LONG ISLAND BIOLOGICAL ASSOCIATION

INCORPORATED 1924

ANNUAL REPORT

OF

THE BIOLOGICAL LABORATORY

FOUNDED 1890

SIXTIETH YEAR

1949
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The Long Island Biological Association

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REPORT OF THE DIRECTOR

Through efforts made during the past few years, the research program of the Laboratory has been developed to a reasonably satisfactory extent, so that this year it did not require much administrative activity to keep it going. More time was therefore available to concentrate on the other needs of the Laboratory, some of which are urgent. Outstanding among them is the necessity of improving the physical condition of our plant.

Since the beginning of the war no major work has been done on our buildings; we have kept up with essential repairs only. But our needs at present go beyond any improvements that can be accomplished in the buildings we now have. In recent years our program has undergone considerable modifications, and new conditions have been created for us by rapid developments in the field of science and by a significant shift of the general interest in scientific research. If the Laboratory is to fulfill the requirements imposed by this new situation, a more extensive development of our facilities is necessary. The following is a list of our most notable requirements:

(1) A lecture hall: for Symposia conferences, and evening lectures; exhibits and teas for the members of the Long Island Biological Association; and recreational activities.

(2) A fund for repair and improvement of old buildings.

(3) A modern fireproof laboratory building, for year-round research projects. This would make the Nichols Laboratory available for summer research.

(4) Dormitory facilities for the year-round research assistants.

The first of these needs has been solved satisfactorily. The Carnegie Corporation has made a grant of $100,000 to the Carnegie Institution of Washington, for a lecture hall at Cold Spring Harbor to be used jointly by the Department of Genetics and the Biological Laboratory. The amount available is ample to build an auditorium that will accommodate the Symposia and evening lectures during the summer and that also can be used for recreational purposes throughout the year. The architect is now preparing the plans, and building can begin at the same time as the construction of the Department of Genetics' new laboratory buildings.

During the past year the Laboratory has lost several good friends with the passing of Mrs. Walter Jennings, Mrs. Acosta Nichols, Mrs. Otto H. Kahn and Mr. John Chase. Mrs. Jennings had been active in support of the Laboratory from the time Mr. Jennings became a member of the Board of Directors of the Long Island Biological Association, and her interest continued to the end. Mr. and Mrs. Nichols, in 1927, gave a fund to the Laboratory for the erection of the George Lane Nichols Memorial Laboratory in memory of their son. This building still serves as administrative headquarters and as the center for year-
round research. Mrs. Kahn showed her interest in the Laboratory by becoming a founder of the Association; and Mr. Chase was one of its patrons.

**Research**

The group under the leadership of Dr. Bruce Wallace, which is investigating heredity in a population exposed to X-rays and radium rays, made an interesting observation important in relation to the dynamic forces operating in evolution. They found that the recessive lethal hereditary changes induced by X-ray exposure are reduced in the population by 50% in only six generations. These "lethals" are by far the most frequent hereditary changes, whether occurring spontaneously or induced by treatment with radiations. They can exist because the units determining hereditary traits are present in pairs, one member contributed by the father and the other by the mother, and the effect of a lethal unit is covered up by the normal member of the pair. The results of Wallace's experiments show, however, that these induced lethals exert some action even when carried together with their normal counterparts, and moreover that this action is sufficiently harmful to make individuals possessing lethals less fitted to survive when in competition with other individuals. This information is of considerable importance for our understanding and evaluation of the forces active in organic evolution, and is of marked interest in predicting what results exposure to radiations may have on the genetic constitution of populations.

From the many interesting results obtained in the experiments with bacteria carried on by Drs. V. Bryson, B. Prytz, and J. Hsie, I shall mention briefly those of more general interest. The Cold Spring Harbor laboratories have been doing pioneer work in research relating to the origin of bacterial resistance to various agents—particularly penicillin, streptomycin, and other antibiotics. This year Hsie undertook an extensive study with two new antibiotics: chloromycetin, and neomycin. The latter is particularly important because it is effective against a group of bacteria that are not affected by penicillin—a group that includes the organism responsible for tuberculosis. Streptomycin is the only other antibiotic known to act on tuberculosis bacilli, and its use is made difficult by the fact that strains of bacteria resistant to streptomycin are found with a very high frequency. The studies made at the Laboratory during the past year have shown conclusively that resistance to neomycin, on the other hand, originates in such a way that a completely resistant bacterial strain will occur only under special circumstances and very rarely. This fact will make neomycin desirable for clinical use, provided a compound of sufficiently low toxicity is developed.

The work of Drs. Bryson and Prytz during the year had as its integrating theme the isolation of bacteria exposed for many generations to poisons and enzyme inhibitors, the investigation of biochemical properties of isolated strains, and a determination of the genetic characteristics of
these strains. The first of these goals has been attained, and progress on the second is detailed in the report of Dr. Prytz. The third objective is under investigation, and already many interesting facts have come to light. Considerable time has been devoted to studying the role of environmental factors, particularly ultraviolet irradiation, influencing the survival of streptomycin-resistant mutants. It has been found that ultraviolet irradiation increases the viability of E. coli mutants capable of growth in 10 units of streptomycin. The survival of these mutants is also conditioned by the presence of certain sugars, and by constituents of the Krebs cycle. Since streptomycin is assumed to act on individual bacterial cells by blocking the terminal respiratory cycle, it is possible that a determination of specific chemical substances that help to overcome the inhibition mechanism will aid in elucidating the mode of action of this antibiotic.

Again this year the largest group among the summer investigators worked with microorganisms—particularly bacterial viruses, bacteria, and fungi. Mark Adams and Gordon Lark, of New York University, found a strain of virus T5, the result of a hereditary change or mutation, which was about 1000 times more resistant to heat in a dilute salt solution than the original strain. Jan Mohr, of the University of Oslo (Norway), and Sol Goodgal, of John Hopkins University, made attempts to induce hereditary changes in bacterial viruses by treatment with ultraviolet radiation and nitrogen mustard. Their results suggested an effect but were not conclusive. W. Braun and K. H. Lewis, of Camp Detrick, Maryland, used a special technique to study the morphology of colonies of a number of mutant strains of colon bacteria, and found that in a majority of cases well-defined differences could be observed, even between mutants exhibiting similar properties such as streptomycin resistance. S. Goodgal also studied the effect of visible light on the survival of mustard-inactivated bread mold (Neurospora) and bacterial virus. He found that light does not affect treated virus, but has some effect on treated mold spores.

Various aspects of metabolism were studied in bacteria by J. S. Gots, of the University of Pennsylvania, in protozoa by I. A. Tittler of Brooklyn College, and in rats by J. A. Stekol of the Institute of Cancer Research, Philadelphia. Again this year, A. W. Bernheimer continued his efforts to find a phage-like virus that would affect protozoa.

H. A. Abramson continued his investigations to simplify the separation by electrophoresis of substances like those that cause ragweed hay fever and asthma. E. Racker, of New York University College of Medicine, studied aldehyde oxidation in connection with problems of alcohol intoxication. B. Jablons, of New York Medical College, conducted experiments dealing with purification of the antipressor principle, called tubulin, present in the mammalian kidney. He studied its effect on peripheral blood vessels of the frog, hamster, and mouse. V. Menkin, of Temple University School of Medicine, made further observations of a necrosin-like substance extracted from clams. S. Granick, of the Rockefeller Institute, worked on the development of a method for introducing large
foreign molecules into the cytoplasm and nuclei of living cells for the purpose of observing their effects on heredity.

Several scientists used their time at the Laboratory for writing. A. Saifer, of Veterans Hospital, Brooklyn, revised for publication a paper entitled “Studies with the quantitative cephalin-cholesterol flocculation reaction,” and prepared the initial draft of a paper on “A series dilution-flocculation method for the determination of gamma globulin and fibrinogen in cerebrospinal fluid.” D. Shemin, of the College of Physicians and Surgeons, wrote a review article and four papers; and U. Fano, of the National Bureau of Standards, worked on lectures and on a book, “Introductory Physics of Atoms and Radiations.”

Symposium

The series of conferences known as the Cold Spring Harbor Symposia on Quantitative Biology was started in 1933 and has been held every summer since then except for three years during the war. The aim of these conferences is to bring together scientists working on different aspects of some particular problem, and to give them an opportunity of discussing their work at leisure in the congenial environment of the Laboratory.

The 1949 Symposium was held June 8 through 16, with an unusually large attendance of 186 scientists, mostly in the field of biochemistry since the subject was “Amino Acids and Proteins.” Amino acids are constituent parts of proteins, which of course are among the most important components of living matter. The symposium meetings dealt with fundamental aspects of the various problems encountered by research workers in studying these compounds. The more we can learn about the component parts of proteins, and particularly about the way in which they are joined together, the sooner it will be possible to work out the details of their chemical structure. These details are important for understanding the functions that proteins perform in living cells. Therefore, most of the symposium sessions were concerned with methods for splitting proteins into simpler compounds in order to determine their structure. In addition to standard chemical analysis, some of these methods involved the application of electric currents, high-speed centrifugation, column separation, X-ray diffraction pictures, and enzymatic action.

An important step forward in this field was reported by Dr. F. Sanger, of the University of Cambridge, who has made a detailed analysis of the proteins contained in insulin. Dr. Kai O. Pedersen, of the University of Upsala, described experiments making extensive use of the ultra-centrifuge; and Dr. Kaj Linderstrom-Lang, director of the Carlsberg Laboratory, Copenhagen, spoke on the breakdown of proteins by enzymes. An ingenious automatic method was described by Drs. W. M. Stein and S. Moore of the Rockefeller Institute in New York, whereby amino acids can be separated from a complex mixture, or from broken-down tissues,
and used to determine the exact amounts present in the material being investigated.

Most of the reports dealt with the analysis of chemical compounds either isolated from living cells or prepared in the laboratory. Some, however, described work done directly with living cells, such as bread mold (Professor S. Emerson, California Institute of Technology), bacteria (Professor J. S. Fruton, Yale University School of Medicine), liver tissue (Professor J. M. Luck, Stanford University), normal and cancerous tissues (Dr. P. C. Zamecnik, Harvard University), and various organisms (Professor J. F. Danielli of the University of London, and Dr. B. P. Kaufmann, Miss Helen Gay, and Dr. Margaret McDonald of the Carnegie Institution at Cold Spring Harbor).

Seven of the speakers were invited from Europe: Dr. Dorothy Crowfoot, Oxford University, England; Dr. J. F. Danielli, University of London; Professor Claude Fromageot, Faculte des Sciences, Paris; Dr. Kaj Lindstrom-Lang, Carlsberg Laboratory, Copenhagen, Denmark; Dr. Kai O. Pedersen, University of Upsala, Sweden; Dr. F. Sanger, University of Cambridge, England; and Dr. R. L. M. Synge, Rowett Research Institute, Bucksburn, Scotland. Since Dr. Synge was unable to get here, his paper was read by Dr. Stanford Moore, one of the American speakers.

Other Europeans attending the symposium came from London and Birmingham, England; Berlin and Frankfort, Germany; Copenhagen, Denmark; and Belgium. In addition, 14 states were represented by the registrants, many of whom were from the New York area. Other Long Island laboratories represented were the Brookhaven National Laboratory at Upton, the National Dairy Research Laboratories at Oakdale, Hofstra College at Hempstead, and the Carnegie Institution at Cold Spring Harbor.

Nine days of fair weather were enjoyed by the group, who held a number of informal gatherings in addition to the three meetings scheduled each day. Two evenings of folk dancing were led by Dr. E. C. MacDowell of the Carnegie staff; and a picnic trip to Jones Beach on Tuesday afternoon, June 14, was organized by the Laboratory.

All papers prepared for the symposium, and much of the material presented in discussions, were published in book form as Volume XIV of the Cold Spring Harbor Symposia on Quantitative Biology. The table of contents of the volume is reproduced on pages 43 and 44.

**Teaching**

The Nature Study Course was given by Dr. Pauline James, of the Department of Nature Study, Cornell University. This course is designed to stimulate interest in nature among young people, by showing them how to observe the many interesting plants and animals around them, by teaching them how to answer the questions raised by their ob-
servations, and by helping them realize that careful and accurate study of the smaller incidents observed by all of us contributes greatly toward expanding our knowledge of natural phenomena. The course was divided into four sections, according to the ages of the pupils, and was attended by sixty-three young people. On the afternoon of the closing day a public exhibition was held to demonstrate the activities of the various classes to parents and friends.

For the fifth successive year an intensive three-week course was offered in techniques and problems of research with bacterial viruses. It was taught by Professor Mark H. Adams of the New York University College of Medicine. There was a capacity enrollment of fourteen students taking both lectures and laboratory, plus three more who attended only the lectures. A series of seven special seminars was arranged in connection with this course.

Lectures

Regular lectures were held throughout the summer, in cooperation with the Department of Genetics of the Carnegie Institution. The speakers were summer members of the Laboratory, and arrangements were made by Dr. Bruce Wallace of the Laboratory staff. A list of speakers and titles is given below:

August 11: Werner Braun, Camp Detrick, Maryland. Dissociation in Brucella abortus.
August 18: Ernst W. Caspari, Department of Genetics. Studies on gene action in Ephestia.

Special Events

Two illustrated lectures of general interest were given during the summer for members of the Association and their friends. On the evening of July 14 Mr. Victor W. von Hagen, explorer and writer, described his experiences in travels through South America. His lecture was entitled, "From the Rio Putumayo to Popayan (Colombia)." On Thursday evening, September 22, Dr. Robert Cushman Murphy, president of the Asso-
ciation and chairman of the Department of Birds at the American Museum of Natural History, spoke on the subject of his travels in New Zealand, with illustrations in color.

A demonstration of the research work being done at the Laboratory and at the Department of Genetics was held in Blackford Hall on Sunday afternoon, September 18, and was well attended by members and friends of the Association. Scientists from the two laboratories explained the exhibits and discussed their work with the guests; and tea was served by the Women’s Committee of the L.I.B.A.

Dining Room

The Blackford Hall dining room was in operation from June 6 to September 6, and accommodated both the members of the Biological Laboratory and the resident members of the Department of Genetics. During the Symposium period it served meals to more than one hundred persons, and during the remainder of the summer to between fifty and sixty persons. Mrs. Lillian Yongen acted as dining-room manager.

Laboratories and Equipment

During the year the Dr. Walter B. James Memorial Laboratory building was re-equipped for work with fruitflies (Drosophila), which are being used in studies of the effects of continuous irradiation on the structure of populations. Thermal controls were installed in two rooms, to keep temperature constant at 20 degrees centigrade. One of these rooms, used for radium treatment, was insulated with sandbags. Air-cooling units were installed in two laboratories. One room was equipped for washing and sterilizing culture containers, and preparing media. Seven low-power binocular microscopes and one high-power research microscope were acquired for this work.

Buildings and Grounds

During 1949 only essential work was done on buildings and grounds. This consisted of putting new roofs on the Dr. Walter B. James Memorial Laboratory and Hooper House, painting the exterior of the James Laboratory and of Williams House, and painting several rooms in the residence buildings.

Finances

The expenses of the Laboratory fall into two well-defined categories: running expenses (administration, operation, and summer activities), and expenses of the full-time research program. The first of these is covered by the dues and contributions of the members of the Long Island Biological Association, the contributions of the Wawepex Society, the interest on securities, and the income connected with various Laboratory activities (rentals, overhead of research projects, courses). This group of expenses includes the upkeep of buildings and grounds, an item for which the need
is particularly great; and in order to meet it our present income will have to be increased.

The expenses of full-time research are covered by grants received from the Jane Coffin Childs Memorial Fund for Medical Research, the National Tuberculosis Association, the Army Chemical Corps, and the Atomic Energy Commission.

Through the initiative of Mrs. Lewis E. Pierson, Jr., a fund of $1793 was raised as a memorial to victims of the recent poliomyelitis epidemic, which was particularly severe in the West Hampton Beach locality. This sum has been turned over to the Laboratory, to be used for fundamental research with viruses, the group of organisms to which the causative agent of polio belongs.

Acknowledgments

It gives me great pleasure to acknowledge the support given to the Laboratory by the members of the Long Island Biological Association. At present only the smaller part of the total expenditures of the Laboratory is covered by the contributions of the membership; but this part of the budget provides for upkeep and overhead expenses, and is most essential for the existence of the Laboratory.

The Women's Committee, under the presidency of Mrs. George S. Franklin, made an important contribution toward the support of the scientific work of the Laboratory; and its House Committee, under the chairmanship of Mrs. Percy H. Jennings, collected contributions for the furnishing of residences. Mrs. Gordon Rentschler contributed a considerable number of pieces of furniture. Mrs. Ashton Hawkins served as chairman of the committee on arrangements and catering for the Open House Tea and Exhibit in September.

Acknowledgment is made of the contribution of the Wawepex Society toward the upkeep of buildings and grounds, of the John D. Jones Scholarship maintained by that Society, and of a special contribution made this year for the reroofing of Hooper House.

The Laboratory recognizes with gratitude the research grants made by the Jane Coffin Childs Memorial Fund for Medical Research, the National Tuberculosis Association, the Army Chemical Corps, and the Atomic Energy Commission; and the contributions for basic research with viruses raised through the efforts of Mrs. Lewis E. Pierson, Jr. The grant of the Carnegie Corporation again provided funds for expenses of the Symposium, particularly for the considerable travel expenses of foreign guests.

With much pleasure I acknowledge the interest shown by the Carnegie Corporation in the work of the Laboratory, expressed once more by a recent grant made to the Carnegie Institution of Washington for construction of a lecture hall to be used jointly by the Department of Genetics and the Laboratory.

M. Demerec
Director of the Laboratory;

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Further experiments involving the effect of irradiated media on bacterial multiplication and mutation have placed this work on a more quantitative basis. It is now clear that the greater resistance of radiation-resistant strains of Escherichia coli to irradiated media can be demonstrated only in populations below a concentration of $10^7$ cells per ml. At high concentrations of cells the radiation-sensitive strain also is resistant to irradiated media. The elimination of bactericidal and bacteriostatic effects of irradiated broth by large inocula suggests that the toxic substances previously described as inactivated by catalase and reducing agents are effectively bound or inactivated by large numbers of cells, so that the toxic threshold is not reached. A parallel is suggested with the work of Dale, in which the total amount of an enzyme destroyed by a fixed dose of X-ray irradiation is relatively constant, but the percentage destroyed is related to the total quantity present.

Attempts have been made to confirm reports of Stone, Wyss, and Haas on mutagenic effects of irradiated media, utilizing the system intensively employed by Demerec and co-workers. This consists of growing cells in small tubes of broth and plating the entire culture at the completion of growth in nutrient agar containing streptomycin. The frequency of cultures containing one or more streptomycin-resistant mutants, as indicated by the formation of a colony in streptomycin agar, can be used to determine the rate of mutation by the formulae of Luria and Delbruck. Nutrient broth was irradiated at a distance 7.6 cm from a 15-watt mercury vapor lamp for a period of seven hours, in quantities of 15 ml per Petri plate. The mutation rates, as calculated by the zero-culture method of Luria and Delbruck, are summarized for a series of separate experiments in the table below. Each experiment is of necessity small because the amount of broth that can be obtained at one time is limited by the allowable depth of the exposed liquid, the length of the mercury vapor tube, and evaporative loss. Zero cultures are those not containing any cells, among the bacteria of fully grown cultures, that can form colonies when the entire culture is plated in 25-unit streptomycin nutrient agar. In this and ensuing work the dihydro form of streptomycin was used.

<table>
<thead>
<tr>
<th>Expt. No.</th>
<th>Total Cultures</th>
<th>Mean Assay ( \times 10^8 )</th>
<th>Zero Cultures</th>
<th>% Zero Cultures</th>
<th>Mutation per ( 10^{10} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-irradiated</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No. 1</td>
<td>17</td>
<td>6.0</td>
<td>15</td>
<td>88</td>
<td>1.5</td>
</tr>
<tr>
<td>No. 2</td>
<td>16</td>
<td>7.5</td>
<td>14</td>
<td>87</td>
<td>1.3</td>
</tr>
<tr>
<td>No. 3</td>
<td>16</td>
<td>3.4</td>
<td>14</td>
<td>87</td>
<td>2.8</td>
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<tr>
<td>No. 4</td>
<td>15</td>
<td>3.1</td>
<td>13</td>
<td>87</td>
<td>3.1</td>
</tr>
<tr>
<td>No. 5</td>
<td>20</td>
<td>3.8</td>
<td>16</td>
<td>80</td>
<td>4.1</td>
</tr>
<tr>
<td>No. 6</td>
<td>20</td>
<td>3.1</td>
<td>15</td>
<td>75</td>
<td>6.4</td>
</tr>
</tbody>
</table>
Conventional statistical methods of comparing standard errors cannot be correctly employed in dealing with very small numbers in the order of magnitude of mutation rates. It can alternatively be calculated that the higher mutation rate obtained in each experiment with irradiated media could occur on the basis of a random difference in \((0.5)^6\) or about 1.6% of repeated duplicate series of experiments. It appears reasonable to conclude that the irradiated medium may be mutagenic, although the effect is only moderate and confirmatory experiments are required.

In the process of obtaining cells resistant to specific poisons and enzyme inhibitors, it is a routine procedure to grow cultures at varied concentrations of chemical, selecting survivors that are capable of further growth at higher chemical levels. It appeared of interest to determine if mutagenic agents would be active at concentrations inadequate to prevent cell multiplication. If this were true, any routine attempt to build up the resistance of a stock to specific chemicals could at the same time be used as a test to uncover new mutagenic agents. Limited tests have not yet revealed new mutagens by examination of bacteria grown in the presence of chemicals. Analysis is made for abnormally high "backgrounds" of streptomycin-resistant or phage-resistant cells. As a type of control, cells were grown in the continuous presence of ultraviolet radiation, an agent known to be mutagenic. These experiments conducted on a larger scale indicate that ultraviolet irradiation will produce mutation at levels compatible with cell multiplication. It would be an oversimplification to assume that growth and viability are normal in these experiments. Lower titers are commonly observed in cell populations grown in the presence of chemical or physical agents. The many abnormal forms observed cytologically after growth under ultraviolet light render it probable that some killing has occurred. The proportion of mutants recovered with ultraviolet treatment is very high, because the method permits establishment of clonal populations as descendants of single mutants.

We have made a study of the effects of ultraviolet light on the expression of streptomycin-resistant E. coli mutants. It was observed that if cultures of E. coli grown in synthetic media are irradiated with ultraviolet at a dose adequate to kill approximately half the cells, there is a great increase in the number of colonies obtained when the surviving cells are plated in nutrient agar containing ten micrograms of streptomycin per ml. The following table shows the numbers of colonies of E. coli observed after plating samples of cells irradiated with different doses of ultraviolet into nutrient agar containing 10 micrograms of streptomycin per ml.
The colonies are derived from a plating of about $8 \times 10^8$ cells in nutrient agar containing 10 micrograms of streptomycin per ml. Most of the colonies obtained are small, and would have been unable to grow at 25-microgram concentrations. Such low-grade mutants have been described by Demerec and are easily distinguished from normal when streaked on streptomycin agar. Approximately 1% of the survivors are normal overlaps.

The experiment represented by the table was designed to test whether mutation is a function of the dose of ultraviolet energy, regardless of survival, or is more closely related to survival level. For this reason, the radiation-sensitive strain B and several other stocks (B/r, B/M, and 43) known to be radiation-resistant were used. The absolute increase in numbers of colonies following low doses of ultraviolet has been found to depend not on mutation, but on selection, and to involve a complex of factors whose existence can only be summarized in this brief report.

The first unusual finding with this material was the fact that at a concentration of approximately $4 \times 10^8$ cells per ml, or more, the number of colonies obtained in 10-unit streptomycin nutrient agar became less as the number of cells plated was increased, the direct opposite of what would be anticipated. This experiment was performed by plating different concentrations of cells without any irradiation or supplementary carbohydrate. It was therefore obvious that when large numbers of cells are plated in streptomycin agar, certain mutants fail to form visible colonies. These mutants must nevertheless be present in the inoculum, and apparently can be made to grow if the environment is modified by the addition of glucose and treatment with a low dose of ultraviolet light. The ultraviolet treatment does not seem to produce a simple streptomycin inactivation, allowing added colonies to grow, since, if irradiation is omitted and lower concentrations of streptomycin are substituted for the 10-unit level, an entirely different result is obtained, with smaller numbers of colonies observed at inhibitory concentrations of the antibiotic.

Any marked increase in survivors following ultraviolet irradiation and plating in 10 units of streptomycin requires the presence during growth of low concentrations of d-glucose, l-fructose, or d-mannose. Preliminary experiments indicate that some other representative related carbohydrates may produce the effect, and that as the concentration of sugar is raised the specificity becomes less critical. Many carbohydrates will increase the survival level of unirradiated cells in streptomycin. According to the Embden-Meyerhof-Parnas scheme of glycolysis, the three sugars, glucose, fructose, and mannose are capable of direct phosphor-
lation to fructose-6-phosphate through the activity of hexokinase. Most other carbohydrates are initially phosphorylated by a different route. These three sugars possess several properties in common, and so it would not be possible at present to state the basis for their biological specificity. Since current concepts of streptomycin inhibition infer that the terminal respiration cycle may be blocked by streptomycin, it is conceivable that an alternative source of some essential metabolite through fermentation might enable low-grade mutants and possibly a few normal cells to survive low doses of streptomycin. Thus inhibition of the effects of streptomycin, enabling the expression of low-grade mutants, may be provided directly by high concentrations of several carbohydrates or by ultraviolet irradiation of cells and low concentrations of a more restricted group of sugars. The influence of high concentrations of carbohydrates has been ascribed by other investigators to an acid inactivation of streptomycin.

The close structural similarity of this antibiotic to sugars suggests to us that the streptomycin-inhibiting effects of certain carbohydrates may occur through a system of competitive or noncompetitive antagonism. The carbohydrate would act by blocking the reactive sites of streptomycin action, or by providing a substitute source of an essential metabolite for which streptomycin acts as a structural analogue. It has been found that ultraviolet-inactivated cells exude extracellular materials detectable by ultraviolet spectrophotometry. The theory that these substances may be causally associated with the reduced effectiveness of streptomycin following irradiation of cells is under investigation. When very low concentrations of carbohydrate are employed it is experimentally evident that the majority of colonies growing in streptomycin nutrient agar are mutants, with variant morphology detected by streaking on MacConkey. Their survival in the presence of glucose and some other sugars is perhaps due to synthesis by the cell of a compound structurally similar to or derivable from the sugar-like portions of the streptomycin molecule. It is of particular interest to note that some strains of E. coli ordinarily described as dependent upon streptomycin for growth will form profuse filaments turbid cultures in streptomycin-free medium containing only nutrient broth and 4% glucose.

This paper is based upon work done for the Biological Department, Chemical Corps, Camp Detrick, Frederick, Maryland, under Contract No. W-18-064-CM-223 with the Long Island Biological Association.
Comparative Biochemical Studies of Mutant Strains of Bacteria

Bo Prytz and Constance Raymond

The biochemical work initiated last year on the chemical and biochemical properties of Escherichia coli and some of its mutants was continued. The bacterial strains studied were: Strain B, sensitive; B/r, radiation-resistant; B/r/sd, radiation-resistant and streptomycin-dependent; B/r/s, radiation-resistant and streptomycin-resistant; B/4, T4 phage-resistant, proline-nondependent; B/4, T4 phage-resistant, proline-dependent; B/M, nitrogen mustard-resistant; B/1,5, T1 and T5 phage-resistant.

The organisms were grown on a synthetic (M-9) medium with glucose as the only carbon source. The cultures were incubated at 37° C with continuous aeration, and harvested by centrifugation at the end of their actively growing stage. In order to free the cells of all chemical contaminants they were washed twice with distilled water by centrifugation and finally brought up to a standard concentration in distilled water by adjustment of the turbidity.

A detailed analytical study was carried out on the cells. This included analyses of total dry weight, total nitrogen, total phosphorus, protein phosphorus, nucleic acid phosphorus, inorganic phosphorus, total nucleic acid, deoxyribose nucleic acid, and ribose nucleic acid. Some of the standardized cultures were also analyzed for the total number of cells per ml of suspension. The following table shows the average results, calculated as per cent of the dry weight found:

<table>
<thead>
<tr>
<th></th>
<th>B</th>
<th>B/r</th>
<th>B/r/sd</th>
<th>B/r/s</th>
<th>B/4</th>
<th>B/M</th>
<th>B/1.5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Per cent of bacterial dry weight</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total nitrogen</td>
<td>10.04</td>
<td>15.45</td>
<td>17.83</td>
<td>26.76</td>
<td>14.38</td>
<td>23.18</td>
<td>12.54</td>
</tr>
<tr>
<td>Total phosphorus</td>
<td>1.82</td>
<td>2.46</td>
<td>4.68</td>
<td>4.73</td>
<td>2.48</td>
<td>3.45</td>
<td>2.14</td>
</tr>
<tr>
<td>Protein phosphorus</td>
<td>0.27</td>
<td>0.28</td>
<td>0.57</td>
<td>0.66</td>
<td>0.42</td>
<td>0.46</td>
<td>0.24</td>
</tr>
<tr>
<td>Nucleic acid phosphorus</td>
<td>1.05</td>
<td>1.53</td>
<td>2.60</td>
<td>2.88</td>
<td>1.52</td>
<td>2.12</td>
<td>1.38</td>
</tr>
<tr>
<td>Inorganic phosphorus</td>
<td>0.30</td>
<td>0.42</td>
<td>0.56</td>
<td>0.81</td>
<td>0.47</td>
<td>0.62</td>
<td>0.45</td>
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<td>Nucleic acid</td>
<td>11.8</td>
<td>16.3</td>
<td>25.9</td>
<td>34.2</td>
<td>16.1</td>
<td>23.7</td>
<td>15.8</td>
</tr>
<tr>
<td>Desoxyribose nucleic acid</td>
<td>2.7</td>
<td>2.1</td>
<td>4.6</td>
<td>6.1</td>
<td>3.2</td>
<td>3.5</td>
<td>2.4</td>
</tr>
<tr>
<td>Ribose nucleic acid</td>
<td>8.5</td>
<td>8.5</td>
<td>15.1</td>
<td>27.0</td>
<td>15.0</td>
<td>19.0</td>
<td>8.73</td>
</tr>
<tr>
<td>Bacterial dry weight</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>mg per ml</td>
<td>1.67</td>
<td>1.25</td>
<td>0.87</td>
<td>0.62</td>
<td>1.47</td>
<td>0.72</td>
<td>1.45</td>
</tr>
</tbody>
</table>

A glance at this table will show that chemical differences appear to exist between the sensitive strain B and some of the mutants, although they are only noticeable when the percentage of total nitrogen is compared with the number of milligrams of bacterial dry weight per ml. The figures indicate that there exists an inverse ratio between these two values, so that a strain with a low total nitrogen possesses a high dry weight, and vice versa. Since the total nitrogen includes protein, nucleic acids,
and nonprotein nitrogen, some compound other than a nitrogenous one must make up the dry weight of the high-dry-weight, low-nitrogen strains. This might be carbohydrate, fat, or organic acids; or it might be that some strains were dehydrated more easily and more thoroughly than some other strains, and that the high dry weight was caused by water of hydration. This latter possibility was eliminated by taking several samples of bacterial suspensions and drying them under high vacuum and at elevated temperature for an extensive period of time. The dry weights reported above, obtained by the standard procedure—that is, drying over concentrated H$_2$SO$_4$ under vacuum at room temperature—did not change with the more drastic and prolonged treatment. Nor did the use of the Karl Fischer reagent indicate any water present in the bacterial residue, and, furthermore, the bacterial residue obtained by drying was not noticeably hygroscopic. A fat extraction was carried out on several of the bacterial residues obtained by drying, but no measurable amount of fatty material was extracted.

Although all the cultures were harvested at approximately the same stage in their growth phase, it appeared desirable to analyze the various strains at different points of the growth cycle. Since the nitrogen-dry weight ratio was the outstanding variable found, this ratio was used to determine the strain differences at three points of the growth phase: namely, in the initial, actively growing stage; at the end of the log phase; and 10 hours later. The analysis included the determination of dry weight, percentage of total nitrogen, percentage of protein nitrogen, and, in several samples, percentage of total reducing sugars on an acid hydrolysate of the bacteria. In no case was there found any correlation between the analytical figures and the growth stage at which the organism was analyzed. The percentage of total reducing substances found, calculated as glucose, amounted on the average to about 1.5% of the dry matter and did not account for the discrepancies noted earlier. The protein nitrogen averaged about 70%–75% of the total nitrogen.

The analytical figures reported here are stated in terms of per cent of dry matter. When the figures are viewed on a basis of per cent of bacterial-suspension volume, no such strain-differences are found. This will indicate that the normal variations found in the determination of bacterial dry weight are quite large, and that there probably are no essential differences in the chemical composition of the various strains analyzed. With our present analytical tools such differences, if present, will be very difficult to demonstrate.

Another approach to the problem of strain differences between a sensitive organism and its mutants is the biochemical, in which the metabolic function of the organisms is analyzed. One such metabolic function is the respiratory enzyme system of living organisms. One specific group of this system consists of the dehydrogenase enzymes, which oxidize various substrates by the removal of hydrogen atoms. The standard procedure used for studying this particular group of enzymes is the Thunberg methylene blue technique, in which the enzyme catalyzes the
oxidation of the substrate—the hydrogen donator—and thereby reduces the methylene blue compound—the hydrogen acceptor—to the colorless leuco base in the absence of oxygen. The speed with which this dehydrogenation takes place under optimum conditions can be used as a quantitative measure of the physiological activity of the cells.

Cultures of E coli, strain B, and the various mutants mentioned above, prepared as previously described, were tested on a series of substrates by the Thunberg technique. Differences observed in time of decolorization showed that the sensitive B strain possessed a higher activity than any of the mutants, and that B/r/s and B/r/sd were lowest in activity.

In order to get a more quantitative picture of the dehydrogenase activities than is possible with the methylene blue technique, a method was worked out using triphenyltetrazolium hydrochloride as the indicator dye. This dye is colorless in the oxidized stage and red when reduced. It has the advantage that the reaction can take place in the presence of atmospheric oxygen, since the reduction is irreversible, and the colored compound, which is insoluble in water, can be extracted and measured photometrically.

Living cells absorb the dye and deposit the red compound inside the cell. This means either that a cell blank has to be run with every sample, so that the dye absorbed by the cells can be taken into consideration in the calculation of the activity, or a means has to be developed whereby the dye can be extracted from the cells. The dye is soluble in many organic solvents, but none of the solvents tried extracted the dye completely and in several cases an emulsion formed when the bacterial suspension was shaken with the solvent. It was finally found that a mixture of acetone and ether accomplished the desired result, leaving a colorless bacterial suspension behind and extracting all the dye.

The method outlined above gives an exact quantitative measure of dehydrogenase activity, stated in absolute values as milligrams of dye reduced in a given time by a standardized cell suspension. The following table shows the average results obtained by this method on a number of substrates:

<table>
<thead>
<tr>
<th></th>
<th>10^−2 mg triphenyl-tetrazolium HCl reduced per mg bacterial N₂ in 10 minutes</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>B</td>
</tr>
<tr>
<td>Glucose</td>
<td>21.0</td>
</tr>
<tr>
<td>Lactate</td>
<td>5.8</td>
</tr>
<tr>
<td>Pyruvate</td>
<td>6.2</td>
</tr>
<tr>
<td>Acetate</td>
<td>3.3</td>
</tr>
<tr>
<td>Formate</td>
<td>6.8</td>
</tr>
<tr>
<td>Glycerophosphate</td>
<td>4.5</td>
</tr>
</tbody>
</table>
It is clearly evident from this table, first, that there is a very active glucose dehydrogenase present in the organisms studied, and second, that in all the cases studied the streptomycin-dependent variant possesses a lower activity than the other strains. Also it would appear that the dehydrogenase activity diminishes as the streptomycin resistance increases, from the resistant strain to the dependent strain. The biological significance of this fact may be that as the streptomycin resistance and dependence increases the organism loses its ability for anaerobic growth and becomes dependent upon the availability of oxygen for its normal growth cycle.

Two more enzymes involved in the oxidation-reduction system of bacteria were investigated. A series of catalase determinations was carried out on each organism, and the monomolecular constant for the reaction was calculated for each organism. No significant differences were found between the sensitive strain B and the mutants.

Peroxidase activity was likewise investigated, and the "Purpurogallin number" calculated. No significant differences were found among the organisms studied.

A very extensive series of respiration studies was carried out with the Warburg respirometer. The results were analyzed statistically in order to see whether or not the variations found between different strains were significant.

The oxygen consumption was measured while a standardized suspension of nonproliferating, resting cells was metabolizing the substrate. In this way it was found that only B/M differed significantly from the sensitive B strain in its respiration on a glucose and pyruvate substrate. In both cases it demonstrated a lower rate of oxygen consumption. This is not caused by a slower metabolism, because, if the amount of glucose that disappears in a given time is measured, B/M metabolizes glucose faster than B. It apparently utilizes some other route for glucose metabolism in addition to aerobic oxidation. On lactate, acetate, and formate substrates no significant differences were found between any of the strains. A limited series of experiments was carried out on glycerophosphate as a substrate. Here it was found that all the strains except B/4 differed significantly from the sensitive B strain. They all possessed a higher rate of activity than either B or B/4. Although glycerophosphate may not necessarily be a normal metabolite in the glucose degradation, appearing only under certain conditions, it does represent a phosphorylated compound and may give an indication of the metabolism of other phosphorylated intermediates.

Study of the intermediary bacterial metabolism will be the means of revealing by which alternative metabolic routes the mutant strains of various bacteria circumvent an otherwise toxic agent.

This paper is based upon work done for the Biological Department, Chemical Corps, Camp Detrick, Frederick, Maryland, under Contract No. W-18-064-CM-223 with the Long Island Biological Association.
Genetic Studies of the Development of Resistance to Chemicals in Mycobacteria

Jen-yah Hsie

The development in M. ranae of resistance to neomycin, dihydrostreptomycin, sodium para-amino salicylic acid (NaPOS), zephiran chloride, and cloromycetin was investigated. It was found that the development of resistance to dihydrostreptomycin in M. ranae takes place in one large step or in several smaller steps, and that resistance to the remainder of the chemical agents mentioned above develops by way of a number of separate steps, each giving increased resistance.

Four steps were required for the development of complete resistance to neomycin. With zephiran chloride, fifth-step selection strains of M. ranae could be completely inhibited by 30 micrograms of zephiran chloride per milliliter of asparagine glycerol agar (AGA) (inoculum about 10^8 cells), whereas the original wild-type strain of M. ranae was completely inhibited by five micrograms per ml.

Chloromycetin in concentrations of 100 units per ml of medium completely inhibited the growth of M. Ranae when the inoculum was about 3×10^3 cells. Some of the survivors on 50 units of chloromycetin per ml of medium required 200 units of chloromycetin per ml to inhibit growth of the same size of inoculum. The human strain of tubercle bacillus, H37Ra, was completely inhibited by 5 units of chloromycetin per ml of Tween 80 liquid medium enriched with 10% Difco. Tb Albumin Medium (inoculum, about 1×10^7 cells). A population of about 10^8 cells of M. ranae could be completely inhibited by 6 milligrams of NaPAS per ml of asparagine glycerol agar, whereas the same size of population of the first-step selection strains required 10 mg NaPAS per ml to inhibit their growth.

Cross-resistance to these chemical agents in the various mutants of M. ranae was studied. It was found that the neomycin-resistant strains of various grades of resistance were as sensitive to dihydrostreptomycin as the original strain, except for a slightly greater viability of the fourth-step neomycin-resistant strain in low concentrations of dihydrostreptomycin (0.5 micrograms per ml AGA). On the other hand, the slightly and fully dihydrostreptomycin-resistant strains showed slight cross-resistance to neomycin only on low concentrations of 0.2 to 0.5 units per ml of medium. All dihydrostreptomycin-resistant strains were as sensitive to one unit of neomycin as the original wild-type strain.

All the mutants showing various grades of resistance to neomycin, dihydrostreptomycin, neomycin-dihydrostreptomycin combination, and sodium p-amino salicylic acid were as sensitive to zephiran chloride as the original wild-type strain. On the other hand, the strains of the four steps of zephiran chloride-resistant mutants were as sensitive to dihydrostreptomycin and NaPAS as the original wild-type strain. They manifested slight cross-resistance to neomycin only on low concentrations of neomycin,
just as the dihydrostreptomycin-resistant strains of various grades did to neomycin. They were as sensitive to one unit of neomycin as the original wild-type strain.

The mutants completely resistant to neomycin, dihydrostreptomycin, and neomycin-dihydrostreptomycin combination, and the four grades of mutants resistant to zephiran chloride showed no higher resistance to NaPAS than the original wild-type strain. On the other hand, preliminary data showed that NaPAS-resistant mutants of first-step selection were as sensitive to neomycin and dihydrostreptomycin as the original wild-type strain, except for slightly greater viability of the NaPAS-resistant mutants in low concentrations of dihydrostreptomycin (0.5 units per ml AGA).

Combinations of neomycin and dihydrostreptomycin, neomycin and zephiran chloride, and dihydrostreptomycin and zephiran chloride manifested an additive effect against M. ranae in vitro. The mixtures of neomycin and zephiran chloride, and dihydrostreptomycin and zephiran chloride, could be kept at 4° C for 30 and 40 days, respectively, without appreciable loss of potency—a desirable characteristic for clinical purposes.

Combinations of NaPAS with neomycin, dihydrostreptomycin, or zephiran chloride manifested against M. ranae in vitro a synergistic inhibitory effect—that is, one which is greater than merely additive.

The rate of mutation of M. ranae to full dihydrostreptomycin resistance was estimated to be approximately $10^{-9}$ per bacterial generation, that of the slightly dihydrostreptomycin-resistant strain usually about five times higher, and that of intermediate types ten to a hundred times higher.

This work was aided by a research grant from the National Tuberculosis Association.
Radiations and Populations
Bruce Wallace

The work reported here was done under Contract No. AT-(30-1)-557, U. S. Atomic Energy Commission. We wish to acknowledge the assistance given by Carol V. Madden, Geoffrey E. Plunkett, Theodore R. F. Wright, Jane Brandt, Gloria Casillo, and Henry Gardner. Dr. James C. King has recently joined our group.

Parallel to recent progress in electronic and atomic technology, there has developed among biologists a concern for the genetic future of our population. This concern arises from a knowledge that ionizing radiations have become an integral part of our industrial, military, and medical techniques, that these radiations induce gene mutations, and that nearly all gene mutations are deleterious.

The research project of which this is the first report is concerned with a determination of the extent and nature of the effects of radiations on the genetic structure of populations. It is hoped that these investigations will be of use in predicting the outcome of exposure of members of our own population to radiations, and, more generally, that our techniques will prove adequate to furnish experimental proof of many of the mathematical theories existent in the field of population genetics.

Analyses of populations of Drosophila melanogaster have been made to determine the fate of mutant genes induced in the original parental flies by short, intense X-ray treatments. Three experimental populations have been studied for thirteen generations. The original males of population No. 1 were exposed to 7000 r; females of population No. 1 and males and females of population No. 2 were exposed to 1000 r. The flies of population No. 3 have never been irradiated. This report will be limited to the fate of recessive lethal gene mutations; for they alone have responded rapidly enough to selective forces to warrant consideration.

In brief, the experimental technique consists of maintaining the populations of flies in cages under conditions which permit a generation to hatch within two weeks (25° C). To determine the genetic structure of a population at any time, eggs are removed from a cage and allowed to develop in culture bottles. A number of males (about 200) that have hatched from these eggs are used to initiate a series of matings, which terminate, if all is normal, in cultures containing curly-winged, lobe-eyed flies and wild-type flies in the ratio of 2 to 1. The wild-type flies of any one culture will all be homozygous for one second chromosome of one of the original males. If one of these chromosomes carries a lethal gene, no wild-type flies will appear in the test culture; if any gene which lowers the viability of the wild-type flies (semilethal, subvital) is present, it will be reflected in the appearance of fewer than the expected number of wild-type flies. If the tested chromosome carries a gene which alters the appearance of the "wild-type" fly, this will be indicated by the altered appearance of all non-curly, non-lobe flies in the culture.
The frequencies of recessive lethals in the samples taken to date are given in the table. From these data several conclusions can be drawn. (1) As a result of spontaneous mutations, second-chromosome lethals are accumulating in population No. 3. On the basis of 1796 chromosomes tested through the thirteenth generation, the rate of accumulation is 0.43% per generation. The frequency of lethals found in this untreated population in the 13th generation is approximately as high as that induced originally in population No. 2 by 1000 r of X-rays. (2) The frequency of lethals observed in population No. 1 decreased for several generations and then increased. Since the lethals observed represent the sum of induced and spontaneous mutations, the fate of the induced mutations can be revealed by subtracting the accumulated spontaneous lethals (see the table, “expected” for population No. 3) from each sample. The adjusted data, given in the table, show that the frequency of induced lethals underwent an initial drop and showed no significant change in later samples; the final frequency is approximately 9%.

<table>
<thead>
<tr>
<th>Sample No.</th>
<th>Population No. 1</th>
<th>Population No. 2</th>
<th>Population No. 3</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>% Lethal</td>
<td>% Lethal</td>
<td>% Lethal</td>
</tr>
<tr>
<td></td>
<td>Lethal adjusted</td>
<td>Lethal adjusted</td>
<td>Expected</td>
</tr>
<tr>
<td>1</td>
<td>131 18.3</td>
<td>152 4.6</td>
<td>133 0.8</td>
</tr>
<tr>
<td>2</td>
<td>156 14.1</td>
<td>164 8.5</td>
<td>52 0</td>
</tr>
<tr>
<td>3</td>
<td>173 11.6</td>
<td>168 4.2</td>
<td>183 2.2</td>
</tr>
<tr>
<td>4</td>
<td>178 10.1</td>
<td>186 6.5</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td></td>
<td></td>
<td>212 1.4</td>
</tr>
<tr>
<td>6</td>
<td>202 10.9</td>
<td>188 4.3</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td></td>
<td></td>
<td>263 4.6</td>
</tr>
<tr>
<td>8</td>
<td>248 13.7</td>
<td>237 5.1</td>
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<tr>
<td>9</td>
<td></td>
<td></td>
<td>285 3.2</td>
</tr>
<tr>
<td>10</td>
<td>226 14.6</td>
<td>226 7.1</td>
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<tr>
<td>11</td>
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<td>283 4.9</td>
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<tr>
<td>12</td>
<td>289 14.5</td>
<td>273 9.2</td>
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</tr>
<tr>
<td>13</td>
<td></td>
<td></td>
<td>385* 5.2</td>
</tr>
</tbody>
</table>

* Tentative.

The constant frequency of induced lethal genes in the fourth and subsequent samples from population No. 1 indicates that these genes are escaping in the eliminative action of natural selection; this escape must result from complete recessivity and/or extremely low frequency of lethal genes at each of the numerous gene loci capable of mutating to lethality.
To determine the nature of the original decrease in lethal gene frequency, we may deduct 9% from the adjusted value for each sample; the resultant figures are the frequencies of lethal genes which are eliminated from the population. The frequencies of genes in the process of elimination fell from an original 8.9% to 4.3% and then to 1.3% in three generations, a decrease of approximately 50% of the original frequency each generation. If one makes calculations on the basis of existing X-ray data, one finds that approximately 10% of the second chromosomes in population No. 1 should have been involved in a translocation-lethal complex. Since translocations in the heterozygous condition lower fertility approximately 50%, it is safe to assume that the lethals which were eliminated from population No. 1 were associated with translocations and hence had a selective disadvantage of approximately .5 in the heterozygous condition.

In conclusion, we may state that approximately one-half of the X-ray-induced lethal genes in population No. 1 were rapidly eliminated because of their association with translocations. The other half, being recessive, have been incorporated into the genetic conglomerate of the population along with spontaneously occurring lethal gene mutations. If any of the induced lethals, other than those associated with translocations, are incompletely recessive, either their original frequency or their rate of elimination is so low that they do not alter the general conclusion presented here.
Abramson, Harold A., Cold Spring Harbor, N. Y.—Simplified preparative electrophoresis was investigated in more detail. As pointed out recently, many attempts have been made to adapt the separation of electrically charged ions like proteins to simple equipment, operable at room temperature. Membranes, sand barriers, jells, and jellies have been used to immobilize the material and prevent convection. It is now possible by means of the technique developed at the Biological Laboratory to carry out at room temperature preparations of proteins and substances similar to those that cause ragweed hay fever and asthma. The apparatus is one of classical design except that it is fitted with a two-way stopcock and one or more tailhole stopcocks. The technique depends upon a modification of the boundaries of the electrophoretic method. It has been shown that the separation depends on the viscosity-density relationship of the supernatant liquid, B, and the liquid P, forming the junction containing the protein. It was possible to use not only glycerol but other liquids of high viscosity; various sugars, urea, and glycols were found to take the place of glycerol with equal success in experimental work. The details of the procedure are found in the following table, which summarizes the differences in preparative procedures with the classical method of Tiselius and the method herein described.

<table>
<thead>
<tr>
<th>Tiselius Method</th>
<th>Method Reported Here</th>
</tr>
</thead>
<tbody>
<tr>
<td>Conductance B = Conductance P</td>
<td>Conductance B $&gt;$ Conductance P</td>
</tr>
<tr>
<td>Viscosity B = Viscosity P</td>
<td>Viscosity B $&lt;$ Viscosity P</td>
</tr>
<tr>
<td>Density B $\leq$ or slightly less than P</td>
<td>Density B $&lt;$ Density P</td>
</tr>
<tr>
<td>pH of B = pH of P</td>
<td>pH of B, at or close to isoelectric point of protein to be separated in solution P</td>
</tr>
<tr>
<td>Temperature controlled at, or near 4° C</td>
<td>Room-temperature fluctuations; no temperature control</td>
</tr>
</tbody>
</table>

Adams, Mark H., and Lark, Gordon, College of Medicine, New York University, New York, N. Y.—In the Annual Report for 1947 it was noted that phage T5 was very unstable in 0.1 N NaCl, being rapidly inactivated at room temperature. The addition of magnesium or calcium salts increased the stability of the virus so that it had to be heated above 70° C before inactivation became rapid. This increased stability appears to be due to the formation of a dissociable complex between the phage particle and the metal cations. In pursuing this problem further, it was discovered that phage T5 spontaneously undergoes mutation to a form which is about 1000-fold more stable in dilute salt solutions than is the parent form. The heat-resistant mutant also forms a complex with metal cations which further increases its heat resistance; but the mutant appears to differ from its parent quantitatively in its ability to form complexes.
Since this is an entirely new type of virus mutant, it is felt that further study of its properties will be of general interest to virus workers.

Bernheimer, Alan W., College of Medicine, New York University, New York, N. Y.—Investigations were conducted with the ciliated protozoan, Tetrahymena geleii, with a view to finding a phage-like virus for this organism. Previous studies, in which virus was sought in filtrates of waters naturally populated with Tetrahymena, were negative. It was considered worth while to attempt to isolate from natural sources strains of the ciliates themselves, and to examine them for the possible presence of a latent virus. Satisfactory methods were developed for finding fresh strains of Tetrahymena and obtaining them in bacterium-free cultures, and application of these methods resulted in the isolation of five new strains from standing water in the vicinity of Cold Spring Harbor. Experiments involving mixing the new strains with one another and with four stock strains, in all possible combinations, failed to indicate the presence of a lytic agent.—In addition, experiments on the photoreactivation of ultraviolet-irradiated Tetrahymena were initiated; these are being continued in New York.

Braun, Werner, and Lewis, Keith H., Camp Detrick, Md.—Special methods for the determination of differences in colonial morphology were applied to the study of various mutants of Escherichia coli. With the help of obliquely transmitted light and special solid media such “2-1 agar” or MacConkey agar, slight differences in colony morphology, otherwise unrecognizable, became apparent. Examination of 36 mutant cultures, differing in biochemical requirements or resistance to various agents, showed that, with only one exception, all-mutants differed in colony-morphology from that of their parent type; most of those tested also differed from one another. Several colony types could be detected in some supposedly pure mutant cultures. Each of these types could be isolated, and gave rise to subcultures with uniform colony types. Such subcultures differed from one another in the rate of microcolony formation (presumably owing to differences in lag period or differences in generation time), but they appeared identical for the mutant characteristic (e.g., streptomycin dependence) for which they had been selected originally. These results indicated that many physiological or biochemical changes are associated with slight changes in colony morphology, and demonstrated the value of using colony morphology as a simple indicator of a population’s homogeneity.—Differences in colony morphology were then utilized for a simple analysis of interactions between mutant populations. The colony morphology of streptomycin-dependent (sd) and streptomycin-resistant (sr) mutants differed significantly; and, using this as a marker, it was observed that the presence of sr mutants may inhibit the growth of sd mutants. This effect was very pronounced in the presence of small amounts of streptomycin and may be due to the destruction or utilization of streptomycin by sr mutants. These observations suggest that, in terms
of streptomycin requirements, some sr mutants may be merely the low members of a series of sd mutants.——After aging on MacConkey agar, colonies grown from some mutant cultures (e.g., B/r) displayed a multitude of papillae and marginal sectors. From the progeny of such a papillated colony, five different colony types could be isolated, of which four gave rise to stable cultures, whereas the fifth proved to be an unstable type that constantly gave rise to two types segregating at the ratio of 2:1. Preliminary studies failed to yield conclusive evidence on the mechanism responsible for this instability.——In addition, an “acriflavine test,” previously utilized as an indicator of antigenic characteristics among pathogenic organisms, was applied to the E. coli mutants. This test permitted the classification of mutants on the basis of “smooth” (S), “intermediate” (I), “rough” (R), or “mucoid” (M) acriflavine reactions. It was noted that changes in resistance or biochemical requirements were frequently associated with changes in acriflavine reactions as well as colony morphology. All tested B and B/r cultures, and mutants derived from them, proved to give I, R, or M reactions. Another E. coli strain (No. 17), previously isolated at Camp Detrick, gave results that resembled the reactions of S types of previously investigated pathogens. It was found to be radiation- and phage-resistant but produced non-S mutants that were phage-sensitive. Mutants resistant to 200 micrograms per ml of streptomycin were also isolated from strain 17, and the mutation rate to sr was found to be comparable to that previously determined for B and B/r cultures at high streptomycin levels.——Finally, preliminary studies were made on the feasibility of using phages as selective agents by maintaining the “S phage-resistant” type in the presence of phage in liquid cultures for prolonged periods. Although the phage-sensitive mutants did not accumulate under these conditions, other phage-resistant variants with altered colony morphology did appear as the cultures aged, so that the population became heterogeneous even though phage-sensitive types were eliminated.

**Goodgal, Sol H., Johns Hopkins University, Baltimore, M.**——The effect of visible light on the survival of mustard-inactivated microconidia of Neurospora crassa and bacteriophage T2r was studied. In a series of three experiments it was found that mustard-inactivated bacteriophage was not reactivated by treatment with visible light, either before adsorption to the B strain of E. coli or during the latent period. Microconidia of Neurospora crassa inactivated with .2% HN2 and then pour-plated at appropriate dilutions may be reactivated (60%-100% increase over dark controls) by prolonged treatment with low-intensity visible light at low temperatures (20°-22° C). At higher temperatures there is a considerable increase (two to fivefold) in the number of viable conidia, and no difference in pour-plates kept in the light or dark. High-intensity visible light applied at any temperature to conidia in liquid complete or minimal medium produces no increase over dark controls. The results are interpreted to mean that the effect of visible light on mustard-inactivated spores
probably involves a different mechanism than the photoreactivation of U.V.-inactivated microconidia or bacteriophage, possibly a localized internal thermal reaction, or an accumulated temperature rise within the Petri dish irradiated with visible light. The thermal reactivation of mustard-inactivated organisms is being further pursued.

Gots, Joseph S., School of Medicine, University of Pennsylvania, Philadelphia, Pa.—As part of a series of studies on purine metabolism of E. coli, investigations were started on the nature of the hypothetical purine precursor, 5(4)-amino-4(5)-imidazolecarboxamide. This compound was identified by Shive, et al (J. Amer. Chem. Soc. 69; 725, 1947) as the non-acetylatable diazotizeable amine which Stetten and Fox (J. Biol. Chem. 161: 333, 1945) found to accumulate under sulfonamide bacteriostasis in E. coli cultures. It was postulated that this compound could be converted to purines by the incorporation of a C1 compound, presumably formate, into the 2 position of the purine ring. Sulfonamides were said to prevent this conversion by preventing the synthesis of pteroylglutamic acid (PGA), the prosthetic group of hypothetical coenzyme required for the reaction. The exact mechanism of the accumulation of this precursor would lead to important information concerning the biosynthesis of purines and the mode of action of sulfonamides at these sites.—The conditions for maximal accumulation of the amine under sulfonamide bacteriostasis of E. coli in ammonium-salts-glucose synthetic medium was determined. It was found that: (1) Glycine was necessary as a precursor for the amine, as originally reported by Ravel, Eakin, and Shive (J. Biol. Chem. 172: 67, 1948). (2) Maximal accumulation was obtained under conditions of 50%-60% inhibition of growth by sulfadiazine. A less inhibited growth system presumably was also less inhibited in the enzyme block involved; a greater inhibited growth system presumably decreased concentration of enzyme and other factors necessary for maximal production. The range of growth inhibitions could be obtained by varying the concentration of sulfonamides or inoculum size, or by the addition of varying concentrations of p-aminobenzoic acid (PABA). (3) No accumulation of the amine could be demonstrated under conditions which could not support the growth of the organisms in the absence of sulfonamides. These conditions included glucose depletion in the medium, as well as resting cell suspension.—Most of the time was spent in determining the substance in yeast extract, which, in the absence of sulfonamide, gave the test for the amine as determined by diazotization after acetylation by the Bratton and Marshall test. It was thought that yeast extract contained a substance which represented the true intermediate that accumulated under sulfonamide action, and the observed amine was a by-product of this accumulation. In testing the known components of yeast extract, it was found that tryptophane in excess could replace yeast extract and that the effect of the latter was due to tryptophane. Upon return to my home laboratory in the fall, it was found that the tryptophane effect was due to indole production by
tryptophanase and was entirely unrelated to the problem. The arylamine produced by tryptophane can be accounted for 100% by indole, since the latter gives the same test. The true arylamine under study, however, does not react to the test for indole.——These studies have been continued during the fall and winter months, with additional information obtained as to the effect of sulfonamide antagonists on relieving the accumulation of the amine.

Granick, S., The Rockefeller Institute for Medical Research, New York, N. Y.—The aim of the work was to find some method or methods for introducing large foreign molecules into cytoplasm and nuclei, for the purpose of observing their effects on heredity. During the summer, various biological materials were examined and methods were considered, as a basis for certain experiments to be undertaken in the near future.

Jablons, Benjamin, 140 West 58th Street, New York, N. Y.—Studies were continued on the purification of the antipressor principle, tubulin, present in mammalian kidney. Chemical identification was begun, and some spectrophotometric analyses made. The action of this principle on the peripheral blood vessels of the frog, and on the circulation of the hamster pouch, the frog and toad mesentery, and the peripheral blood vessels of the mouse ear was investigated. Through the courtesy of Dr. Granick, comparative studies were made on some of these preparations with the vasodepressor principle stored in the liver, ferritin. The anti-adrenergic effect of tubulin was studied against adrenalin and noradrenalin, as well as by means of the mouse protection test. The resistance to adrenalin by various species of mice was determined as a prerequisite to the mouse protection test. Other studies begun were the separation of necrosin from protein residues of kidney tissue used in the separation of tubulin and determination of its effect on the hamster pouch, as well as the effect of hyaluronidase to facilitate transillumination of the membranous pouch for microscopic studies. The effect of certain detergents was likewise investigated.——The incidental finding of the stimulating effect of tubulin on the development of tadpoles opened up a possible new field of genetic influence. In addition, with the aid of Mr. Harry White of the Carnegie Institution, apparatus was constructed of Lucite to facilitate study of the hamster pouch and the unanesthetized mouse ear.

Menkin, Valy, Temple University School of Medicine, Philadelphia, Pa.—A number of observations were made on soft-shelled clams, Mya arenaria, to supplement the data obtained in 1948 at Cold Spring Harbor and published in April, 1949, in Physiological Zoology. The extracts of macerated clam tissue were originally made by extracting in 0.6% NaCl, which is hypotonic for clam tissue. The work was repeated by extracting with 3% NaCl solution, which is isotonic. No difference was found in
the results. A number of experiments were done to determine whether there is a toxic diffusible factor in the extract of clams. No such evidence was found.—By treating the extract with acetone, an acetone-protein precipitate may be obtained. This precipitate is in turn taken up with distilled water. It is then treated with N acetic acid and saturated (NH₄)₂SO₄. The precipitate obtained is discarded and the supernatant treated again with saturated (NH₄)₂SO₄. The resulting solution is evaporated and concentrated in a water bath, between 40° and 50° C. Crystals of (NH₄)₂SO₄ are then obtained which have also some toxic material, presumably necrosin-like, adsorbed on them, at least in very small amounts. When low concentrations (10-14 mg) of these crystals are administered to clams, they fail to shorten their life span, but rather prolong it. On the other hand, very high concentrations of these (NH₄)₂SO₄ crystals (e.g., 100 mg) on which toxic necrosin-like material is adsorbed are definitely toxic to the clams and shorten their life span. The (NH₄)₂SO₄ crystals per se show essentially no toxicity.—That the (NH₄)₂SO₄ crystals obtained from the macerated tissue extracts of clams probably carry adsorbed on them a small amount of nitrogenous material seems to be indicated by the following test. The crystals are dissolved in distilled water and are then precipitated with 10% BaCl₂. The precipitate is filtered, and the filtrate is rendered alkaline to pH 7 or 8 with NaOH. The NH₃ is driven off by heat and then the Ninhydrin reaction is applied to the solution. It is found to be strongly positive, in contrast to the negative test with the crystals of (NH₄)₂SO₄ per se. This indicates that the (NH₄)₂SO₄ crystals of the extract of macerated clam tissue seem to carry an alpha-amino group adsorbed on themselves.

Mohr, Jan, and Goodgal, Sol, University of Oslo, Norway, and Johns Hopkins University, Baltimore, Md.—An attempt was made to induce host-range mutants in the bacteriophages T2r and T6r by the use of ultraviolet radiation and nitrogen mustard. Before treatment with the mutagenic agent the bacteriophage was adsorbed to the B strain of E. coli at 37° C for 7-13 minutes under conditions of single infection. The dose of U.V. employed was usually 3600 ergs/mm². Nitrogen mustard (HN2) was applied at concentrations of .05% to 1.25% for five minutes at 22°-23° C and then washed away by means of centrifugation at 0° C. Assays were taken as soon as possible from the experimental and untreated controls. In order to detect host-range mutants the phage T6 was plated on B/6, and only the clear plaques above a certain size (1 mm) were counted, using a low-power microscope with a micrometer. The plaque count was found to be strongly influenced by even small variations in plating technique. Best results were obtained by using a small volume (1.2 ml) of very soft agar and allowing the Petri dishes to remain in position until the plates were counted. Even with this uniform plating technique the difference in plaque count between duplicate plates was several times as great as when T6 was plated on B. The proportion of T6h to T6 was determined by plating on B/6 and B. Treatment with
U. V. or mustard was found to produce an increase in host-range plaques. The average increase with U.V. light (95%-99.8% inactivation) was found to be about 100-fold, and with nitrogen mustard (30%-90% inactivation) about four-fold. The increase of host-range mutants in the experimentals may be due to the induction of mutations; but other possible explanations, such as selection or an accumulation of spontaneous mutations during the multiplication of T2 or T6 on B-contaminated B/2 or B/6, were not excluded.

Racker, E., College of Medicine, New York University, New York, N.Y. — Tetraethylthiuram disulfide, known also under the name of antabuse, is used for the treatment of alcoholism. We have found that in vitro this compound inhibits aldehyde dehydrogenase prepared from beef liver, as described previously (J. Biol. Chem. 177: 883, 1949). A marked inhibition was obtained with concentrations of the drug as low as 10\(^{-5}\) to 10\(^{-6}\) M. This in vitro effect helps to explain the high acetaldehyde blood levels found in patients under treatment with antabuse after they had ingested even small quantities of alcohol. The in vitro inhibition of aldehyde oxidation is noncompetitive and occurs only if the drug is added to the enzyme before the substrate.——A similar inhibition of aldehyde dehydrogenase was found to occur in the presence of chloral-hydrate. This compound has also been used to potentiate the toxic effects of alcohol. The inhibition was found to be competitive and released by the addition of excess substrate. These two findings point to the main pathway of alcohol detoxification by its oxidation to acetaldehyde and then to acetic acid. If the oxidation of acetaldehyde is inhibited, this substance accumulates and symptoms of its toxicity appear.——A new substrate for the aldehyde dehydrogenase was found in betain aldehyde. This substance is formed as an intermediate in the oxidative steps from cholin to betain. Tetraethylthiuram disulfide also inhibits the oxidation of betain aldehyde to betain.——Part of the summer was spent in the library collecting material for a review article on carbohydrate metabolism. This review is written in collaboration with Dr. S. Ratner and will appear in Annual Reviews of Biochemistry, 1950.

Saifer, A., Veterans Hospital, Brooklyn, N. Y.——During my stay at the Laboratory I revised for publication a paper entitled “Studies with the quantitative cephalin-cholesterol flocculation reaction. I. Effect of variation in temperature. The protein patterns of normal and diseased sera.” This paper was accepted by the American Journal of Medical Sciences for publication within the next few months.——I also completed the initial draft of a paper entitled “A serial dilution-flocculation method for the determination of gamma globulin and fibrinogen in cerebrospinal fluid.” This was presented before the Division of Biological Chemistry at the 116th national meeting of the American Chemical Society at Atlantic City on September 18, 1949, and will be submitted for publication shortly.
Stekol, J. A., Lankenau Hospital Research Institute and Institute for Cancer Research, Fox Chase, Philadelphia, Pa.—In continuation of our earlier work, weanling rats 25 days old (Wistar strain, born and raised at the Lankenau Hospital Research Institute) were fed synthetic diets in which the sole source of protein nitrogen was supplied by a mixture of chemically pure amino acids. The diets were complete with respect to all nutritional components except for methionine, cystine, and vitamin B₁₂. The purpose of the experiments was to ascertain whether the addition of methionine to this diet would result in normal growth. Rats fed the basal diet failed to grow. Addition of methionine to the diet stimulated growth, but it was less than one-third the rate of growth expected (normal growth is about 4-4.5 grams per day for a male rat). Further supplement of the diet with vitamin B₁₂ in addition to the methionine produced excellent growth. Thus, these results, in conjunction with our earlier data on vitamin B₁₂, suggest that vitamin B₁₂ is an essential component of diet in the rat, and that it is apparently involved in the utilization of amino acids in general and in the metabolism of methionine in particular. Histopathological examination of weanling rat kidneys showed hemorrhagic lesions in all animals that were deprived of vitamin B₁₂ in the diet. The kidney damage was identical with that produced in weanling rats by severe choline deficiency.

Tittler, Irving A., Brooklyn College, Brooklyn, N. Y.—As a continuation of studies on nutritional patterns in Tetrahymena geleii, grown aerobically in pure culture, experiments were carried out to investigate (1) the influence of yeast extract on growth, (2) the influence of glucose (as an added carbon source) on growth, and (3) the influence of ammonia nitrogen on growth. Results indicated that growth is proportional to yeast-extract concentration from 0.1% to 10%, and to added glucose up to 1.3%. Little difference existed between these concentrations; possibly because of osmotic effects. After six days' incubation in 1% yeast extract with 1% glucose, the pH dropped to 4.5, the organisms were no longer viable, and the presence of a keto acid (which thiamine studies and dinitrophenyl-hydrazine indicate is pyruvic acid) was demonstrated. The evidence for this will be presented elsewhere. The availability of other carbohydrates remains to be determined.—Ammonia nitrogen exerted no significant effect on growth. It is likely that other factors were limiting, so that added ammonia, even if utilizable, would not increase growth in the presence of low yeast extract. With excess yeast extract, adequate nitrogen is available, so that, here too, an ammonia supplement will not give added growth. What is required to determine whether ammonia is utilized is the specific yeast-extract concentration at which growth factors are not limiting but nitrogen is deficient. Since yeast extract is high in nitrogen, this is unlikely.
COURSE ON BACTERIOPHAGES

June 27-July 16, 1949

Instructor: Mark H. Adams, College of Medicine, New York University.
Assistant: Maryda Swanstrom, New York University.

An intensive three-week course, dealing with techniques and theories of current interest in research on bacterial viruses, was offered for the fifth year. Fourteen students were enrolled for the course, and three more attended the lectures and seminars. Several new experiments were added to the schedule, covering photometric methods of following virus growth, methods of concentrating and purifying viruses, and photoreactivation of ultraviolet-activated bacteriophage. The course was housed in the Davenport Laboratory.

The following students were enrolled:
Dr. G. Bertani, Carnegie Institution, Cold Spring Harbor, N. Y.
Miss C. Dissosway, Carnegie Institution, Cold Spring Harbor, N. Y.
Mr. Chas. F. Ehret, Argonne National Laboratory, Chicago, Ill.
Dr. W. F. Goebel, Rockefeller Institute, New York, N. Y.
Mr. Sol H. Goodgal, Johns Hopkins University, Baltimore, Md.
Dr. A. L. Kappus, Marquette University, Milwaukee, Wis.
Mr. G. Lark, University of Chicago, Chicago, Ill.
Dr. Jan Mohr, Rockefeller Foundation Fellow, Norway
Mr. Amos Norman, Columbia University, New York, N. Y.
Miss Nao Okuda, Carnegie Institution, Cold Spring Harbor, N. Y.
Mr. R. M. Renfro, Naval Medical Research Institute, Bethesda, Md.
Miss Margot Sands, Carnegie Institution, Washington, D. C.
Dr. Wm. Sleator, Washington University, St. Louis, Mo.
Mr. Norton Zinder, University of Wisconsin, Madison, Wis.

Of the 61 individuals who have taken the phage course during the past five years, 35 have been research workers with the doctorate, and most of the remainder have been graduate students working for advanced degrees. In addition, a number of laboratory technicians have received training in the specialized techniques of phage research.

Special seminars given by research workers for the benefit of the students in the phage course were as follows:
C. F. Ehret—Genetics of Paramecium.
S. Granick—Study of intermediary metabolism in Chlorella.
W. F. Goebel—Somatic antigens of Shigella sonnei and their interaction with bacteriophage.
E. Racker—The effect of virus infection on brain metabolism.
M. H. Adams—Luria reactivation and photoreactivation of ultraviolet-inactivated phage.
S. Goodgal—Photoreactivation of Neurospora spores.
NATURE STUDY COURSE


The Nature Study Course was conducted as nearly as possible according to the principles set forth in former years, and was guided by the objectives listed in previous reports of the course. Every effort was made to acquaint the youngsters with their natural environment, toward the end that they may grow up to adapt themselves intelligently and successfully to that environment.

The location of the Biological Laboratory within easy access to many different ecological situations, varying from spring-fed freshwater streams and lakes to salt-water beaches and brackish swamps, as well as open fields and heavily wooded hills, allowed opportunity for unlimited field-work. Consequently, all classes spent considerable time in the field in addition to working in Wawepex Laboratory, from which the course was directed.

The unusually large number of young students enrolled made it necessary to divide the Juniors into two groups. The twenty-one six-year-old youngsters met on Monday and Wednesday from 9:00 to 11:00 a.m., and the twenty seven-and eight-years-olds met on Tuesday and Thursday from 2:00 to 4:00 p.m. The Intermediate group, aged nine to eleven years, met on Tuesday and Thursday from 9:00 to 11:00 a.m., and the seven Seniors met on Monday and Wednesday from 2:00 to 4:00 p.m.

The field work of the six-year-old group was limited to nearby areas, within walking distance of the Laboratory. Efforts were made to bring them into contact with as many forms of living things as possible, and to help them form habits of accurate observation. Frequent opportunity was given for sharing experiences with other youngsters of their own age.

The seven-and-eight-year-old group and the Intermediate group took part in all field work, and showed unusual eagerness and interest in their activities. Some collections were made, but more detailed studies were carried on in the field and in the laboratory. In the Intermediate group, some of the youngsters more interested in ornithology came for early-morning bird walks. During the regular classes, the three younger groups had a number of opportunities to study nesting and feeding habits of some of the birds common on Long Island in the summer.

Other activities of the two middle groups included work on insects, rocks, beach life at high tide and at low tide, fresh-water pond life, and woodland habitats. In the study of pond life, the use of the microscope proved extremely valuable as well as fascinating for these groups, since it helped to establish the concept of a microscopic world. Likewise, the setting up of aquaria and terraria indoors brought out the need for an understanding of the interrelations that exist in the natural habitat, if that is to be duplicated successfully.
In the Senior group, most interests were general, and so many of the fields explored by the Intermediates were also studied by the Seniors. One boy was especially interested in reptiles and amphibians, and carried out a project in this field. Another, who expressed an interest in dragonflies and damselflies, succeeded in starting a collection of these insects. All members of the group helped in successfully establishing a large aquarium with local fish, plants, and insects.

Staff members of the New York State Fish Hatchery near the Biological Laboratory kindly conducted the various classes on a tour of the hatchery, explaining the activities and their purpose. Methods and reasons for restocking streams proved to be of great interest, as did inspection of the growing trout.

The two middle groups also visited the Roosevelt Bird Sanctuary at Oyster Bay, where Mr. James Callaghan, the director, demonstrated and explained purposes of the exhibits in the Trailside Museum. He then took the youngsters along the Nature Trail, where they were able to see several birds not previously seen during the course. The older students were especially interested in the exhibits, and after close inspection returned to Wawepex Laboratory and adapted some of the ideas to their own demonstrations.

The Nature Study course closed on July 28 with a public exhibition of the activities of the different groups during the five-week course. The youngsters had shown great interest in preparing their exhibits for their parents and friends, and eagerly pointed them out on the day of the demonstration. At three o’clock, students and visitors alike were shown two short movies, “The Life History of the Sunfish,” and “The Life History of the Snapping Turtle.” Refreshments were then served by the Laboratory.

The following students were enrolled in the course:

Abramson, Barbara  Garver, Maud  Melrose, Bobby
Abramson, Sandy  Goebel, Ann  Melrose, Peter
Adams, Gay  Grace, Catha  Menkin, Lucy
Barkalow, Bruce  Granick, Donna  Miller, Victor
Baty, Carl  Gubelmann, William  Moffitt, Meredith
Berry, Rosina  Hall, Barbara Ann  Moos, David
Bonime, Karen  Hall, Sarah Jane  Morris, Grinnell, Jr.
Buckley, Christine  Heydt, Sheila  Morris, Stephen
Buckley, Sarah  Kaiserman, William  Nields, Elizabeth
Clarke, Duffield  Laverne, Avi  Nields, John
Cleaveland, Edwards  Laverne, Daniel  Noyes, Sandy
Coudert, Allison  Laverne, Jeremy  O’Connell, Raymond
Cox, Sandra  Littauer, Andrew  Page, Jane
Critchlow, Buell  Lomasney, Lynne  Palmer, Constance
Fates, Robin  Loomis, Alfred  Pierce, Elizabeth
Gardner, Wendu  Martin, Peter  Pierce, Martin
Garver, Joan  Martin, William  Saifer, Madlyn
COLD SPRING HARBOR SYMPOSIA ON QUANTITATIVE BIOLOGY

Current volume: XIV. Amino Acids and Proteins. 217 + xii quarto pages, 105 figures. 1949. Table of contents is listed below:

Bull, Henry B.—Hydrolysis of proteins
Craig, Lyman C., Gregory, J. Delafield, and Barry, Guy T.—Studies on polypeptides and amino acids by countercurrent distribution.
Danielli, J. F.—Studies on the cytochemistry of proteins.
Emerson, Sterling—Competitive reactions and antagonisms in the biosynthesis of amino acids by Neurospora.
Fromageot, Claude—The quantitative analysis of amino acids in proteins; insulin and lysozyme.
Fruton, Joseph S., and Simmonds, Sofia—The metabolism of peptides.
Hughes, Walter L., Jr.—Protein mercaptides.
Kaufmann, Berwind P., Gay, Helen, and McDonald, Margaret R.—Localization of cellular proteins by enzymatic hydrolysis.
Keston, Albert S., and Udenfriend, Sidney—The application of the isotopic derivative technic to the study of protein structure.
Klotz, Irving M.—The nature of some ion-protein structure.
Linderstrom-Lang, K.—Structure and enzymatic break-down of proteins.
Luck, J. Murray—The liver proteins.
Pedersen, Kai O.—Size relationship among similar proteins. Association and dissociation reactions of protein units.
Sanger, F.—Some chemical investigations on the structure of insulin.
Shemin, David—Some aspects of the biosynthesis of amino acids.
Smith, Emil L., and Lumry, Rufus—Some considerations of the interaction of the metal peptidases with their substrates.
Stein, William H., and Moore, Stanford—Chromatographic determination of the amino acid composition of proteins.
Synge, R. L. M.—Physical and chemical studies of gramicidin and some implications for the study of proteins.
Zamecnik, Paul C., and Frantz, Ivan D., Jr.—Peptide bond synthesis in normal and malignant tissue.

Previous Volumes

* Out of print.
LABORATORY STAFF

Albers, Carl—Engineer
*Bass, Emma—Maid
*Brandt, Jane—Research Assistant
Bryson, Vernon—Geneticist
Cosillo, Gloria—Technical Assistant
Cuneo, Helen—Research Assistant
Demerec, M.—Director
*Demerec, Rada—Research Assistant
Doermann, Harriet—Research Assistant
Dorsey, Henry—Laborer
Farrington, Margaret—Technical Assistant
*Fedoroff, Alex—Stockroom Man
Franzese, Eleanor—Clerical Assistant
Gardner, Henry—Technical Assistant
Hahn, Leona—Research Assistant
Hsie, Jen-yah—Bacteriologist
*James, Pauline—Nature Study Instructor
Kelner, Albert—Bacteriologist
King, James C.—Research Associate
Klem, Dorothy V.—Secretary
Madden, Carol V.—Research Assistant
Mann, Margaret—Research Assistant
Millemann, Raymond—Research Assistant
Plunkett, Geoffrey, Jr.—Research Assistant
Prytz, Bo—Chemist
Rae, William S.—Superintendent of Grounds
Raymond, Constance—Research Assistant
Reddy, William—Laborer
*Sokoloff, Alexander—Technical Assistant
Wallace, Bruce—Geneticist
Wright, Theodore—Research Assistant
Yongen, Eileen—Research Assistant
*Yongen, Lillian—Dining Hall Manager
*Summer
SUMMER RESEARCH INVESTIGATORS

Abramson, Harold A.—Cold Spring Harbor, N. Y.
Adams, Mark—New York University College of Medicine, New York, N. Y.
Bernheimer, Alan—New York University College of Medicine, New York, N. Y.
Braun, Werner—Army Medical Corps, Camp Detrick, Frederick, Md.
Colowick, Sidney P.—University of Illinois College of Medicine, Chicago, Ill.
Goodgal, Sol H.—The Johns Hopkins University, Baltimore, Md.
Gots, Joseph S.—University of Pennsylvania School of Medicine, Philadelphia, Pa.
Granick, S.—The Rockefeller Institute for Medical Research, New York, N. Y.
Jablons, Benjamin—New York Medical College, New York, N. Y.
Lark, Gordon—University of Chicago, Chicago, Ill.
Lewis, Keith H.—Army Chemical Corps, Camp Detrick, Frederick, Md.
Lieb, Margaret—Columbia University, New York, N. Y.
MacDuffee, Robert C.—Army Medical Center, Washington, D. C.
Mayr, Ernst—The American Museum of Natural History, New York, N. Y.
Menkin, Valy—Temple University Medical School, Philadelphia, Pa.
Mohr, Jan—University of Oslo, Norway
Pearlman, Gertrude—The Rockefeller Institute for Medical Research, New York, N. Y.
Racker, E.—New York University College of Medicine, New York, N. Y.
Ratner, Sarah—New York University College of Medicine, New York, N. Y.
Ruffner, Norman K.—New York University College of Medicine, New York, N. Y.
Saifer, A.—Veterans Hospital, Brooklyn, N. Y.
Shemin, David—College of Physicians & Surgeons, Columbia University, New York, N. Y.
Sleator, William—Washington University School of Medicine, St. Louis, Mo.
St. Lawrence, Patricia—Columbia University, New York, N. Y.
Swanstrom, Maryda L.—New York University College of Medicine, New York, N. Y.
Tittler, Irving A.—Brooklyn College, Brooklyn, N. Y.
REPORT OF THE SECRETARY

The 57th meeting of the Board of Directors of the Association was held on January 19 at the Down Town Association in New York City, with President Robert Cushman Murphy presiding. The Secretary reviewed the minutes of the 56th meeting of the Board in September, and the acts of the Executive Committee at its meeting in December. The Director of the Laboratory in his report summarized the aims and the most recent accomplishments of the several research programs being pursued with bacteria. He discussed briefly the sources of financial support for these programs, the plans for the 1949 Symposium and summer session, and the condition of the Laboratory's residence buildings. This report called forth enthusiastic response concerning the progress of the Laboratory. The Treasurer distributed copies of a statement of income and expenses for 1948, a list of securities, and the proposed budget for 1949, and commented thereon. His report was unanimously accepted.

A meeting of the Executive Committee was held on June 6, in the George Lane Nichols Memorial Laboratory, Cold Spring Harbor. The entire committee was present. Dr. Demerec reported on the building program of the Carnegie Institution for the Department of Genetics, and on the possibility of a coordinated building program for the Biological Laboratory. Mrs. Franklin introduced a discussion of summer functions for the neighborhood. It was agreed to offer two lectures, and to hold a tea and demonstration in September. President Murphy reported on his efforts to secure new members, and there was some discussion of this problem. In order to favor larger attendance at the Annual Meeting and the July Board of Directors meeting, it was agreed to hold them on the 12th rather than the 26th of July this year.

The 26th Annual Meeting of the Association took place on July 12 in Blackford Hall, Cold Spring Harbor. The chief acts of the Association since the previous Annual Meeting were reviewed by the Secretary and approved by the members present. A nominating committee, consisting of Dr. Mayr, Mrs. Franklin and Dr. Bryson, was appointed by the President to propose names for election to the Board of Directors in the Class of 1953. The following were nominated and re-elected to the Board: H. A. Abramson, M. Demerec, Henry Hicks, Dudley H. Mulls, Stuart Mudd, Robert Cushman Murphy, and John K. Roosevelt. It was proposed and voted that in future the Nominating Committee be appointed in advance of the Annual Meeting. The Director of the Laboratory reported on the full-time research program, the successful Symposium meetings in June, and the various aspects of the summer program then in progress. He gave a brief analysis of the financial condition of the Laboratory, and pointed out the conspicuous need for a new lecture hall and for improvement of summer residence accommodations. The report of the Treasurer, distributed in advance, was voted accepted; Mr. Morris pointed out that finances were in good order and had been audited.

The 58th meeting of the Board of Directors followed the Annual
Meeting on July 12. The Secretary reported the minutes of the January meeting of the Board and the June meeting of the Executive Committee. Dr. Demerec reviewed his plans to secure support for a building program for the Laboratory. On behalf of the Women's Committee, Mrs. Franklin reported well-developed plans for the Open House Tea and Exhibit to be held on September 18. By unanimous vote, the current members of the Executive Committee were re-elected for 1949-50: Mrs. George S. Franklin, Messrs. E. C. MacDowell, Grinnel Morris, William B. Nichols, Arthur W. Page, and John K. Roosevelt. Mrs. Franklin introduced a discussion of the nomination and term of office of members of the Board of Directors. This question was referred to the Executive Committee for further consideration.

E. Carleton MacDowell, Secretary.

SCHOLARSHIPS

During the summer of 1949 the following scholarships were awarded:

John D. Jones Scholarship—Drs. Jack Schultz and J. A. Stekol
Temple Prime Scholarship—Dr. Irving A. Tittler and Gordon Lark.
Dorothy Frances Rice Scholarship—Caroline Dissosway and
Nao Okuda
MAIN AND COMPANY

Certified Public Accountants

New York, U. S. A.

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, 1950. 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 are considered necessary in the circumstances.

In our opinion, the accompanying balance sheet and statements of income and expense, net worth, and supporting schedules, together with the notes thereon, present fairly the position of the Long Island Biological Association at April 30, 1950 and the results of its operations for the year ended on that date.

Main and Company
Certified Public Accountants.

New York, N. Y.
June 15, 1950.
LONG ISLAND BIOLOGICAL ASSOCIATION

BALANCE SHEET

APRIL 30, 1950

ASSETS

General and Endowment Fund

Cash:
In banks $ 24,205.97
On hand 100.00 $ 24,305.97

Investments (market value
$33,448.01) 31,745.47

Accounts receivable:
National Tuberculosis Association $ 171.86
United States Department of the Army 13,969.88
Miscellaneous 1,459.23 15,600.97

Land, buildings and equipment, at cost or appraisal values:
Land $ 86,466.52
Improvements to land 2,898.01
Buildings 101,265.00
Land and buildings leased from Wawepex Society 49,700.00
Equipment 57,940.32 298,269.85 $369,922.26

Special Funds
Cash in bank $ 382.12
Investments (market value
$15,229.14) 15,710.00 16,092.12

Total $386,014.38

NOTE: In accordance with the Association’s established practice, the above balance sheet does not include the inventory at April 30, 1950 of the published volumes of the Association’s yearly Symposia of Quantitative Biology, nor has any depreciation or amortization on buildings and equipment been recorded on the Association’s records. Additions and improvements to buildings and equipment have been charged against current operations in conformity with the Association’s usual practice.
**LIABILITIES AND NET WORTH**

**General and Endowment Fund**

**Liabilities:**
- Accounts payable $10,513.77
- Special grants:
  - Josiah Macy, Jr. Foundation $647.66
  - The Jane Coffin Childs Memorial Fund for Medical Research $1,536.83
  - Polio Basic Research Fund of Long Island $1,793.00
  - United States Atomic Energy Commission $13,206.67

**Total Liabilities** $27,697.93

- Reserve for scientific research 3,000.00

**Endowment Fund:**
- Dr. William J. Matheson Bequest 20,000.00
- Net worth $319,224.33 $369,922.26

**Special Funds**

- Blackford Memorial Fund:
  - Principal $5,000.00

- Charles Benedict Davenport Memorial Fund:
  - Principal $4,934.75
  - Unexpended income 290.25 5,225.00

- Charles Benedict Davenport, Junior, Fund:
  - Principal 1,037.12

- Temple Prime Scholarship Fund:
  - Principal $2,500.00
  - Unexpended income 50.00 2,550.00

- Dorothy Frances Rice Fund:
  - Principal $2,238.32
  - Unexpended income 41.68 2,280.00 16,092.12

**Total** $386,014.38
# LAND, BUILDINGS AND EQUIPMENT

**April 30, 1950**

## Land:
- Purchased with funds raised through public subscription: $69,466.52
- Henry W. de Forest land: 5,000.00
- Airslie land: 12,000.00

Total: $86,466.52

## Improvements to land:

<table>
<thead>
<tr>
<th>Item</th>
<th>Cost</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pipe line</td>
<td>$1,860.39</td>
</tr>
<tr>
<td>Road</td>
<td>746.64</td>
</tr>
<tr>
<td>Light and telephone poles</td>
<td>290.98</td>
</tr>
</tbody>
</table>

Total: $2,898.01

## Buildings:

<table>
<thead>
<tr>
<th>Building</th>
<th>Cost</th>
</tr>
</thead>
<tbody>
<tr>
<td>Airslie building</td>
<td>$5,000.00</td>
</tr>
<tr>
<td>Blackford Hall *</td>
<td>19,000.00</td>
</tr>
<tr>
<td>Cole Cottage</td>
<td>2,105.00</td>
</tr>
<tr>
<td>Davenport Laboratory</td>
<td>8,500.00</td>
</tr>
<tr>
<td>Henry W. de Forest Building</td>
<td>15,000.00</td>
</tr>
<tr>
<td>Reginald G. Harris House</td>
<td>8,500.00</td>
</tr>
<tr>
<td>Dr. Walter B. James Laboratory</td>
<td>13,500.00</td>
</tr>
<tr>
<td>George L. Nichols Memorial Laboratory</td>
<td>13,700.00</td>
</tr>
<tr>
<td>Williams House</td>
<td>11,300.00</td>
</tr>
<tr>
<td>Urey Cottage</td>
<td>2,660.00</td>
</tr>
<tr>
<td>Machine shop and garage</td>
<td>2,000.00</td>
</tr>
</tbody>
</table>

Total: 101,265.00

Land and buildings leased from Wawepex Society under lease expiring in 1979:

<table>
<thead>
<tr>
<th>Item</th>
<th>Cost</th>
</tr>
</thead>
<tbody>
<tr>
<td>Land</td>
<td>$13,500.00</td>
</tr>
<tr>
<td>Buildings:</td>
<td></td>
</tr>
<tr>
<td>Hooper House</td>
<td>$13,200.00</td>
</tr>
<tr>
<td>Jones Laboratory</td>
<td>10,000.00</td>
</tr>
<tr>
<td>Osterhout Cottage</td>
<td>5,500.00</td>
</tr>
<tr>
<td>Wawepex Laboratory</td>
<td>7,500.00</td>
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</table>

Total: 49,700.00

## Equipment:

<table>
<thead>
<tr>
<th>Category</th>
<th>Cost</th>
</tr>
</thead>
<tbody>
<tr>
<td>General</td>
<td>$38,577.27</td>
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<tr>
<td>Biophysics</td>
<td>16,849.90</td>
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<tr>
<td>Physiology</td>
<td>2,513.15</td>
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</tbody>
</table>

Total: 57,940.32

Total: $298,269.85

*Built on land leased from Wawepex Society*
STATEMENT OF NET WORTH
For the Year Ended April 30, 1950

Balance, May 1, 1949  $317,309.17

Add:
Excess of income over expense for the year ended April 30, 1950  1,915.16

Balance, April 30, 1950  $319,224.33
STATEMENT OF INCOME AND EXPENSE
For the Year Ended April 30, 1950

Income:
Contributions:
Dues and contributions $ 6,986.32
Carnegie Corporation (grant for annual Symposia) 6,000.00
Wawepex Society 2,000.00 $14,986.32
Symposia:
Book sales $10,466.89
Registration fees 24.74 10,491.63
Dining Hall 10,883.30
Rooms and apartments 8,393.40
Research fees 5,827.85
Interest and dividends on investments 1,034.01
Profit of sale of securities 64.69
Other income:
Summer course tuition $ 650.00
John D. Jones Scholarship 250.00
Nature study course 393.40
Annual distribution from Walter B. James Fund 170.00 1,463.40
Total income $53,144.60
## Expense:

**Symposia:**
- Publication of annual Symposia on Quantitative Biology: $11,153.82
- Expense of participants and lecturers: 3,776.96

<table>
<thead>
<tr>
<th>Expense</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dining Hall</td>
<td>10,151.36</td>
</tr>
<tr>
<td>Rooms and apartments</td>
<td>1,924.85</td>
</tr>
<tr>
<td>Research expenses</td>
<td>1,768.47</td>
</tr>
<tr>
<td>Summer course expense</td>
<td>480.85</td>
</tr>
<tr>
<td>Distribution of John D. Jones Scholarship</td>
<td>250.00</td>
</tr>
<tr>
<td>Buildings and grounds maintenance:</td>
<td></td>
</tr>
<tr>
<td>Salaries</td>
<td>5,475.55</td>
</tr>
<tr>
<td>Materials and Supplies</td>
<td>5,516.55</td>
</tr>
<tr>
<td>Heat, light and water</td>
<td>1,837.22</td>
</tr>
</tbody>
</table>

**Buildings and grounds maintenance:**

<table>
<thead>
<tr>
<th>Item</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Salaries</td>
<td>$5,475.55</td>
</tr>
<tr>
<td>Materials and Supplies</td>
<td>5,516.55</td>
</tr>
<tr>
<td>Heat, light and water</td>
<td>1,837.22</td>
</tr>
</tbody>
</table>

**General and Administrative:**

<table>
<thead>
<tr>
<th>Item</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Salaries</td>
<td>$3,365.90</td>
</tr>
<tr>
<td>Insurance</td>
<td>599.86</td>
</tr>
<tr>
<td>Printing and stationery</td>
<td>614.33</td>
</tr>
<tr>
<td>Telephone, telegraph and postage</td>
<td>332.82</td>
</tr>
<tr>
<td>Miscellaneous</td>
<td>980.90</td>
</tr>
</tbody>
</table>

**Total expense**

| Total expense                       | 48,229.44|

**Provision for reserve for scientific research**

| Provision for reserve for scientific research | 3,000.00 |

**Excess of income over expense**

| Excess of income over expense         | $1,915.16 |
# STATEMENT OF SPECIAL GRANTS

For the Year Ended April 30, 1950

<table>
<thead>
<tr>
<th>From Whom Received</th>
<th>Balance, May 1, 1949</th>
<th>Transaction May 1, 1949 to April 30, 1950</th>
<th>Balance, April 30, 1950</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Due to Association (Accounts Receivable)</td>
<td>Unspent Balance of Grant</td>
<td>Amounts Received</td>
</tr>
<tr>
<td>Josiah Macy, Jr., Foundation</td>
<td>$ 839.44</td>
<td></td>
<td></td>
</tr>
<tr>
<td>The Jane Coffin Childs Memorial Fund for Medical Research</td>
<td>604.76</td>
<td>$ 2,000.00</td>
<td></td>
</tr>
<tr>
<td>National Tuberculosis Association</td>
<td>199.06</td>
<td>5,980.00</td>
<td></td>
</tr>
<tr>
<td>Polio Basic Research Fund of Long Island</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>United States Atomic Energy Commission</td>
<td>$ 8,099.59</td>
<td>56,510.00</td>
<td></td>
</tr>
<tr>
<td>United States Dept. of the Army</td>
<td>6,339.24</td>
<td>14,181.02</td>
<td></td>
</tr>
<tr>
<td><strong>Totals</strong></td>
<td><strong>$14,438.83</strong></td>
<td><strong>$80,464.02</strong></td>
<td><strong>$59,848.18</strong></td>
</tr>
</tbody>
</table>