.

The Laboratory recognizes with gratitude the research grants made by the National Tuberculosis Association, the Army Chemical Corps, the Atomic Energy Commission, the Office of the Surgeon General of the Army, and the Office of Naval Research, and also the grants made for the expenses of the Symposia by the Carnegie Corporation of New York, the National Foundation for Infantile Paralysis, the National Science Foundation, and the Association for the Aid of Crippled Children.

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

M. Demerec
Director of the Laboratory

REPORTS OF LABORATORY STAFF

GENETIC AND BIOCHEMICAL STUDIES OF BACTERIA

V. Bryson, L. Geronimus, H. Davidson, H. Deiches, and P. D. Skaar

Experiments have been performed using the turbidostat in an analysis of population changes that occur during the continuous cultivation of bacteria at 37° C. With 35-ml *Escherichia coli* populations photometrically maintained at constant density, quantities of nutrient broth up to ten liters have been automatically added over a period of several days to provide for uninterrupted growth. It has been found that under conditions of rapid cell multiplication, the measurement of mutation rate is made difficult by the simultaneous occurrence of selection. The three systems employed for study were mutations to bacteriophage resistance, using phages T3 and T5, and reversion from proline dependence to nondependence in strain E12-100. At population densities of approximately fifty million cells per ml, the proportion of T5-resistant mutants in a population remains low, whereas the proportion of T3-resistant cells increases exponentially at rates which may produce more than a hundredfold increase of the proportion of mutants in 12 hours. The rapid increase is observed in both wild-type populations and cultures of the auxotrophic mutant, E12-100. In the latter strain, the prototrophs also accumulate rapidly and exponentially.

Several interpretations may be advanced to explain these findings. It could be assumed that the increase is the consequence of a selective deposit of mutant individuals on surfaces of the growth tube, perhaps arising from a stickiness of the mutant cell. The adherent bacteria would produce local sources of additional mutants that could wash off into the medium, thereby decreasing the proportion of wild-type cells and providing opportunity for further adherent clones. This interpretation is unlikely in view of recent experiments wherein a clean growth tube was substituted without eliminating the rapid increase of mutant cells. Instead, the mutant bacteria seem to be better fitted than their normal parents for conditions of continuous growth. Under such conditions, the selection of spontaneously occurring T3-resistant mutants rapidly results in a new type of bacterial population. Thus we see that resistance to phage may confer an advantage even when phage is absent.

The investigations of last year on the mutagenic action of manganous ion have been extended to include the influence of other chemicals on manganous-induced genetic change. The test system employed was the induction of mutations in *E. coli* from streptomycin dependence to nondependence as studied by Demerec and Hanson. As a working hypothesis, it was assumed that substances acting intracellularly to lower the concentration of manganous ion by oxidation would depress mutation. Kenten and Mann (1952) have reported that manganous ion can be oxidized by plant peroxidase systems in the presence of hydrogen peroxide and a hydrogen donor. Nonenzymatic reduction of manganese can then be effected by oxalate, a substance found by Demerec and Flint to increase the mutagenic activity of manganous chloride solutions.

Experiments were therefore set up attempting to influence mutation rate by the addition of hydrogen peroxide, or various hydrogen donors known to act in peroxidase systems.

Since flavin enzymes can reduce atmospheric oxygen to hydrogen peroxide, appropriate substrates for these enzyme systems were also included as test substances. Substrates employed were *dl*-alanine, xanthine, formaldehyde, histamine, cadaverine, and putrescine. At concentrations of .05%, the polyamines were effective in reducing the number of manganous-induced mutations to streptomycin nondependence. Decamethylenediguanidinc, an inhibitor of diamine oxidase, and the antihistamine pyribenzamine also effectively reduced mutation. The result obtained with these organic bases, all used as hydrochlorides, suggested several possibilities: (1) bacterial oxidation of the bases produces ammonium ion, which competes with manganous for sites of activity, even as sodium ion is presumed to do; (2) cations resulting from the dissociation of bases act competitively to reduce the effectiveness of manganous ion; (3) organic bases have a specific antimutagenic action in the manganous-treated cells; or (4) solutions of organic bases tested as hydrochlorides have a pH unfavorable for manganous-induced mutation. Ammonium ion was then found to be less active on a molar basis than histamine dihydrochloride in depressing mutation. The remaining possibilities were explored by means of experiments using neutralized and unneutralized solutions of histamine dihydrochloride.

The rationale of these experiments was as follows: if histamine acts as a cation, then raising the hydrogen ion concentration to the vicinity of pH 7 should have no effect upon its activity; for histamine is a strong base and is about 99% dissociated at neutrality. Therefore the cation concentration could change only by a fraction of one per cent. If, however, histamine enters the cell as undissociated base, raising the pH should result in a great increase in the antimutagenic action of histamine, for the increase of undissociated base would be large (e.g., 1.0% is ten times 0.1%). Finally, if acidity is the significant factor, there should be a decrease of antimutagenic effect with a rise in pH. The addition of 0.0025 molar NaOH to a 0.05% histamine solution (a change of pH from 5.0 to 6.6) resulted in a marked diminution of the antimutagenic effect, showing that acidity is the main factor involved. An increase in the concentration of manganous ions does not overcome the effect of hydrogen ions and therefore the inhibition is not a matter of simple competition. However, simple competition cannot be expected between a divalent cation and a hydrogen ion when a macromolecule approaches its isoelectric point as the pH is lowered. In such a case, the ability of hydrogen ions to compete with a divalent cation increases exponentially. Experiments have shown that the mutagenic effectiveness of manganous chloride disappears suddenly in the pH zone between 5.5 and 5.0. This suggests a rapid rise in the effectiveness of hydrogen ion as a competitor of manganous ion, and therefore a close approach to the isoelectric point of some substance which binds manganous ion. Since many proteins (but no nucleic acids or phospholipids) have an isoelectric point at about pH 5, it may be inferred

that manganous ion is concentrated on the *E. coli* cell by being bound by the same surface proteins which give the cell its isoelectric point at about pH 5. This hypothesis could be tested by the use of gram-positive bacteria whose low isoelectric point (approximately pH 2) should make the antimutagenic effect of acid less evident in the vicinity of pH 5.

In an additional study, the resistance of *E. coli* mutants to freeze-drying (lyophilization) has been investigated. A large amount of information is already available for the comparison of different bacterial species. No systematic survey has been attempted to determine whether certain specific mutants, or classes of mutants, are particularly resistant to the process. To study the significance of genotype more extensively the following mutant strains of *E. coli* were chosen for comparison: two standard laboratory strains (B and K-12); the radiation-resistant mutant of B (B/r); a streptomycin-resistant mutant of B (B/S); a streptomycin-dependent mutant of B/r (B/r/Sd-4); a neomycin-resistant small-colony mutant of B (B/NM_s); a neomycin-resistant large-colony mutant of B (B/NM_l); a methionineless mutant of K-12 (K-12-58-161); a complex strain of K-12 that is thiamineless, threonineless, and leucineless, is unable to ferment lactose, maltose, xylose, mannitol, galactose, or arabinose, and is also resistant to streptomycin and phage T1 (K-12-1177); a viomycin-resistant mutant of B (B/VM); and a manganous-induced phage-resistant mutant of B/r (B/r/1).

The experiments that have been performed with these strains have not been a test of survival over long periods of storage following lyophilization. They serve only to indicate the relative survival of cells maintained for periods up to one week at 37° C after the lyophil process. The suspending medium (skim milk) was deliberately chosen as not ideal, in order to bring out differences in viability that might not be detected under optimum conditions. Wide fluctuations in the survival of specific strains were observed in consecutive experiments. This suggests that the experiments were not as well controlled as may be possible. However, it was noted that strain K-12-58-161 was ranked first or second in viability in each of eight independent lyophilizations.

Further work involved the use of *Serratia marcescens*, a chromobacterium, closely related to *Escherichia coli*. It is refractory to most antibiotics, and occasionally is pathogenic. Since Syzbalski has reported that specific strains of *E. coli* may be made sensitive to certain antibiotics by the process of becoming resistant to others (collateral sensitivity), we attempted to select a strain of Serratia that could be used in the laboratory with greater safety. Unfortunately, collateral sensitivity to polymyxin B could not be shown in chloramphenicol-resistant strains, or vice versa. The potential usefulness of extensive collateral sensitivity appears to be limited by its restriction, as a phenomenon, to relatively few bacterial strains.

Another project with utilitarian implications was the application of the gradient-plate method to the demonstration of antiradiation effects of chemicals. It has long been known that the damaging effects of radiation are to some extent reversible. A notable example is the process of photoreactivation

discovered by Kelner. Another well-known means of counteracting radiation damage is the application of compounds containing sulfhydryl groups to the irradiated cells. Routine laboratory methods to perform the tests are rather laborious and time-consuming. Incorporation of chemicals to be screened for antiradiation effects into gradient plates provides a rapid technique. A cencentration of 0.2% cysteine in the upper layer of a plate gave some protection to *E. coli* that had been spread on the plate surface and exposed to 120 ergs per mm^2 of ultraviolet light. The advantage is that the concentration of chemical need not be precise, since three zones may be allowable on a single plate: a left-hand zone of chemical inhibition, a central zone of chemical protection, and a right-hand zone of radiation inhibition, depending on the depth of chemical in the gradient plate.

In a previous report (1949) we presented evidence that the number of colonies of *E. coli* appearing in nutrient agar plates containing 10 units of streptomycin per ml was increased by low doses of ultraviolet radiation (50% survival), and that the effect did not depend on the induction of mutation. We further observed that the number of colonies appearing was *inversely* proportional to the concentration of cells plated, suggesting a competition in crowded populations. Since fewer colonies were produced when the inoculum was reduced by one-half than when a concentration of ultraviolet was employed yielding 50% survivors, it was assumed that the radiation effect was different from the dilution effect. It has now been found that no essential difference exists, since the killing effect of ultraviolet *alone* is not an adequate control for estimates of ultraviolet damage. Posttreatment of irradiated cells with streptomycin, an integral part of the experimental procedure, results in a synergistic toxicity which reduces the viable population to a level permitting the growth of numerous colonies. The effect of radiation is here selective, and not mutagenic. Proflavine may be substituted for ultraviolet radiation as the toxic agent, with a similar effect.

A cooperative project concerned with the influence of toxic agents on bacterial cytology has been performed with the supervision and extensive aid of Dr. E. D. DeLamater. The details of this work are described more fully in the report of Dr. Szybalski, who participated closely in the experiments. In brief, it was found that certain antibiotics, including terramycin, aureomycin, streptomycin, erythromycin, magnamycin, neomycin, and viomycin, produced reversible mitotic arrest in *B. megaterium* if not used in excessive concentrations. Some polyploidy was noted. There was also a great increase in the stainability of nuclear components assumed to be DNA by the application of specific cytochemical dyes.

Considerable attention has been devoted to the study of complex phenotypes obtained by treating *E. coli* with mutagenic agents and then screening the induced phage-resistant mutants for evidence of other changes. In last year's report we described the numerous auxotrophic properties of different T1-resistant mutants. Further work has been done on the patterns of reversion from auxotrophy to prototrophy, in an effort to determine whether

the multiple phenotypic changes result from one genetic event with pleiotropic consequences, or from simultaneously induced multiple mutations. With the recent demonstration by Calef that B/r strains can be crossed with K-12, analysis of the nature of complex mutants is now being approached by the more direct method of bacterial recombination.

Thirty-four auxotrophs obtained by selecting for T1 resistance after treatment with manganous chloride have been chosen for breeding analysis. Some of these are deficient for more than one growth factor, several are slow fermenters, and one is incapable of fermenting mannitol. These represent about 30% of a randomly isolated T1-resistant population; whereas comparably treated populations not selected for T1 resistance contain only about 5% auxotrophs. Over half of these thirty-four T1-resistant auxotrophs have been crossed to T1-sensitive K-12 strains auxotrophic for other growth factors. If the associated changes to auxotrophy and phage resistance in the B/r parent are due to a single gene change, all prototrophic recombinants should be phage sensitive. If they are due to separate mutations, some phage-resistant prototrophs should be expected.

Two of the phage-resistant mutants are resistant to T1 but not to T5. In each case, no phage-resistant prototrophs could be recovered when these mutants were crossed to phage-resistant K-12. One of these tested mutants is tryptophaneless, and the results of the cross were consistent with those of crosses involving other B/r/1 tryptophaneless mutants. The conclusion that these two particular effects are due to a single gene change is thus supported. The other B/r/1 in this series is deficient for another growth factor, and probably represents a change at another locus.

The main interest here, however, centers on the thirty-two B/r/1,5 mutants, for this is the type whose frequency is significantly increased by manganous treatment. In every cross, the separability of auxotrophy and T1,T5 resistance could be demonstrated. In addition, the two requirements of one doubly auxotrophic mutant could be separated, as could the single instance of mannitol insufficiency. These results suggest, therefore, that pleiotropy may be excluded as a factor in bringing about the observed excess of complex phenotypes; and a clustering of genetic changes in particular cells is indicated. Within the limits imposed by the mechanics of bacterial crosses, no tendency could be observed for these changes to be clustered on a subcellular level, that is, for associated mutations to be closely linked.

BACTERIAL GENETICS AND ACTION OF ANTIMICROBIAL AGENTS

WACLAW SZYBALSKI

The work reported here was aided by a research grant from the Office of Naval Research, Nonr-1154(01).

Cytological studies on the influence of various inhibitors on the bacterial nucleus (in cooperation with E. D. DeLamater and M. E. Hunter, University of Pennsylvania). Bacteria, aside from being in general smaller, do not differ from other cells or organisms in their essential structure and their nuclear division mechanism. The purpose of this investigation was to analyze the action of various inhibitors on the mitotic activity of bacteria. It was reasoned by Levan (Cold Spring Harbor Symp. Quant. Biol. 16, 409, 1951) that "if similar deviations were induced in mitosis of yeast as were known to occur in higher plants after such [c-mitotic type] treatment, it would be an indication that the Feulgen-positive bodies observed in yeast were really chromosomes undergoing mitosis." Similar reasoning could be applied to bacteria.

Two representative bacterial species were employed, *Bacillus megaterium* and *Micrococcus cryophilus*. All methods used for staining have been previously described (DeLamater, Stain Technology 26, 199, 1951). Toxic substances were incorporated into the medium according to the gradient-plate technique (Szybalski, Science 116, 46, 1952). The concentration of the drugs used was adjusted so as to demonstrate both growth and inhibition on the same plate. The following substances were employed: benzimidazole, camphor, colchicine, formaldehyde, isoniazid, sodium *p*-aminosalicylate (PAS), oxytetracycline (terramycin), chlortetracycline (aureomycin), erythromycin, carbomycin, streptomycin, neomycin, and viomycin.

The observed cytological effect of inhibitory concentrations of benzimidazole and isoniazid on *B. megaterium* was a progressive reduction in density of chromosome staining, due apparently to interference with DNA synthesis. With the same organism, formaldehyde, camphor, and colchicine were observed to produce transient diploidy as ascertained by chromosome counts. These effects are less obvious than certain others now to be described.

The effect of the tetracyclines (terramycin and aureomycin) was most thoroughly studied. Similar results were obtained with *B. megaterium* and *M. cryophilus*, and so only the first will be described. In preparations made after 2-4 hours of exposure to 1-5 times the inhibitory concentrations of these antibiotics, a very high percentage of nuclei was found in metaphase, suggesting an arrest of nuclear division at this particular point. The effect of the drug on the divisional apparatus appears to be as follows. The chromosomes increase in size and density and seem to fill the whole nuclear sac. This chromosomal mass elongates, assuming a sausage-like shape. It lies axially in the cell, which elongates correspondingly without crosswall formation. Within such dense chromosomal masses, individual chromosomes cannot usually be defined. The centrioles become much enlarged. Their migration to

the poles is impeded, which is particularly evident under the influence of PAS. Centrioles arrested in polar positions continue to reduplicate, so that numerous centrioles accumulate in the cytoplasm at the poles of the elongated nuclear sac.

Further evidence of the transient polyploid conditions was obtained by studying recovery stages upon inactivation of the drug. The action of the tetracyclines is merely bacteriostatic, since a high percentage of the cells survives. As recovery proceeds, the dense, elongated nuclei become lobulated owing to regularly spaced constrictions in the seemingly thickened and toughened nuclear membrane. Within individual lobules, of which there may be as many as eight per nucleus, three chromosomes constituting the individual haploid complements can be counted. Segregation of individual haploid complements thus appears to occur by constriction of the nuclear sac. Haploid complements may proceed directly to metaphase with the chromosomes remaining in the contracted state, or the chromosomes may elongate to form interphase nuclei. In nuclei proceeding to metaphase, it is postulated that the necessary centrioles are derived from the centriolar multiplication previously described.

The effects of erythromycin, carbomycin, neomycin, viomycin, and streptomycin are essentially similar but not identical, resembling that of the tetracyclines.

The action of these drugs on bacteria seems to be parallel to their effect on larger cells, as shown for several mitotic poisons and antibiotics by various investigators. No implication is intended, however, that the deranged nuclei necessarily constitute the primary site of action of the toxic agents studied.

Genetic recombination in Bacillus megaterium. DeLamater and Hunter (J. Bact. 65, 739, 1953) have presented cytological evidence of the formation of conjugation tubes in a strain of *Bacillus megaterium*. This suggests a fusion of two parental cells which may produce progeny having mixed genetic characteristics of both parents. The simplest way to test this possibility is to prepare mutant strains having some selective markers, such as drug resistance or nutritional deficiency. For instance, if a strain resistant to drug "A" and another strain resistant only to drug "B" are seeded on an agar plate containing both drugs, no growth should occur because the drugs will reciprocally inhibit both strains. If, however, a few cells resistant to drug A *or* drug B had the possibility of exchanging hereditary factors through genetic recombination, then clones might be produced which would be either resistant to both drugs or sensitive to both drugs. The former would produce colonies in the presence of the combined drugs. Analogous experiments with *E. coli*, using growth inhibitors, have been performed by Lederberg (J. Bact. 59, 211, 1950).

Our experiments made use of strains of *B. megaterium* resistant to the following drugs: amicetin, bacitracin, cinnamycin, erythromycin, isoniazid, micrococcin, PAS, streptomycin, and oxytetracycline. In addition, histidine-deficient and aspartic acid–deficient strains and their streptomycin-resistant derivatives were employed. Three types of crosses were performed: (1) be-

tween two deficient mutants on minimal agar; (2) between the wild-type strain (*B. megaterium*) and the streptomycin-resistant mutants of the amino acid–deficient strains, on streptomycin-containing minimal agar; and (3) between strains singly resistant to the above-listed antibacterial agents, on Penassay agar (Difco) supplemented with the corresponding pairs of toxic drugs.

Crosses of the first type always gave negative results, that is, no colonies appeared on the minimal agar and there were no indications of genetic recombination. The second and the third types of cross gave very irregular results. Here the apparent rate of recombinations varied from zero up to values of 10^{-6} when cells were allowed to grow for 15 minutes to 12 hours in the liquid medium (Difco nutrient broth—16 g/l) before plating. Preliminary opportunity for contact before plating seems to be necessary because the actively growing cells would otherwise all be killed in the presence of toxic agents before having a chance to recombine with each other to produce a few doubly resistant viable types. Attempts to trace the source of the irreproducibility of results by changing the age of the cultures, the time of cell contact before plating, and other parameters, were without success. Because of variability in results and because of the rarity of successful crosses, the work on this problem was temporarily discontinued.

Genetic recombination between strains B and K-12 of Escherichia coli. The feasibility of crosses between *E. coli* strains B and K-12 opens a new field for exact genetic studies employing strains B and B/r, widely used in quantitative bacteriology, and hitherto considered as sterile. The following strains were used in the present work: *E. Coli* K-12 strains 58161 M_2–F+, 58161-CS2 M_2–F–, J13-3 M_2–H–, W1177 Th–L–B_1–F–, W1177-CS11 Th–L–B_1–F+, J4-5 H–T_1–, J4-13 T_1–S/G–, J5-3 M_1–P_1–, J5-7 P_1–S/G–, J5-10 H–P_1–, J6-2 H–T_1–P–, J6-6 H–T_1–, J7-2 M_1–P_1–L–, J7-12 H–M_1–P_1–, J7-22 M_1–P_1–, J8-1 H–P–I+V–, J11-6 H–M_2–P–, and Y-87 M_2–; B/r strains 12-22 T–(s), M-4 T–(l), R4-88 H–(s), 12-23 H–(s), 12-91 H–(l), and IMN M_2–A–; and a strain (H–M–) obtained from F. J. Ryan. Abbreviations denote the deficiencies (*A*—arginine, B_1—vitamin B_1, *H*—histidine, *I+V*—isoleucine and valine locus, *L*—leucine, *M*—methionine, *P*—proline, *S/G*—serine or glycine, *T*—tryptophane, *Th*—threonine). Known identities of some loci are indicated by subscript numerals. The letters (*s*) and (*l*) designate mutagen-stable and -labile loci, whenever mutagen stability has been tested. All the B strains and two K-12 strains are of the F– mating type; the rest of the K-12 strains are F+. Mutation to prototrophy in the listed strains is satisfactorily low. Each of the B/r strains was plated with K-12 F+ strains on minimal agar of the following composition: glucose, 1 g; K_2HPO_4, 7 g; KH_2PO_4, 2 g; sodium citrate · $5H_2O$, 0.5 g; $MgSO_4 \cdot 7H_2O$, 0.1 g; $(NH_4)_2SO_4$, 1 g; agar, 15 g; demineralized water, 1 liter.

The compatibility of crosses is determined by an infective hereditary factor F. Only crosses between F+ and F– or between F+ and F+ are fertile. The F– × F– cross is completely sterile. Strain B behaves like the F– parent, as indicated by a general failure of B × B and B × K-12 (F–) recombinations, whereas crosses between B and K-12 (F+) are in general successful.

Some of the last-named crosses were extremely fertile, approaching the frequency of 10^{-5}, while others were almost if not completely sterile. Only some of the observed sterility is ascribable to a possible allelism of common parental deficiencies. For example, IMN27 (M^-) will demonstrably cross with W1177, but not with the methionine-deficient strains 58161 or Y87. Some of the other strains (e.g., Ryan's H^-M^-) gave significant but very low recombination rates when crossed to several K-12 mutants.

Persistence of prototrophy upon subculture and the segregation of unselected markers attest to the integrity of all recombinants that have been carefully studied. Some observations, however, indicate instability of certain recombinants and persistent heterozygosity. For example, on EMS agar containing maltose the recombinant colonies between maltose-positive strain 58161 and a maltose-negative B/r strain were heavily sectored. When transferred to the surface of EMB-maltose agar they produced both *mal*$^+$ and *mal*$^-$ colonies. Many recombinant colonies showed morphological sectoring on minimal agar also. Syntrophism, or possibly some other type of synergism, obscured some of the crossing experiments and in a few cases simulated the formation of recombinant colonies between strains B and K-12(F^-). On subculturing, such colonies proved not to be true prototrophs. It was possible, however, to obtain successful crosses between the B/r strain and some rare K-12 $\times$ B/r recombinants. For instance, the streptomycin-resistant strains 12-22 and 12-23 produced streptomycin-resistant prototrophs when crossed with one of the 12-91 $\times$ 58161 recombinants on streptomycin-containing (200 μg/ml) minimal agar. This 12-91 $\times$ 58161 strain possessed a B/r phenotype and its F+ property was lost after a few subcultures. It was not possible to infect this or any other B strain with the compatibility factor F+.

Allelic relationships between mutagen-stable and -labile loci in E. coli strain B. Amino acid–requiring bacterial mutants (auxotrophs) may revert to amino acid–independent prototrophs when tested on synthetic media. The rates of these mutations are generally increased by the action of ultraviolet light or other mutagens. Demerec described the phenomenon of "mutagen stability" when he found that in some particular strains the rate of reversion to prototrophy could not be increased above the spontaneous level by treatment with several mutagenic agents. More detailed studies have suggested that rates of both spontaneous and induced mutation to prototrophy in some nutritional mutants of *E. coli* are determined by the allelic form of the gene present at a certain locus; each allele either is affected by a particular mutagen in a specific way (mutagen labile) or is not affected at all (mutagen stable).

Crosses between strains B and K-12 permit indirect genetic analysis of the allelic relationships between corresponding loci. An indirect approach was necessary because of the failure to obtain efficient crosses within the B strains. It was assumed that allelism between two loci in the B strain would result in lack of recombination with a particular K-12 strain carrying a similarly located deficiency, provided the control crosses with the markers removed, or on supplemented agar, did show recombination. Eleven mutagen-stable and

-labile strains of B/r, with independently isolated requirements for either histidine, tryptophane, or proline, were crossed with twenty-two strains of K-12 which exhibited similar deficiencies. The results of a few more informative crosses (number of recombinants per 10^9 cells) are arranged in the table.

B/r \ K-12	J5-3 M_1—P_1— H^+	J5-10 $M_1^+P_1$— H—	J7-12 M_1—P_1— H—	J7-22 M_1—P_1— H^+	58161 M_2—P^+ H^+	J11-6 M_2—P— H—	J13-3 M_2—P^+ H—
R4-88 H—(s)	345	0	4	346	180	0	0
12-23 H—(s)	400	150	240	120	170	540	10
12-91 H—(l)	60	0	1	110	44	0	0

The independently isolated histidine loci of several K-12 strains (J5-10, J7-12, J11-6, J13-3) seem to be allelic to histidine loci of two B/r strains (R4-88, 1291) but not to the third (1223).

Thus two mutagen-stable histidineless loci in B/r are not allelic, but one of these (R4-88) is allelic to a mutagen-labile histidineless marker of the strain 1291. This may indicate that mutagen lability depends on the allele rather than on a specific locus.

The low number of recombinants in crosses J7-12 $\times$ R4-88 and J7-12 $\times$ 1291 exemplifies another frequent observation. More careful analysis of this phenomenon suggests that it may be a consequence of pseudoallelism. A similar conclusion may be drawn from experiments with several other markers.

RADIATIONS AND POPULATIONS

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Previous annual reports (1950-1953) have dealt with our studies of irradiated populations of *Drosophila melanogaster*. These studies have indicated that the effect of "deleterious" gene mutations on populations carrying the mutated genes is not simply related to the deleteriousness of the mutations as measured by ordinary techniques. The presence of many lethal genes in a population does not indicate per se that the average individual of this population is less viable or "fit" than individuals of another population in which there are fewer such genes. Similarly, an increase in the frequency of lethals within a population over a period of time does not necessarily reflect a corresponding decrease in the average viability of the individuals of this population. The frequency of deleterious mutations has remarkably little to do with "fitness"; "fitness" is determined by so many factors that it is relatively independent of frequencies of seemingly deleterious genes.

During the past year further investigations of population gene pools have been carried out. The purposes of these investigations were to effect comparisons between gene pools of different populations and to discern organizational patterns within these gene pools. The experimental techniques and data are discussed at length because of the technical nature of the problem; a generalized discussion of the type used in past reports would not properly emphasize the experimental bases for some of the more important conclusions. Specifically, this analysis attempts to study the interaction of genes from different populations without rendering these genes homozygous by special breeding techniques. The results demonstrate differences between gene pools of the various "normal" populations studied and suggest one system of organization brought about by selection acting within populations.

Flies from four different populations of *D. melanogaster* were used in this experiment. Two of the populations were our experimental populations No. 1 and No. 3; each of these had been started a hundred or more generations previously from an Oregon-R strain of flies. The two populations differ in that the original flies of population No. 1 were subjected to a large dose of X-rays. The other two populations were recent derivatives of natural populations. Dr. Paul Levine of Harvard University sent us flies from his "M" population, a laboratory population two or three generations old, started with flies from an Amherst, Massachusetts, compost heap. Dr. J. C. King of this Laboratory supplied us with flies from his Syosset, Long Island, population, a population that had been in the laboratory for about thirty-five generations. The notations *1*, *3*, *M*, and *S* will be used frequently in referring to these populations below.

Flies from the four populations were mated and remated systematically in

order to obtain individuals carrying definite combinations of genes from one, two, three, or all four populations. Complete details of the matings need not be reported here, nor need we consider the technicalities of gene recombination; it must be mentioned, however, that males transmitting crossover chromosomes were obtained through backcrosses to hybrid females. The genotypes of individuals studied are represented schematically by indicating the matings that led to their formation. The system can be illustrated by 1M×SS individuals (read as [1 times M] times [S times S]): the mothers of these individuals were obtained by mating males and females of the Syosset population; the fathers were special hybrid males obtained by crossing flies from population 1 with flies from Amherst. There are fifty-five combinations that can be made by such matings among four populations: 11 × 11, 11 × 13, 11 × 1M, 11 × 1S, 11 × 33, 11 ×3M, 11 × 3S, 11 × MM, 11 × MS, 11 × SS, 13 × 13, 13 × 1M, 13 × 1S, 13 × 33, 13 × 3M, 13 × 3S, 13 × MM, ... etcetera . . . . MS × MS, MS × SS, and SS × SS. In addition to these fifty-five combinations, we studied six additional ones (13 × 13, 1M × 1M, 1S × 1S, 3M × 3M, 3S × 3S, and MS × MS) in which ordinary hybrid males were used as male parents.

The experiment consisted of determining the relative abilities of individuals of different genotypes to survive on a limited amount of food. Forty freshly hatched larvae of each genotype were placed in each of 20 vials containing 2 cc of medium; adults were counted shortly after they commenced to emerge from pupae, and counts were continued until all had emerged. Unfortunately, 2 cc of medium allows such complete development that the total counts of these flies were virtually meaningless; the first counts, however, which were made at comparable times for all sixty-one genotypes, showed clear-cut differences in developmental rates. The discussion that follows will be restricted to these first counts.

The mating system outlined above gives an experimental basis for distinguishing between the role of ordinary dominance-recessive relationships between homologous chromosomes and the functional relationship of genes derived from a given population. Alleles at a given locus on homologous chromosomes are generally referred to in terms of homozygosity or heterozygosity; for this study these terms have been used to indicate that the two alleles are from the same or different populations, respectively. The proportion of all loci that are heterozygous by our definition can be calculated for each of the sixty-one genotypic combinations; 11 × 3M individuals, for instance, are 100% heterozygous whereas 13 × 1M individuals are 75% heterozygous. Individuals designated 0% heterozygous—all loci derived from the same population, as in 11 × 11 or SS × SS—were obtained by matings between different strains isolated from these populations.

In addition to analyzing the relationships between alleles on homologous chromosomes, the experimental procedure allows an analysis based on proportions of chromosomes having loci derived from two different populations. Any chromosome transmitted by an interpopulation hybrid individual is assumed to carry loci derived from the two different populations, and is there-

fore "synthetic." Individuals of the different genotypes listed above are 0%, 50%, or 100% synthetic, depending upon whether neither, or one, or both parents were hybrids. The six extra combinations referred to earlier comprised a special case. The male parents used in these crosses were hybrid males that could not transmit crossover chromosomes; individuals of these genotypes, therefore, received nonsynthetic chromosomes but synthetic chromosome sets from their fathers.

The effects of heterozygosity and syntheticness on developmental rates are shown in Table 1, which lists the numbers of adult flies present at the first counting. The figures are given for groups of five vials; therefore the original number of larvae in each group was 200. The most important points demonstrated by these data are: (1) Interpopulation hybrids (100% heterozygous, 0% synthetic) developed faster (more flies in the first count) than any other type. (2) Individuals arising from intrapopulation matings (0% heterozygous, 0% synthetic) were the second-fastest developers. (3) Development rates become slower as syntheticness increases or, to a smaller extent, as heterozygosity decreases.

Two hypotheses can account for the role of synthetic chromosomes in determining the rate of development. (1) Intranuclear interactions: One can postulate that certain physiological processes are controlled by genes A and B in one population and by C and D in another. AABB and CCDD individuals (0% heterozygous, 0% synthetic) and ABCD individuals (100% heterozygous, 0% synthetic) develop rapidly because the necessary physiological reactions are adequately cared for. Individuals carrying synthetic chromosomes may have AACD, ABBD, and other combinations of genes that lead to a physiological imbalance. (2) Intrachromosomal interactions: This hypothesis postulates that individual chromosomes acquire a functional organization under the pressure of selection operating within local populations. Since the functional organization of a chromosome in one population need not be precisely that of the homologous chromosome in another population, recombination between the two may impair the efficiency of this organization.

The data presented in Table 1 suggest that the second of these hypotheses accounts for some of the retardation of developmental rates. First, the developmental rates of individuals of the six extra genotypes were no slower than those of the corresponding individuals (50% heterozygous, 50% synthetic) of the main experiment. The important difference between these two classes of individuals is that in the main experiment each 50% heterozygous–50% synthetic individual carried a nonsynthetic haploid set of nonsynthetic chromosomes, whereas in the extra tests each one carried nonsynthetic chromosomes in synthetic combinations (chromosome 2, for instance, may have come from population 1, and chromosome 3 from Syosset). These results indicate that the different parts of any intranuclear interacting system are localized on individual chromosomes; if this were not the case, these systems should be disrupted by segregation of chromosomes as well as by recombination within individual chromosomes. Second, under the intranuclear hypothesis there would be good reason for expecting individuals of the class

50% heterozygous–100% synthetic (main experiment) to develop faster than the other two classes of the 100% synthetic type. The basis for this expectation is the possibility of forming ABCD individuals within the 50% heterozygous–100% synthetic class through the union of AC and BD gametes; this possibility does not exist in the case of 75% heterozygous–100% synthetic or 100% heterozygous–100% synthetic individuals. The observed developmental rates of these three classes of individuals are contrary to this expectation. In summary, the data presented here suggest that natural selection within local populations acts to effect an intrachromosomal functional organization that can be disrupted by interpopulation recombination.

Quite apart from the question of gene organization or gene action is the problem of distinguishing between the structures of different gene pools. At one time, similarity of the individuals of two populations was considered ample proof of basic similarities of genes within the two populations. Later, with the appreciation of the high frequency of concealed recessive genes, it became customary to analyze and compare populations in terms of frequencies of these recessives. For technical reasons these analyses generally dealt with the frequencies of recessive lethals. It is often overlooked that the "normal" genes of these analyses need not be identical and therefore any one test may fail to indicate extensive differences between two populations. The current analyses discussed above allow extended comparisons of gene pools of the four different populations. In these comparisons the genes are not rendered homozygous by special and highly artificial breeding systems but are heterozygous for alleles of the same or of different populations; all the individuals of this experiment were normal "wild-type" flies.

The individuals studied comprised sixty-one different genotypes; each genotype represented a certain combination of genes drawn from the gene pools of four different populations. For any two populations there are seventeen pairs of combinations that allow comparison of the action of genes from one gene pool with the action of those from another in the same combinations. In studying populations 1 and 3, for instance, we can compare the following classes: 1I $\times$ 1I *vs.* 3I $\times$ 3I, 1I $\times$ 1M *vs.* 3I $\times$ 3M, 1I $\times$ 1S *vs.* 3I $\times$ 3S, and so forth. In each instance the basic assumption is made that the genes of population 1 and population 3 are the same and that therefore the comparable combinations should evoke identical reactions. Significant differences in the developmental rates of the two members of a pair of combinations is evidence that the gene pools of the two populations are not identical. Table 2 lists in general terms the seventeen different combinations. The two populations to be compared are called *x* and *y* and the other two populations *a* and *b*. For any specific comparison, *x* and *a* are chosen from the series 1, 3, M, and S so that they fall to the left of *y* and *b*, respectively. In comparing populations 1 and 3, as in the above example, population No. 1 is *x*, population No. 3 is *y*; Amherst, then, must be *a* and Syosset must be *b*. The general schemes for the three pairs of combinations given as examples are xx $\times$ xx *vs.* yy $\times$ yy, xx $\times$ xa *vs.* yy $\times$ ya, and xx $\times$ xb *vs.* yy $\times$ yb.

The results of the comparisons of the seventeen different gene combinations for each of the six possible pairs of populations are shown in Table 3. The two columns on the left show the pairs of populations being compared (x and y). The next seventeen columns indicate whether or not there were significant differences in the developmental rates of the two types of flies. The last column indicates whether or not the results of the seventeen comparisons as a whole can be considered significant.

Table 3 brings out several important points. First, the last column shows that there is extremely little likelihood that the gene pools of populations 1 and 3, 1 and Amherst, 3 and Amherst, or 3 and Syosset are alike. Second, in spite of the improbability that these pairs of gene pools are alike, this fact is not obvious from every one of the combinations in which comparisons were made; actually, a minority of the tests gave significant results. Third, among those combinations in which significant results are observed the relative effects of the genes of the different pools can change abruptly. In the comparison of 1 and 3, the three pairs of combinations 1I $\times$ 1I *vs.* 3I $\times$ 3I, 1I $\times$ 1M *vs.* 3I $\times$ 3M, and 1S $\times$ MM *vs.* 3S $\times$ MM agree in demonstrating that genes from population 1 result in faster developmental rates than do those from population 3; in the pair of combinations 1I $\times$ MS *vs.* 3I $\times$ MS, however, the relative effects of these genes on developmental rates are reversed. Fourth, the particular pairs of combinations that distinguish between two populations are highly specific. The combinations 1S $\times$ MM and 3S $\times$ MM distinguish between populations 1 and 3; 1M $\times$ MS and 3M $\times$ MS, an analogous pair involving the same gene pools in the same proportions, does not make the distinction. Within Table 3 there are ten pairs of combinations which show significant differences between two populations and which have analogous pairs elsewhere within the table; of these ten only two (1I $\times$ 1S *vs.* M3 $\times$ MS and 1I $\times$ 3S *vs.* MM $\times$ 3S) are both significant and analogous. Fifth, the failure of the tests to reveal differences between No. 1 and Syosset and Amherst and Syosset must result from insufficient tests; Syosset cannot be identical with both Amherst and population 1 because 1 has been shown to differ significantly from Amherst. Apparent similarity between two populations in any series of tests of this sort almost certainly means that the methods used and the gene combinations available for study were insufficient to reveal existing differences.

The experiments described in this report bring to a close the detailed analyses of experimental populations No. 1 and No. 3, analyses that have extended over a period of 120 generations or more. In brief, the histories of the populations are as follows. The original flies of the two populations carried identical mixtures of quasi-normal second chromosomes obtained from an Oregon-R strain of flies. The original flies of population 1 were exposed to a single dose of X-rays—7000 r for males and 1000 r for females. The frequencies of lethals in these two populations were studied from the first generation. During the early generations, induced lethals were eliminated from No. 1 at a rapid rate, a rate easily explained by the association of about one-half of these lethals with translocations, and the semisterility of translocation

heterozygotes. After this initial loss of lethals, a drop from about 20% to 10%, the frequency of lethals rose in this population. The rate of this increase until generation 30 or 35 in population No. 1 was the same as that at which spontaneous lethals accumulated in No. 3. At about generation 35 the frequency of lethals in No. 1 leveled off at 20%-25%, and then slowly decreased over the next 40 generations until it reached 15% or so. The average frequency of lethals observed from generation 108 to generation 117 was about 15%. The average frequency in the untreated control population (No. 3) during this same interval was 25%. A genetic test that determines the viability of individuals carrying random combinations of chromosomes from a population has been used in studying these two populations since the 30th generation; these tests have revealed that random combinations of second chromosomes from population No. 1 result in more viable individuals than comparable combinations of chromosomes from No. 3. This greater viability of chromosome combinations from No. 1 has persisted in spite of the changes in frequency of lethals that this population has undergone. These two populations have been used in studies on variation arising through recombination, and to illustrate new techniques for estimating the frequencies of sub- and super-vitals within populations. Finally, the study described in this report has shown that the gene pools of the two populations can be distinguished not only by frequencies of recessive lethals but also by the interactions of all the genes in combination with genes from other gene pools. It appears that little more can be learned from continued intensive analyses of this type. The two populations will be maintained for other studies.

TABLE 1

Numbers of adults present in the first count of flies carrying different combinations of genes from four populations. Definitions of "heterozygous" and "synthetic" are given in the text.

Per cent Synthetic	Per cent Heterozygous 100%	75%	50%	0%
0%	148.46 ±2.25	— —	— —	142.38 ±2.80
50%	136.44 ±2.15	— —	139.63 ±2.13	— —
100%	140.58 ±3.96	135.77 ±2.00	133.42 ±3.53	— —
	Extra Combinations			
50%	— —	— —	142.25 ±3.10	— —

TABLE 2

Seventeen pairs of gene combinations that allow extensive comparisons of two populations. The populations to be compared are designated x and y; the remaining two populations are a and b.

1.	xx×xx	*vs.*	yy×yy
2.	xx×xa	*vs.*	yy×ya
3.	xx×xb	*vs.*	yy×yb
4.	xa×xa	*vs.*	ya×ya
5.	xa×xb	*vs.*	ya×yb
6.	xb×xb	*vs.*	yb×yb
7.	xa×aa	*vs.*	ya×aa
8.	xb×bb	*vs.*	yb×bb
9.	xa×bb	*vs.*	ya×bb
10.	xb×aa	*vs.*	yb×aa
11.	xa×ab	*vs.*	ya×ab
12.	xb×ab	*vs.*	yb×ab
13.	xx×aa	*vs.*	yy×aa
14.	xx×bb	*vs.*	yy×bb
15.	xx×ab	*vs.*	yy×ab
16.	xa×xa*	*vs.*	ya×ya*
17.	xb×xb*	*vs.*	yb×yb*

*From the six additional combinations.

TABLE 3

Summarized results obtained by comparing the rates of development of identical combinations of genes from two different populations. The two populations being compared are x and y; s denotes cases in which the x combination developed significantly faster than the combination involving y; *s* denotes cases in which the y combination developed faster; S indicates that the probability of observing the array of 17 probabilities in the individual combinations was less than .001.

x	y	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	Total
1	3	s	s	-	--	--	--	--	--	--	s	--	--	--	--	*s*		--	S
1	M	--	--			s	--	s	--	--	*s*	--	--	--	--	s	-	--	S
1	S	--	--	--	--	--	--	--	--	--	*s*	--	--	--	--	--	--	--	--
3	M	*s*	--	*s*	--	s	--	--	--	*s*	-	s	--	-	--	--	--	--	S
3	S	*s*	--	*s*	-	-	--	--	*s*	-	--	--	--	--	--	--	*s*	--	S
M	S	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	-

THE GENETICS OF RESISTANCE TO INSECTICIDES

James C. King

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The project of investigating the genetics of resistance to insecticides was undertaken on the assumption that fundamental knowledge about the subject would be of help to entomologists dealing with problems of control, who in recent years have had to cope with the increasingly perplexing problem of the development of resistance by wild populations to omst of the newer residual insecticides. During the first year (Annual Report, 1953, pp. 36-38) the program of developing lines of *Drosophila melanogaster* was undertaken in a manner which we hoped would give not only resistant lines but also information on the nature of the process by which inherited resistance develops within a population. Two different stocks were used—Oregon-R, which had been maintained in the laboratory for some thirty years, and a wild stock collected in a grocery store in Syosseet, New York, in July, 1952. Ten selected lines were set up—four stemming from Oregon-R and six from Syosset. These lines were established and maintained by breeding survivors from among flies that had been treated with an aerosol of DDT dissolved in tributyrin. Three levels of selective intensity were used. Some lines were carried using survivors of doses that killed 50% of the treated flies, others the survivors of doses killing approximately 95%, and still others the survivors of doses killing about 99%. One of the Oregon-R lines and all the Syosset lines were carried in duplicate. Thus we hoped to determine (1) whether different stocks would respond differently to selection, (2) whether different levels of selective intensity would evoke different responses, and (3) whether the same stock treated in the same manner would necessarily respond similarly.

At the end of the first year the ten lines had been carried through eight to twelve generations of selection. It was impossible at that time to demonstrate statistically that any one line had increased its resistance to DDT significantly, but it was possible to show that the Syosset lines, taken together, gave lower mortalities than the control flies more often than a hypothesis of random fluctuation would have predicted. The response of the Oregon-R lines, however, did not differ from that of their controls.

During the second year of the project the program of treatment and selection has been continued, and the various lines have now been carried through twenty-three to thirty generations. As the data have accumulated, more refined methods of statistical analysis have been employed, and it is now possible to see that definite patterns of response to selection have emerged.

Resistance is measured by plotting the percentages of mortality on a probit scale against time-dose on a logarithmic scale. A regression line is

fitted to the points, and the point on the time scale corresponding to the intersection of the regression line and the fifth probit (50% mortality) is the LD_{50}—the exposure in minutes which will kill 50% of the treated flies. The most precise means of fitting the regression line is the maximum-likelihood method. This involves elaborate and cumbersome calculations. A simplified approximation of the method, developed by Litchfield and Wilcoxon, has been adopted, since in cases where the two methods have been used and compared the results are approximately the same.

Two of the selected Syosset lines have now achieved a tolerance for DDT strikingly different from that of their unselected control. The LD_{50} of the Syosset control has fluctuated between three and nine minutes; a value of about six is probably a satisfactory estimate. The two selected lines SyS-1001 and SyS-1002, after twenty-five and twenty-three generations of selection, respectively, have been showing LD_{50} figures of from twenty-one to twenty-seven minutes—three to four times the control figures. The differences are highly significant statistically, with no overlapping of the 95% confidence intervals. These two lines are carried at the 50% level of selective intensity. The four Syosset lines carried at the two higher levels have not responded to selection in any such clear-cut way. One of them shows no evidence of increased resistance; the other three do indicate increased resistance, but there is very considerable fluctuation from generation to generation and in no case has one of these lines given as high an LD_{50} as those shown by SyS-1001 and SyS-1002.

Among the Oregon-R lines there has been almost no evidence of response to selection. ORS-1001, which is carried at the 50% level of selective intensity, had given no indication of increased resistance after twenty-two generations. Since this indicated that the Oregon-R stock was much less responsive than the Syosset, the Oregon-R lines carried at higher levels of selective intensity—which also were showing no response—were discontinued. With generation twenty-three the ORS-1001 line showed a slight increase in resistance, which has continued through generation twenty-five. It now gives an LD_{50} of about fourteen minutes, and there is every indication that this difference is real. It is very interesting, however, that the Oregon-R line took so much longer to respond to selection than the corresponding Syosset lines. The latter began to show a definite increase in resistance at about the eleventh generation.

In order to get evidence concerning the nature of the genetic factors producing resistance, a cross was made between SyS-1001 F_{14}, which had an LD_{50} of twenty-one minutes, and unselected Syosset with an LD_{50} of approximately six minutes. The F_1 of this cross gave an LD_{50} of about thirteen—very nearly half-way between the parent strains. Untreated F_1 flies were inbred, and the F_2 gave an LD_{50} of seven minutes—not significantly different from the control.

In answer to the questions previously raised, we can now say (1) that different stocks do respond differently to the same type of selection, and (2) that different levels of selective intensity produce different responses in

the same stock, at least within the same period of time. There is also evidence that the same stock subjected to the same level of selective intensity does not necessarily respond in the same way. Of the two Syosset lines carried at the highest level of selective intensity, one (SyS-1) increased slightly in resistance from generation one to generation four, but showed no further increase from generation four to generation twenty. It then dropped back to a figure indistinguishable from that of the control. The other (SyS-2) showed no increase until generation ten. From ten to twenty-six it has been fluctuating rather widely, but displaying a resistance definitely greater than that of the control.

SyS-1001 and SyS-1002 show essentially the same gain in resistance, as compared with the control, and one might conclude that they have responded to selection in an identical way. That this is not the case is clearly demonstrated by the results of crosses between the two lines. One such cross was made between SyS-1001 F_{18} and SyS-1002 F_{17}. At that point both lines were given LD_{50}'s of approximately twenty minutes. The F_1 of the cross also gave an LD_{50} of twenty minutes. In the F_2, however, the LD_{50} dropped to ten minutes. The same type of cross was repeated between F_{23} and F_{22} when the selected lines were showing LD_{50}'s of approximately twenty-five minutes. Here again the F_1 showed an LD_{50} of twenty-five, the F_2 a drop to fifteen. The only explanation of these data is that the two selected lines have arrived at the same high level of resistance by sorting out and retaining different combinations of potential genetic factors for resistance. In the F_1, where every individual has one complete haploid set of chromosomes from each line, there is no change in resistance. In the F_2 individuals, where the chromosomes exist in all manner of reshuffled recombinations, the two systems no longer interlock, various extreme combinations appear, and the average resistance (mean tolerance, i.e., LD_{50}) falls.

All the evidence accumulated in the present work on resistance indicates that the character, although hereditary, is not produced by a single pair of alleles or a small number of pairs. The slowness with which a line responds to selection, the fact that the 50% level of selective intensity is more effective than much higher levels, the intermediate resistance of the F_1 of a cross between a resistant and a susceptible line, and the drop in resistance from the F_1 to the F_2 in a cross between two lines which do not differ in resistance, all indicate that we are dealing with a multifactorial or polygenic system.

What we seem to have is a large number of different hereditary factors which, when accumulated in certain distinct combinations in a population, produce resistant individuals. To some extent these factors must be dominant, because a cross between a resistant and a nonresistant population gives an F_1 intermediate in resistance. But the dominance relationships must be complex, and must depend on combinations of factors, for the resistance of the F_2, where the chromosomes exist in reshuffled combinations, is still lower. That the dominance of resistance factors is not simply additive is further shown by the fact that in the F_1 of two resistant strains, resistance is no higher than in the parents, although the drop in the F_2 shows clearly that

different factors must have been brought together. In other words, the cumulative effects of the hereditary factors of resistance are produced by non-additive interactions among them.

It is also clear that the drop in resistance from F_1 to F_2 cannot be explained by the relaxation of selection which is necessary in following a cross of this sort. One given line (SyS-1002Rx), taken from SyS-1002 at F_{19} and allowed to breed without further selection, showed no significant drop in resistance after four generations.

How are we to explain the slowness with which a population responds to selection for resistance? One might say that the factors for resistance are not present in natural populations and that one must wait for them to arise by mutation before one can select for them. This is, of course, a possibility. But, unless we assume that treatment with DDT induces such mutations (which is extremely unlikely) if the mutation rate is high enough to give us several mutant alleles at different loci in ten or twenty generations, it would already have produced such alleles in the population before we started our selection.

It seems more likcly that response to selection is slow because what is needed to produce resistant individuals is the chance throwing of rare genetic combinations. Once these appear, their frequency rises in the population because of the adaptive advantage of the individuals carrying them, even though some of them are lost from generation to generation through the process of gamete and zygote formation. Some of these combinations may be rare merely because of the scarcity of the genetic elements which must go into them. Others may be rare because linkage relations keep them from appearing until the occurrence of appropriate but infrequent crossovers. So it is likely that different strains respond to selection at different rates for at least two reasons: (1) they may differ in the amount of genetic heterogeneity at the various loci involved; and (2) the necessary recombinations may be less likely in one strain than in another because of fortuitous differences in linkage relations. These factors could very well explain the absolute and the varying numbers of generations necessary for response in different stocks.

A third possible reason for slowness of response is that the genetic system of a given stock may resist the accumulation of factors making for resistance. (Cf. King, 1954, The genetics of resistance in *Drosophila melanogaster*, Journal of Economic Entomology 47, in press.)

There are two other factors which may contribute to the slow response. The measuremcnt of resistance is imprecise. There are fluctuations in results from treatment to treatment, giving large errors for any figures obtained. Therefore a rather large difference in resistance is necessary before it is possible to demonstrate it statistically. Furthermore, mortality or survival in a given treatment is not dependent on genetic factors alone. Some flies survive because by chance they do not receive a dose as great as the mean dose for all the flies in that treatment. Others die because by chance they receive a dose higher than the mean. Some flies survive because they happen to be in especially favorable physiological condition; and among the dead are some who

were by chance not in such good fettle. So among the survivors selected for breeding there is always a proportion who are there for reasons quite unconnected with their genotypes. These phenomena tend to reduce the speed with which response to selection can be produced and detected.

The information on resistance which has been ferreted out so far is fragmentary. It is not sufficient to form the basis for an ironclad prescription for effective pest control. Continuing investigation will undoubtedly give us more precise information and allow us to establish or reject our present hypotheses and to confect more accurate and complete ones. Nevertheless, the light we now have can be used to cast some slight glimmer on control problems. It seems incontrovertible that we must think in terms of populations and not in terms of resistant and nonresistant individuals. The fact that in the laboratory a 50% level of selective intensity is more effective than a level between 90% and 100% suggests that an excellent way of developing resistant strains in the field would be to use low doses of insecticide over wide areas for long periods of time. It would be worth investigating whether restricting the insecticide to high doses in carefully limited spots and for short periods would not minimize the development of resistance in the wild population. The intermediate tolerance of the F_1 of a resistant times a nonresistant cross, and the further decline in the F_2, suggest that to avoid the development of resistant populations in the field we should keep the number of treated survivors as low as possible and the relative number of untreated individuals high. These suggestions are the most obvious which can be made on the basis of our very incomplete knowledge. Further knowledge should certainly give a firmer ground on which to build suggestions for a program for minimizing the development of resistance in the field.

EFFECTS OF LSD25

H. A. Abramson, L. H. Geronimus, C. Kornetsky, and L. J. Ingraham

Although much scientific investigation is at present in progress all over the world on the way in which the brain cells act to produce physiological and mental activity, not even the mechanisms in the simplest of drugs in everyday use, like the barbiturates, are understood. We are engaged in a program to understand each part of the processs which underlies the cellular metabolism of the brain, not only from the point of view of the brain cells themselves, but also from the point of view of the organism as a whole, that is, the human being—why he acts the way he does, and how abnormal behavior may be brought back into more socialized channels. The work at present may be divided into two parts. One part deals with the cells. The other part deals with man himself.

Half of all hospital beds in the United States are required for the treatment of mentally disturbed patients. About 30% of these patients are suffering from disorders having an unknown etiology and showing no anatomical lesions. These disorders include, among others, the manic-depressive phychoses, pure paranoia, and schizophrenia (dementia praecox). The victims of these mental diseases are generally persons who are potentially vital, and often important, members of society. These conditions may very well be a result of a remediable metabolic defect. Consequently, not only has the search for a physical basis for schizophrenia and related diseases been intensified in recent years, but the need for expansion at the present time appears urgent.

A new avenue of approach to the problem has been made possible by the discovery of a drug which, in extremely small amounts, can produce in normal people mental states which resemble schizophrenia. This drug, lysergic acid diethylamide (LSD25), is a comparatively simple derivative of the ergot drugs used for centuries to control hemorrhage after childbirth. LSD25 can be used to provide a model, readily studied in the laboratory, not only of the behavioral aspects of schizophrenia, but, also, perhaps, of the metabolic deficiencies accompanying this most common of mental disorders. We are studying both the psychological and the biochemical effects of LSD25 and related compounds.

The approach to the basic metabolic problem has been concerned largely with the effect of LSD25 and other ergot derivatives upon the respiration of brain homogenates (preparations ground to a point where the cells are largely destroyed). In experiments using the Warburg-Barcroft apparatus, LSD25, in concentrations between 1×10^{-5} and 1×10^{-4} molar, can partially inhibit the oxygen consumption of homogenates both of fresh guinea pig brain tissue and of frozen bovine and human brain. At least as far as guinea pig brain is concerned *d*-lysergic acid and ergonovine, which are closely related to LSD25 chemically, have no effect. Ergotamine and dihydroergotamine have, if anything, a greater inhibitory effect than does LSD25 (Table I). However, these two drugs are relatively complex and may act at a different point in the metabolic chain. Moreover, a difference can be detected between

TABLE 1

Effect of LSD25 and other ergot derivatives on respiration of guinea pig brain homogenate. Experiment performed in duplicate. Concentration of all drugs, 0.00025 M. Time, 2½ hours. Final pH, 7.6.

	Oxygen Consumption (Microliters)					
	d-Lysergic Acid	LSD25	Ergonovine	Control	Ergotamine	Dihydroergotamine
First Set of Vessels	269.7	267.8	282.0	301.5	224.0	227.9
Second Set of Vessels	271.4	258.5	272.0	280.2	.....	236.0
Total	541.1	525.3	554.0	581.7	≅450.0	463.9

LSD25, on the one hand, and ergotamine and dihydroergotamine on the other, by means of experiments with minced guinea pig brain. Guinea pig brain minces may be expected to behave more like intact tissue than do homogenates. Therefore, it is noteworthy that, whereas a mince exposed to LSD25 shows a decrease in oxygen consumption immediately, just as does a homogenate, a mince exposed to either ergotamine or dihydroergotamine shows initially very little, if any, decrease in oxygen consumption. With these latter drugs, an hour or more must elapse before an effect comparable to the one obtained with homogenates can be seen. Consequently, the over-all effect in an experiment lasting an hour and a half is that LSD25 is more active than even ergotamine and dihydroergotamine (Table 2). Attempts are now being

TABLE 2

Effect of LSD25 and other ergot derivatives on respiration of guinea pig brain minces. Concentration of all drugs, 0.00004 M. Time, 1½ hours. Final pH, 7.2–7.4.

	Per Cent Inhibition of Oxygen Consumption	
	Expt. A	Expt. B
Ergotamine	6.5	
Dihydroergotamine	0	
LSD25	10.2	12.1
Ergonovine		0
d-Lysergic Acid		0

made to isolate the particular enzyme system or systems in brain which are susceptible to LSD25.

Another approach to the search for the mode of action of LSD25 involves the study of certain strains of microorganisms called auxotrophs. These

strains each require the addition of a specific growth factor to the medium for multiplication. We are studying the effect of LSD25 and closely related drugs upon the growth of auxotrophs requiring factors whose chemical formulae resemble parts of the LSD25 molecule. Thus far we have found a strain of *Escherichia coli* requiring nicotinamide for growth which seems to be able to grow to a limited extent in a medium that has been supplemented with either LSD25 or *d*-lysergic acid instead of nicotinamide.

The behavioral part of the problem has so far been confined to the evaluation of a large body of data, on the effect of LSD25 on normal human subjects, amassed by a group of psychiatrists, phychologists, and physiologists at the Mount Sinai Hospital, New York City, working with the same principal investigator.

By subjecting some of these data to the procedures of content analysis, we have reached the following conclusions. Under the conditions of observation, LSD25 in small doses produces many symptoms which are very common in the neurotic states in man. These are represented by symptoms and signs referrable to the autonomic nervous system. As the dose of the drug is increased, approaching 100 micrograms (one five-millionth of a pound) by mouth, the pattern of reactivity alters. At this higher dose level, normal individuals show confusion, distortions of perception, and psychotic symptoms similar to those already noted in the psychiatric literature. Figure 1 demonstrates the relationship between these neurotic and psychotic signs and the dose level. As the dose is further increased, the psychotic pattern prevails, and the lack of communication so typical of the schizophrenic state may supervene.

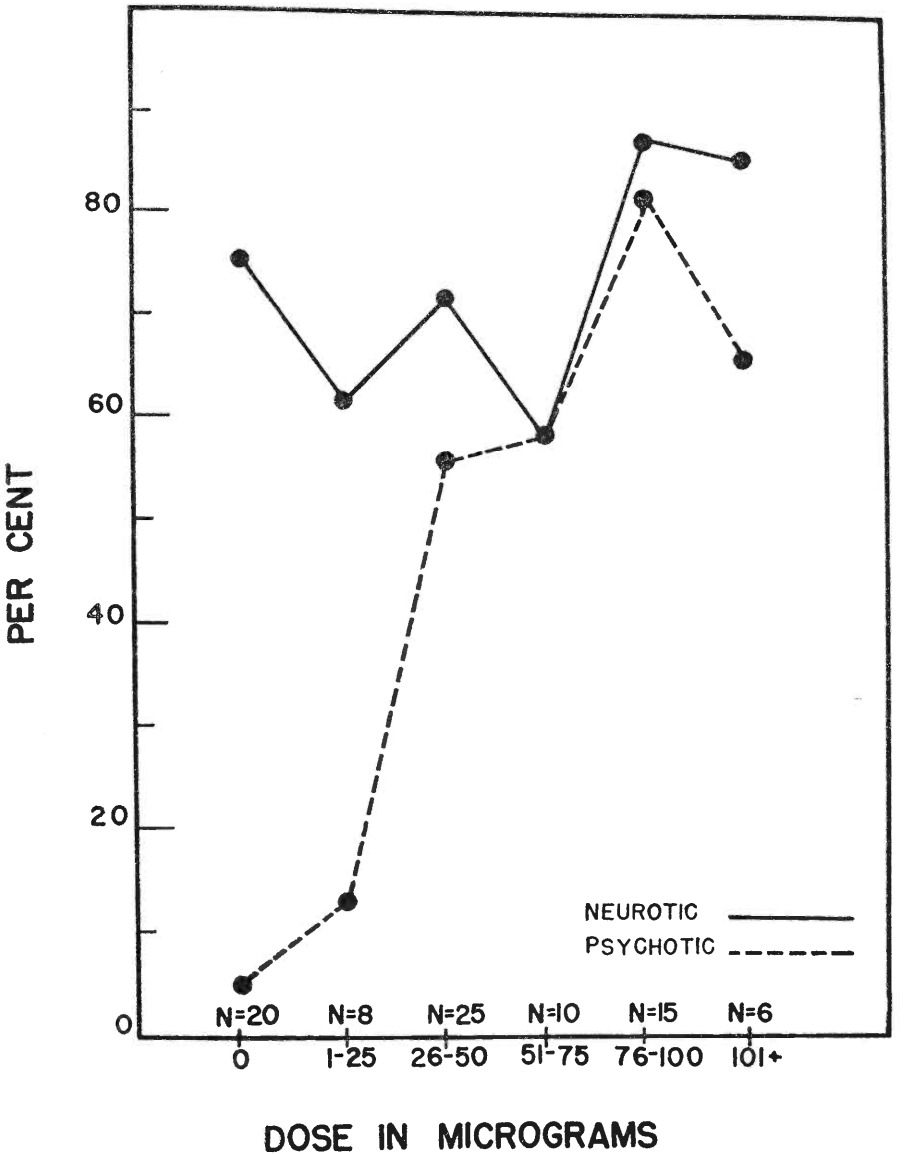

Fig. 1. Percentages of subjects showing neurotic and psychotic symptoms at different levels of LSD dosage.

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REPORTS OF SUMMER INVESTIGATORS

ABRAMSON, HAROLD A., AND JARVIK, MURRAY E., New York, N.Y.—During the past year, in connection with group research on the production of experimental psychoses in man by lysergic acid diethylamide, a good deal of experimental data have been obtained. During the summer a survey was undertaken to ascertain what parts of the symptomatology produced by the drug would be amenable to further analyses, and to possible uses in psychotherapy. Toward the end of the summer the Cold Spring Harbor project was joined by Dr. Conan Kornetsky of the Public Health Service, who is now in residence for the year. Our studies at Cold Spring Harbor, therefore, are continuing with the main project and experimental work being carried out at The Mount Sinai Hospital, New York City.

DELBRÜCK, M., AND KEYNAN, A., California Institute of Technology, Pasadena, Calif.—A study was made of transient growth responses to light of the sporangiophores of Phycomyces sp. The sporangiophores (spph) are unicellular, multinucleate stalks, carrying the spherical sporangium at the top. The spph are about .05 millimeters in diameter and several centimeters tall. They grow at a rate of about .05 millimeters per minute. This growth in length is accomplished by an actual growth in area of the primary wall of a zone of 2 mm just below the sporangium.

The growth rate is approximately the same at any constant light intensity. If the light intensity or the direction of the light undergoes a short transient change ("stimulus"), there may occur a transient change in the growth ("response"). If the direction of illumination is from above, or if it is symmetric from two or more sides, and if the stimulus involves a change of intensity only, then the response consists in a transient change in growth *rate*. A maximal response consists in a doubled growth rate during the period from four to eight minutes after the stimulus. If the stimulus involves a change in the symmetry of illumination, then the growth response may be assymmetric, resulting in a tropic response. The tropic response is directed towards the source of light. It is caused by a greater growth response of the far side of the spph (with respect to the light source) than of the near side. Several previous investigators have proved that the greater response of the far side is due to the cylindrical lens properties of the spph. Castle (1933) has shown that the far side absorbs 10%–20% more of the incident light than the near side.

The feature of greatest interest in these responses is the enormous range of light and dark adaptation of the spph. After a sojourn in complete darkness for a period of about 90 minutes the spph's will respond vigorously to a stimulus lasting a second and of an intensity several hundred thousand times lower than would be needed in the presence of a constant illumination of moderate intensity. We would like to study the kinetics of this "adaptation," quantitatively and in detail, and to discover its underlying mechanism. We believe that current theories of such adaptation phenomena are quite wrong and are based on very inadequate experimental data.

In our experiments during the summer we studied microscopically the following aspects of vertical and lateral growth responses:

1) Growth-rate responses under conditions of periodic symmetric bilateral stimulation.

2) Periodic tropic responses under conditions of periodic unilateral stimulation, alternately from left and right (LR cycles).

3) Periodic bilateral stimulation with LR cycles of the same period superimposed: (a) both cycles in phase, intensity ratio of the two types of stimulation varied; (b) stimulus ratio fixed, phase relation varied.

4) LR cycles suddenly shifting from a low to a high intensity level or vice versa.

5) LR cycles of small amplitude superimposed on a continuous bilateral illumination.

6) Single unilateral stimulus at a defined sensitivity level. The defined sensitivity level was obtained by a preceding regime of 30 minutes of bilateral high-intensity exposure followed by 30 minutes of complete darkness.

7) Rhythmic behavior in the dark and under conditions of simultaneous constant illumination from above and one side.

8) Blaauw and van Heyningen (1925) reported the remarkable finding that growth responses very similar to the light-induced ones occurred in response to exposure to ionizing radiations. Several attempts were made to duplicate these findings, with uniformly negative results. The cause of this discrepancy is unknown.

9) Attempts to control the initiation by the mycelium of spph formation. It would be very desirable to control the time of initiation and the character (diameter, growth rate, sensitivity) of the spph. Growth of the mycelium above, below, and between sheets of dialysis membranes was tried, as well as desiccation, excess humidity, transfer to stale media, etc. These experiments did not yield any useful clues.

Our experiments led to the following principal conclusions: (1) There is no absolute or relative refractory period, following any response, analogous to those observed in true excitatory phenomena. Under conditions of periodic stimulation, responses are observable in response to stimuli following each other at two-minute intervals. Previous reports on refractory periods as long as 30 minutes or more are based on a confusion with adaptations to changes in intensity level. (2) LR cycles of stimulation afford a sensitive means for measuring changes in sensitivity.

DELBRÜCK, MARY BRUCE, Pasadena, Calif.—Observations were made on the Formicidae of the Cold Spring Harbor region. The following ants were collected, classified, and installed in artificial nests for a period of two months, where a daily study was made of their habits. *Lasius alienus americanus* (Formicinae), *Tetramorium caespitum* (Myrmicinae), *Monomorium minimum* (Myrmicinae), *Solenopsis molesta* (Myrmicinae), *Tapinoma sessile* (Dolichoderinae), *Crematogaster linealata* (Myrmicinae), *Camponotus pennsylvanicus* (Formicinae), *Aphaenogaster rudis* (Myrmicinae), *Acanthomyops interjectus* (Formicinae), *Formica subintegra*, and a slave ant, *Formica fusca* Linne (both Formicinae).

In all cases some workers with a number of immature forms were taken, and in most cases a fertile queen was secured as well, so that, save with the Aphaenogaster (which was unable to control molds in its nest) and the Formicas, it was possible to observe egg-laying, care of the larvae and pupae by the workers, transformance of pupae to adult worker and adult winged queens and males, nest construction, and feeding.

In addition, various questions concerning these ants were studied: (a) Tolerance between different nests of ants of the same species. This ranged according to species, the range of behavior including: voluntary merging of two nests, eventual merging after several days of mild animosity, expulsion of one from the nest site, and immediate and violent war ending with the death of large numbers of individuals. (b) Conditions under which a colony of ants will accept a queen of the same species but of a foreign nest. Foreign queens were introduced into Lasius, Solenopsis, Monomorium, Camponotus, and Acanthomyops nests, and in some cases were accepted immediately, or after a few days; in others they were killed. Whether the nest already possessed one of its original queens, or an "accepted" queen, or was queenless, was a factor. (c) Behavior of ants in moving their brood from a short distance back into the nest. The establishment of a scent trail, the duration of effectiveness of this trail, the nest and species specificity of the trail, and the factor of direction of light in conjunction with the trail, were studied.

GOLDFARB, A. ROBERT, Chicago Medical School, Chicago, Ill., and ABRAMSON, HAROLD A., New York, N.Y.—A special fraction of ragweed obtained by purification with methyl alcohol has been studied by means of paper chromatography. These studies confirm earlier work by electrophoretic analysis. By continuing the precipitation with 90 per cent methyl alcohol, further purification was achieved and a more potent fraction of ragweed extract is now available for study. It is planned to undertake a nation-wide survey of the usefulness of this fraction in the therapy of ragweed hay fever and asthma.—Our technique of purification by removal of pigments has been extended to oak-pollen extracts as well as to timothy-pollen extracts, with results similar to those obtained with giant ragweed pollen.

LENNOX, E. S., Fellow of National Foundation for Infantile Paralysis, University of Illinois, Urbana, Ill.—Two separate lines of research were pursued: (1) preliminary investigation of the small bacteriophage phi-X-174, and (2) a continuation of previous research on the protection of *E. coli* strain B by visible light from subsequent ultraviolet irradiation. Phage phi-X-174 grows well on *E. coli* strain C (122 *E. coli* BTCC), on *E. coli* strain H (from Hershey), and on *Shigella dysenteriae* Y6R. Stocks were usually made on C in nutrient broth without added salts. The lysates, filtered through Mandler filters, were found to be stable. There are host-range and plaque-type mutants (easily recognized on agar with vital dyes). Some mutants selected for plaque type or host range are quite unstable. The latent period on C in nutrient broth at 37° C is about 14 minutes with a fairly slow rise. The burst size is between 400 and 500. The ultraviolet killing is exponential over four decades with an ultraviolet cross section about one-third that of

bacteriophage T2. Attempts to demonstrate photoreactivation failed.—The experiments on ultraviolet killing of *E. coli* B showed that the previous light history of the cells had a strong influence. Bacteria were grown in the dark to a density of 10^8 per cc, washed, taken up in saline phosphate buffer, and then aerated in the dark for about 12 hours at 37° C. Aliquots (light sample) were exposed to a strong source of light rich in 3600 A radiation. Then both dark and light samples were exposed to various doses of ultraviolet light. An aliquot of the dark bacteria which were exposed to ultraviolet was then exposed to the same amount of light as the light aliquot. Killing was greatest (for all ultraviolet doses) in the dark sample. Previous light gave marked protection against subsequent ultraviolet but did not reduce the killing as much as light given after ultraviolet. The kinetics of this phenomenon were not clear, and depended upon the medium in which the cells were grown and on the plating medium; moreover, the results varied erratically in different batches of the same medium.

LURIA, S. E., University of Illinois, Urbana, Illinois.—Most of the summer was spent proofreading and indexing a book on *General Virology*. My only experimental work, done in the laboratory of Dr. A. D. Hershey, was on the isolation and chromatographic analysis of the deoxyribonucleic acid synthesized by *Escherichia coli* strain B after infection with phage T2 in the presence of proflavine. The infected bacteria synthesized enough DNA to supply 200 phage particles, and this DNA was of the viral type, containing hydroxymethylcytosine instead of cytosine. It was concluded that proflavine, which prevents liberation of infectious phage, does not act by preventing the synthesis of specific phage DNA.

MANDELL, JOSEPH D., California Institute of Technology, Pasadena, Calif.—Work was carried out on the inactivation of bacteriophage T4*r* by specific serum in distilled water. Experiments were conducted to study the effect described by N. K. Jerne and Lis Skovsted in *Annales de l'Institut Pasteur* (t. 84, no. 1, 1953). A sample of anti-T4*r* horse serum sent by Jerne was used. Both the rapid inactivation of T4*r* by antiserum diluted in distilled water as compared with antiserum diluted in buffered saline, and the dependence of this inactivation in small volumes on a cofactor, reported by Jerne and Skovsted, were confirmed. The inactivation rate constant for T4*r* in buffered saline was found to be about 1500–2000 per minute, whereas in distilled water with cofactor rate constants of 200,000–500,000 per minute were measured.—The rate constants for T2*r* and T6*r*, phages serologically related to T4*r*, were also measured. T2*r* and T6*r* were found to have about the same rate constants, namely, approximately 350 per minute in buffered saline and approximately 35,000 per minute in distilled water plus cofactor.—Gelatin was found to be a potent cofactor, giving the same maximal cofactor activity over a range of 4000 μg to 1 μg per ml.—Kinetic studies are now in progress with the T4*r*–anti-T4*r* system in distilled water with gelatin as a cofactor. The experiments are not yet satisfactorily reproducible, and the factors responsible for the uncontrolled variation are being sought. One complication that initially was not controlled in our experiments was the

instability of highly diluted antiserum in distilled water when stored in the refrigerator in small volume—even in the presence of 100 μg per ml of gelatin.

MESSINA, A. R., Department of Micropaleontology, American Museum of Natural History, New York, N.Y.—During the week spent at the Biological Laboratory, two principal objectives were pursued. The first of these was to determine the type of bottom sediment in which living foraminifera flourish, and the second to collect and maintain them in culture at the Laboratory of Micropaleontology. Fourteen stations were established, from the foot of the dock at the Biological Laboratory, through Cold Spring Harbor and Oyster Bay, out to the south shore of Long Island Sound beyond Lloyds Point. Samples from each of these fourteen stations were collected and examined. It became apparent from this study that living foraminifera were most abundant in those areas where the bottom was fine, soft, and rich in organic debris. Hard, clean, rocky bottoms did not show any living populations. Selective samples from the collection were brought back to the department laboratory. Further observations and study of the group are still being pursued.

SANDOW, ALEXANDER, Department of Biology, Washington Square College, New York University, New York, N.Y.—My work was concerned with the preparation of a paper, "Some observations on iodoacetate rigor of muscle," which was read at the XIXth International Physiological Congress, held at Montreal, August 31 to September 4, 1953. I also began the writing of the corresponding full-length paper, which has now been submitted for final publication. This work involved analysis of a large amount of experimental data accumulated in my New York laboratory and an extensive study of the literature on the various actomyosin models of the muscular contractile system. As a result of this study there germinated certain ideas concerned with utilizing the state of iodoacetate rigor as a new model of the living contractile system. These ideas are now being tested and are bearing fruit in current experimental research (see abstract "Mechanochemical responses of iodoacetate rigor muscle," now in the press of the *Federation Proceedings*).

COURSE ON BACTERIOPHAGES

June 22–July 11, 1953

Instructor: MARK H. ADAMS, New York University College of Medicine.

Assistant: EVELYN WADE, New York University.

The Cold Spring Harbor phage course has now been given for nine consecutive years. This advanced and highly specialized course deals with techniques and current research problems in the field of bacterial viruses. As usual, this year it attracted students from many parts of the United States and some visitors from abroad who wished to become acquainted with this rapidly developing branch of biology. Fourteen students attended, most of whom had the doctoral degree.

E. A. Bevan, Ph.D., Dartmouth College, Hanover, N.H.
Everett Bracken, Ph.D., Vanderbilt University, Nashville, Tenn.
Frank Fenner, M.D., Australian National University, Canberra, Australia.
Lippman Geronimus, Ph.D., Biological Laboratory, Cold Spring Harbor, N.Y.
Arthur P. Harrison, Jr., Ph.D., Vanderbilt University, Nashville, Tenn.
Charles Hurwitz, Ph.D., Veterans Administration Hospital, Albany, N.Y.
Howard Ko, Purdue University, Lafayette, Ind.
David R. Krieg, University of Rochester, Rochester, N.Y.
Mary E. McClain, Ph.D., University of California, Berkeley, Calif.
Julius Marmur, Ph.D., Rockefeller Institute, New York, N.Y.
Eli Reichmann, Ph.D., Harvard University, Cambridge, Mass.
Bethsabee de Rothschild, Columbia University, New York, N.Y.
Robert Rowen, New York University, New York, N.Y.
Murray Seldeen, New York University, New York, N.Y.

In connection with the course a series of lectures was given by students and research workers. The speakers and topics are listed below:

M. H. Adams—Photodynamic inactivation of bacteriophage.
V. Bryson—Mutational patterns in bacteria.
M. Delbrück—Phototropism in fungi.
F. Fenner—Studies in epidemiology of virus diseases.
F. Fenner—Theories of antibody formation.
A. Garen—The adsorption of phage to host cells.
A. D. Hershey—The economy of DNA in phage-infected cells.
J. Marmur—Transformation of pneumococci.
E. Reichmann—Theory and biological application of light scattering.
J. D. Watson—The structure of deoxyribose nucleic acid.

COURSE ON BACTERIAL GENETICS

July 15–August 4, 1953

Instructors: V. Bryson, M. Demerec, and E. M. Witkin, in collaboration with Jean Hemmerly, E. L. Labrum, W. Szybalski, and N. Zinder.

Assistant: Ingbritt Blomstrand.

The course on selected methods in bacterial genetics, first given in 1950, was offered for the fourth time to advanced graduate and postdoctoral 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:

Zlata Demerec, Carnegie Institution, Cold Spring Harbor, N.Y.
John A. Growich, Jr., Ph.D., Lederle Laboratories, Pearl River, N.Y.
Charles Hurwitz, Ph.D., Veterans Administration Hospital, Albany, N.Y.
Donald B. Johnstone, Ph.D., University of Vermont, Burlington, Vt.
Alex Keynan, Ph.D., Israeli Institute of Biological Research, Israel.
David R. Krieg, University of Rochester, Rochester, N.Y.
Frederick C. Neidhardt, Harvard University, Cambridge, Mass.
W. B. Redmond, Ph.D., Veterans Administration Hospital, Atlanta, Ga.
Robert Rowen, New York University, New York, N.Y.
Kenneth B. Tate, Chas. Pfizer & Co., Brooklyn, N.Y.
Fanny B. Warnock, State University of Iowa, Iowa City, Iowa.

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

E. W. Caspari—Genes and mitochondria.
E. D. DeLamater—Bacterial cytology.
M. Delbrück—The fluctuation test.
M. H. Adams—Recombination in bacteriophage.
M. Demerec—Spontaneous and induced mutability in bacteria.
E. M. Witkin—Delayed appearance of induced mutants in bacteria.
N. Zinder—Principles of bacterial transduction.
W. Hayes—Bacterial recombination. (Tape recording.)

NATURE STUDY COURSE

June 29–July 31, 1953

Instructor: PAULINE JAMES, Department of Biology, Pan American College, Edinburg, Texas.

Assistants: MRS. MELVIN R. WOOD, Kingsville, Texas.
BRADFORD WARNER, JR., New York, New York.

The five-week Nature Study Course was conducted in a manner similar to that of previous years. Emphasis on field natural history brought out underlying principles of ecology. Group field trips were made to the many different habitats in the area as well as to other places of interest, including the New York State Fish Hatchery, the Roosevelt Bird Sanctuary at Oyster Bay, and the Bird and Mammal Exhibits at the Bronx Zoo. A number of projects were carried on in Wawepex Laboratory, from which the course was directed. The advanced group showed special interest in a study of the local trees and in clearing and arranging a small spring stream for stocking trout.

The great increase in enrollment made it necessary to divide certain age groups and offer an extra class in order to accommodate all students wishing to participate. This made a total of five classes.

The twenty-four Beginners, six years of age, met on Monday and Wednesday from 9:00 a.m. until 11:00 a.m. The twenty-five younger Juniors, seven and eight years old, met on Tuesday and Thursday from 8:00 a.m. until 10:00 a.m., while the twenty-five Juniors who were nine years old met on Tuesday and Thursday from 10:00 a.m. until 12:00 noon. The nineteen Intermediates, aged ten and eleven, met on Tuesday and Thursday from 2:00 p.m. until 4:00 p.m.; and the nine Seniors, aged twelve or over, met on Monday and Wednesday from 2:00 p.m. until 4:00 p.m.

The Nature Study Course closed on July 31, 1953, 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:

Adams, Gay
Adams, Lucy
Adee, Diane
Allen, John Parker
Arena, Joseph
Asmussen, Christopher
Barnes, Bear
Bartlett, Marshall
Bernstein, Henry
Braman, Bill
Buckley, Larry
Carll, Alan
Carll, David
Christian, Lynn
Clark, Sheldon
Cleaveland, Peter
Delbrück, Jonathan
Dewey, Michael

Donaldson, Sally
Elder, Bobby
Gersh, Frank
Glenn, Susan
Gottlieb, Gail
Grassburger, Frank
Guille, Jimmie
Hewitt, Penny
Hewitt, Vicki
Higgins, Michael
Hoguet, Constance
Itter, William
Jackson, Susanne
Jacobsen, Christian
Jeffery, Stephen
Johnson, Malcolm
Johnson, Mathilde
Johnson, Pamela
Keane, Robert
Keim, Donald
Kepler, Ann
Kepler, Steven
Kernan, Mary
Kingsley, Karen
Kipp, John
Kipp, Ricky
Krusa, David
Langley, Christopher
Leach, Charles
Macy, Victoria
Mathers, Michael
Mathers, William
McDonald, Frances
McIlwain, John
MacKay, Robert
Meirs, John
Melzig, Ricky
Miller, Livingston, Jr.
Montgomery, David
Mullen, Joseph
Norton, William
Osborn, Earl
Osborn, Oliver
Page, Jane
Page, Walter
Pierce, Elizabeth
Pierce, Josiah
Pivnick, Carol
Post, Dorothy
Post, Rosalie
Proskauer, Stephen
Radsch, Tom
Ricardi, Barbara
Ricardi, Richard
Robertson, Anne
Robertson, John
Ross, Charles
Ross, Joe
Rousmaniere, Jimmy
Rummer, Bill
Salkaln, Timmy
Sallee, John
Schieffelin, Julie
Schieffelin, Lindsay
Shemin, Louise
Schock, Steven
Seydel. Gregg
Short, Winthrop, Jr.
Skuda, Dane, Jr.
Stone, Elizabeth
Storrs, David
Titus, Jonathan
Towers, John
Truslow, Sophia
Vacquier, Victor
Walker, Elizabeth
Watkins, David
Warner, Miner Hill
Werner, Peter
Werner, Roger
White, Lynn
White, Stephen
Williamson, Anne
Williamson, John

COLD SPRING HARBOR SYMPOSIA ON QUANTITATIVE BIOLOGY

Current volume: XVIII (1953). Viruses. 318 quarto pages. Table of contents listed below.

THE TRANSITION FROM THE INFECTIVE TO THE VEGETATIVE STATE

HERSHEY, A. D.—Functional differentiation within particles of bacteriophage T2.

MARKHAM, ROY—Chemistry of some functional components of viruses.

PUCK, THEODORE T.—The first steps of virus invasion.

WEIDEL, WOLFHARD—Phage receptor systems of *E. coli* B.

STRUCTURE OF VIRUSES

LANNI, FRANK, and LANNI, YVONNE THÉRY—Antigenic structure of bacteriophage.

SCHÄFFER, WERNER, and MUNK, KLAUS—Studies on the complement-fixing antigen of the fowl plague virus.

LARK, KARL G., and ADAMS, MARK H.—The stability of phages as a function of the ionic environment.

WILLIAMS, ROBLEY C.—The shapes and sizes of purified viruses as determined by electron microscopy.

ANDERSON, THOMAS F.—The morphology and osmotic properties of bacteriophage systems.

JESAITIS, MARGERIS, and GOEBEL, WALTHER F.—The interaction between T4 phage and the specific lipocarbohydrate of phase II *Sh. sonnei.*

BIOCHEMICAL STUDIES OF VIRUS INFECTIONS

KOZLOFF, LLOYD M.—Origin and fate of bacteriophage material.

COHEN, SEYMOUR S.—Studies on controlling mechanisms in the metabolism of virus-infected bacteria.

HOST-CONTROLLED VARIATIONS OF VIRUSES

LURIA, S. E.—Host-induced modifications of viruses.

BOWEN, G. H.—Studies of ultraviolet irradiation phenomena—an approach to the problems of bacteriophage reproduction.

STENT, GUNTHER—Mortality due to radioactive phosphorus as an index to bacteriophage development.

ZINDER, NORTON D.—Infective heredity in bacteria.

BARON, L. S.—Genetic transfer by means of Vi phage lysates.

VIRUSES IN TISSUE CULTURE

DULBECCO, RENATO, and VOGT, MARGUERITE—Some problems of animal virology as studied by the plaque technique.

YOUNGNER, J. S.—Some observations on the behavior of poliomyelitis viruses in tissue culture.

SYVERTON, JEROME T., and SCHERER, WILLIAM F.—Applications of strains of mammalian cells to the study of animal viruses.

FENNER, FRANK—Host parasite relationships in myxomatosis of the Australian wild rabbit.

PREVIOUS VOLUMES

*Vol. I (1933) Surface Phenomena, 239 pp.
*Vol. II (1934) Growth, 284 pp.
*Vol. III (1935) Photochemical Reactions, 359 pp.
*Vol. IV (1936) Excitations, 376 pp.
*Vol. V (1937) Internal Secretions, 433 pp.
*Vol. VI (1938) Protein Chemistry, 395 pp.
*Vol. VII (1939) Biological Oxidations, 463 pp.
*Vol. VIII (1940) Permeability and the Nature of Cell Membranes, 285 pp.
Vol. IX (1941) Genes and Chromosomes, 315 pp.
*Vol. X (1942) The Relation of Hormones to Development, 160 pp.
Vol. XI (1946) Heredity and Variation in Microorganisms, 314 pp.
Vol. XII (1947) Nucleic Acids and Nucleoproteins, 279 pp.
Vol. XIII (1948) Biological Applications of Tracer Elements, 220 pp.
Vol. XIV (1949) Amino Acids and Proteins, 217 pp.
Vol. XV (1950) Origin and Evolution of Man, 425 pp.
Vol. XVI (1951) Genes and Mutations, 521 pp.
Vol. XVII (1952) The Neuron, 338 pp.
*Out of print.

LABORATORY STAFF

* Adams, Mark H.—Bacteriologist, Instructor
Barrett, Audrey E.—Research Assistant
* Bryson, Constance—Technical Assistant
Bryson, Vernon—Geneticist
* Burtch, Ethel—Typist
* Civan, Mortimer—Technical Assistant
‡ Corey, Perl Roy—Carpenter
Cosillo, Gloria—Research Assistant
‡ Deiches, Helen L.—Research Assistant
‡ Elliot, Arthur H.—Laborer
Elliot, Dorothy W.—Technical Assistant
Farrington, Margaret—Technical Assistant
Franzese, Eleanor—Secretary
‡ Fricke, Dorothy N.—Research Assistant
Gardner, Henry—Technical Assistant
Geronimus, Lippman H.—Bacterial Physiologist
* Glass, Alan—Technical Assistant
Haworth, Barbara Jane—Research Assistant
Hepler, Joseph Q.—Bacteriologist
Hershey, Harriet D.—Research Assistant
Ingraham, Laura—Research Assistant
* James, Ina Pauline—Nature Study Course Instructor
‡ King, Alfred—Laborer
King, James C.—Geneticist

‡ KLEM, DOROTHY V.—Secretary
KORNETSKY, CONAN—Psychologist
‡ LOWELL, FRANCIS—Superintendent of Buildings and Grounds
‡ LOWELL, LILLIAN—Technical Assistant
MACIURA, STEPHEN—Carpenter
MADDEN, CAROL V.—Research Assistant
* MAYR, CHRISTA—Technical Assistant
MERLINO, ALDO—Laborer
MERLINO, JOSEPH—Laborer
MURDOCK, ROSAMOND L.—Research Assistant
REDDY, WILLIAM—Laborer
SCHEFFLER, GUDRUN—Business Manager
SCHNUR, GLORIA—Stenographer
SKAAR, PALMER D.—Geneticist
* STACKE, JEANNE A.—Stenographer
‡ STUART, BARBARA—Research Assistant
SZYBALSKI, WACLAW T.—Bacteriologist
TREANOR, ELLEN T.—Maid
THURSTON, ROBERT K.—Superintendent of Buildings and Grounds
* TURNER, NELLIE—Cook
* TURNER, HARRY—Assistant Cook
TUTTLE, SUSAN A.—Research Assistant
VANDER SCHALIE, MARGIE—Research Assistant
* WADE, EVELYN—Assistant Instructor
WALLACE, BRUCE—Geneticist
* WOOD, CYNTHIA K.—Assistant Instructor

* *Summer or temporary.*
‡ *Resigned during the year.*

SUMMER RESEARCH INVESTIGATORS

ABRAMSON, HAROLD A.—New York City
ARMSTRONG, JOHN C.—New York City
DELBRÜCK, MARY BRUCE—Pasadena, California
DELBRÜCK, MAX—California Institute of Technology, Pasadena, California
GOLDFARB, A. ROBERTS—The Chicago Medical School, Chicago, Illinois
KEYNAN, A.—California Institute of Technology, Pasadena, California
LENNOX, EDWIN—University of Illinois, Urbana, Illinois
LURIA, S. E.—University of Illinois, Urbana, Illinois
MANDELL, JOSEPH—California Institute of Technology, Pasadena, California
MESSINA, ANGELINA—The American Museum of Natural History, New York, N.Y.
SANDOW, ALEXANDER—Washington Square College of Arts and Sciences, New York, N.Y.
SHEMIN, DAVID—College of Physicians and Surgeons, Columbia University, New York, N.Y.

REPORT OF THE SECRETARY

The 30th Annual Meeting of the Association was held in the Lecture Hall at Cold Spring Harbor on July 28, 1953, with President Ames presiding and twenty-one members present. The Secretary's report, reviewing the chief acts of the Association during the year, was voted approved. Dr. Demerec, in his report as Director of the Laboratory, spoke first of two events of major importance that had occurred within the past year: the opening of the new Lecture Hall on May 29, 1953, and the purchase of about ten acres of property adjoining the Laboratory from the Estate of Mary E. Jones. Dr. Demerec gave a brief review of the progress of the research program, and reported on the Symposium on "Viruses" held in June, the courses on Bacterial Viruses and Bacterial Genetics, and the Nature Study Course for children. On the subject of finances, he referred to the year's deficit of about $5400, explaining that this was due primarily to the fact that two consecutive Symposium volumes had come out and been paid for within the same fiscal year. During the year $12,000 had been borrowed to pay for the property purchased, and efforts were being made to sell six or eight acres of the Association's land on Moore's Hill in order to repay the loan. Dr. Demerec reported that within the preceding five weeks fifty-five new members had joined the Association, and he expressed gratitude to the many friends who during the year had been contributing members. He also acknowledged the contributions of the Wawepex Society, and the grants received from private foundations and from government organizations in support of the scientific activities of the Laboratory. The Treasurer's report for the year ending April 30, 1953, was approved. Mr. Henry Hicks was nominated and elected Board Member Emeritus. The following members were named by the Nominating Committee and re-elected to the Board of Directors to serve until 1957: Harold A. Abramson, M. Demerec, Stuart Mudd, Robert Cushman Murphy, and John K. Roosevelt. Two directorships were left temporarily vacant. By means of resolutions, adopted by the affirmative votes of a majority of the members present, the Certificate of Incorporation and the By-Laws of the Association were amended so as to change the date of the Annual Meeting from the last Tuesday in July to the fourth Tuesday in June. Dr. Max Delbrück presented a general review of the activities of the Laboratory, and made an appeal for improvement of the facilities available for summer investigators.

The 66th meeting of the Board of Directors was held in the Lecture Hall on July 28, 1953, following the Annual Meeting. President Ames reported that negotiations were under way for the sale of three two-acre lots owned by the Association on Moore's Hill Road. It was resolved, by unanimous vote of those present, that the corporation consent to the proposed annexation of certain territory, including the property owned by the Association, into the Incorporated Village of Laurel Hollow. The Executive Committee for the year was elected as follows: Amyas Ames, Mrs. George S. Franklin, E. C. MacDowell, Grinnell Morris, William B. Nichols, Arthur W. Page, and Mrs. Walter H. Page.

At a meeting of the Executive Committee, held at Cold Spring Harbor on October 29, 1953, Mr. Duncan B. Cox and Mr. Nevil Ford were proposed and elected as members of the Board of Directors to fill the vacancies in the class of 1957.

A special meeting of the Board of Directors was held on October 29, 1953, for the purpose of considering and voting on offers for the purchase of four parcels of land owned by the Association. By means of a resolution, adopted by affirmative vote of a majority of the whole number of directors, the President or Vice-President was authorized to complete the sale of these pieces of property.

A meeting of the Executive Committee was held in Dr. Demerec's office in Cold Spring Harbor on January 31, 1954. Dr. Demerec reported that the highly favorable conditions under which the Laboratory's research program had been operating could not be expected to continue much longer, since research grants are increasingly contingent on the ability of the institution conducting the research to provide the salaries of principal investigators. The problem could be met by the establishment of a special research fund, which would guarantee the salary of one or more principal investigators for a period of years. Without the assurance of such backing, it will become problematical whether necessary research funds can be secured or worthy investigators retained or replaced. A preliminary discussion of the possibility of collecting such a fund led to the recommendation that, as a first step, the research program and specific objectives be formulated in terms that would speak for themselves of the the importance and obvious need for the results of the proposed investigations. On the recommendation of the Treasurer and the Finance Committee, the sale and purchase of certain securities was approved by the affirmative votes of the five members present.

The 67th meeting of the Board of Directors was held in the Lecture Hall at Cold Spring Harbor on January 31, 1954. The minutes of the last regular meeting of the Board were accepted as distributed; and the list of 266 contributors reported by the Secretary was ratified, making them annual members for the current year. The Director of the Laboratory then enlarged upon the theme discussed in the Executive Committee meeting. He pointed out that, although the research of the Laboratory receives support from five different sources, four of these are government agencies, which may be subject to economy measures, and that therefore it would be desirable to broaden the base of support still further. He spoke of the need for accumulating a research fund, which would assure the salary of a first-class research scientist for a period of several years, and would improve the Laboratory's chances of securing additional support through grants. Dr. Demerec mentioned the research project being carried on at the Laboratory by Dr. Abramson. He reported an inquiry received from the administration of the College of Medicine of New York University about the possibility of their establishing a cancer laboratory on land leased from the Association. This possibility received favorable consideration in discussion. The proposed budget for 1954-55 was discussed and accepted.

AUDIT CERTIFICATE

MAIN AND COMPANY
CERTIFIED PUBLIC ACCOUNTANTS
NEW YORK

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

We have made an examination of the accounts of the Long Island Biological Association for the year ended April 30, 1954. 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.

Effective May 1, 1953 the accounting principles followed by the Association were changed so as to reflect in its accounts the value of the inventory of published volumes of the yearly Symposium on Quantitative Biology. In effecting such change net worth was increased by $32,624.04 representing the value of the inventory at May 1, 1953. The aforementioned change had no significant effect on the results of operations for the year ended April 30, 1954.

In our opinion, the accompanying balance sheet and statements of income and expense and net worth, and supporting schedule, together with the note thereon, present fairly the position of the Long Island Biological Association at April 30, 1954 and the results of its operations for the year ended on that date in conformity with generally accepted accounting principles applied on a basis consistent with that of the preceding year except as explained in the foregoing paragraph.

MAIN AND COMPANY
Certified Public Accountants

New York, N. Y.
June 8, 1954

LONG ISLAND BIOLOGICAL ASSOCIATION

BALANCE SHEET

April 30, 1954

ASSETS

General and Endowment Fund			
Cash:			
In banks	$ 38,533.53		
On hand	100.00	$ 38,633.53	
Investments (market value $23,282.78)		21,961.39	
Accounts receivable:			
Josiah Macy, Jr. Foundation	$ 83.72		
United States Department of the Army:			
Chemical Corps	8,526.40		
Office of Surgeon General	12,412.89		
United States Department of the Navy:			
Office of Naval Research	3,249.63		
Miscellaneous	1,788.00	26,060.64	
Inventory of books, at cost	$ 36,657.35		
Less: Reserve for obsolescence	3,600.00	33,057.35	
Land, buildings and equipment:			
Land	$ 84,873.21		
Improvements to land	2,898.01		
Buildings	101,265.00		
Land and buildings leased from Wawepex Society	49,700.00		
Equipment	57,940.32	296,676.54	$416,389.45
Special Funds			
Cash in bank		$ 1,167.12	
Investments (market value $15,710.95)		15,710.00	16,877.12
Total			$433,266.57

LIABILITIES AND NET WORTH

General and Endowment Fund

Liabilities:			
Accounts payable		$ 1,806.42	
Accrued payroll		742.80	
Special grants and contracts:			
The Jane Coffin Childs Memorial Fund for Medical Research	$ 405.27		
Geschickter Fund	1,672.51		
National Tuberculosis Association	1,303.39		
United States Atomic Energy Commission	14,743.36	18,124.53	
Total liabilities		$ 20,673.75	
Deferred income		11,500.00	
Reserve for Scientific Research		9,000.00	
Endowment Fund:			
Dr. William J. Matheson Bequest		20,000.00	
Net worth		355,215.70	$416,389.45

Special Funds

Blackford Memorial Fund:			
Principal		$ 5,000,00	
Charles Benedict Davenport Memorial Fund:			
Principal	$ 4,934.75		
Unexpended income	780.25	5,715.00	
Charles Benedict Davenport, Junior, Fund:			
Principal		1,037.12	
Temple Prime Scholarship Fund:			
Principal	$ 2,500.00		
Unexpended income	205.00	2,705.00	
Dorothy Frances Rice Fund:			
Principal	$ 2,274.96		
Unexpended income	145.04	2,420.00	16,877.12
Total			$433,266.57

LAND, BUILDINGS AND EQUIPMENT
April 30, 1954

Land:			
Purchased with funds raised through public subscription		$52,198.22	
Land purchased from Estate of Mary E. Jones		15,674.99	
Henry W. deForest land		12,000.00	
Airslie land		5,000.00	$ 84,873.21
Improvements to land:			
Pipe line		$ 1,860.39	
Road		746.64	
Light and telephone poles		290.98	2,898.01
Buildings:			
Airslie building		$ 5,000.00	
Blackford Hall*		19,000.00	
Cole Cottage		2,105.00	
Davenport Laboratory		8,500.00	
Henry W. de Forest building		15,000.00	
Reginald G. Harris House		8,500.00	
Dr. Walter B. James Laboratory		13,500.00	
George L. Nichols Memorial Laboratory		13,700.00	
Williams House		11,300.00	
Urey Cottage		2,660.00	
Machine shop and garage		2,000.00	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, 1954

Balance, May 1, 1953	$322,449.26
Add:	
Adjustment to record inventory of published volumes of yearly Symposia of Quantitative Biology as at May 1, 1953	32,624.04
Excess of income over expense for the year ended April 30, 1954	142.40
Balance, April 30, 1954	$355,215.70

STATEMENT OF INCOME AND EXPENSE

For the Year Ended April 30, 1954

Income:			
Contributions:			
Dues and contributions	$ 6,909.90		
Carnegie Corporation (Grant for publication of Annual Symposia)	6,000.00		
Wawepex Society	1,900.00		
John D. Jones Scholarship	500.00	$15,309.90	
Symposia:			
Book sales	$16,155.01		
Registration fees	270.00	16,425.01	
Dining hall		9,304.50	
Rooms and apartments		12,512.07	
Research fees		10,970.80	
Interest and dividends on investments		906.69	
Other income:			
Summer course tuition	$ 1,866.00		
Nature study course	891.20		
Beach permits	615.00		
Annual distribution from Walter B. James Fund	165.00		
Profit on sale of securities	130.08	3,667.28	
Total income			$69,096.25

Expense:			
Symposia:			
Cost of books sold and publication expense	$ 6,794.41		
Expense of participants and lecturers	4,214.00	$11,008.41	
Dining hall		9,308.72	
Rooms and apartments		3,783.31	
Research expenses		1,397.51	
Summer course expense		789.11	
Expense of patroling beach		250.00	
Loss on sale of land		1,852.62	
Interest on loan		391.19	
Uncollectible accounts receivable		326.75	
Distribution of John D. Jones Scholarship		360.00	
Buildings and grounds maintenance:			
Salaries	$11,064.80		
Materials and supplies	5,407.42		
Heat, light and water	3,279.04	19,751.26	
General and administrative:			
Salaries	$ 5,543.16		
Insurance	2,154.64		
Printing and stationery	917.91		
Telephone, telegraph and postage	346.78		
Other	1,172.48	10,134.97	
Provision for obsolescence of inventory of books		3,600.00	
Provision for reserve for scientific research		6,000.00	
Total expense			68,953.85
Excess of income over expense			$ 142.40

STATEMENT OF GRANTS AND CONTRACTS FOR SPECIAL RESEARCH
For the Year Ended April 30, 1954

From Whom Received	BALANCE, MAY 1, 1953		TRANSACTIONS MAY 1, 1953 TO APRIL 30, 1954			BALANCE, APRIL 30, 1954	
	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			$ 8,797.19	$ 6,930.93	$ 193.75		1,672.51
Josiah Macy, Jr. Foundation	$ 231.40		880.00	552.32	180.00	$ 83.72	
National Tuberculosis Association		.76	7,248.06	5,402.71	542.72		1,303.39
United States Atomic Energy Commission	4,030.88		49,637.00	29,883.96	978.80		14,743.36
United States Department of the Army:							
Chemical Corps:							
Studies of Bacterial Resistance	8,254.92		23,357.87	21,603.72	1,962.41	8,463.18	
Studies on Mouse Leukemia	3,022.12		4,916.76	2,925.10	(967.24) *	63.22	
Office of Surgeon General	3,533.26		10,323.15	13,477.05	5,725.73	12,412.89	
United States Department of the Navy:							
Office of Naval Research	715.68		6,151.57	7,785.52	900.00	3,249.63	
Totals	$19,788.26	$406.03	$111,311.60	$88,561.31	$9,516.17	$24,272.64	$18,124.53

* Represents decrease in income to Association resulting from chargeback by United States Department of the Army of prior year's income.

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