

Biological Laboratory

OF THE LONG ISLAND BIOLOGICAL ASSOCIATION

COLD SPRING HARBOR, NEW YORK

annual report
1960-61

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Cover: Concentrating biochemical samples by use of a rotary flash evaporator.

Review of the Year

A Message to the Members

During the past year, the program of the Laboratory was strengthened by the expansion of our year-round research program through the addition of Dr. H. Edwin Umbarger to our year-round staff. Dr. Umbarger and his colleagues, working in the area of microbial physiology, broaden our year-round research efforts in genetics. Their first report (in a later section) indicates the utility of bringing geneticists and biochemists together, in the collaborative venture that has been initiated between Dr. Umbarger's group in microbial physiology and Dr. Margolin's group in microbial genetics.

Another major step forward has been the completion of the expansion and modernization of the Dr. Walter B. James Laboratory. The building, now in full use, provides excellent modern research spaces for our present staff. In addition to completion of new research quarters, the first step in our program of renovation of facilities for our summer program is completed, and these modernized facilities were available during the past year.

Our XXVI Annual Symposium, held in June, 1961, on the topic "Cellular Regulatory Mechanisms" brought some 400 participants, including 25 guests from abroad. The tremendous demand for participation in this Symposium by scientists throughout the world was met in part by the use of closed-circuit television for those who could not be accommodated in our auditorium.

Our summer program of courses has been enhanced with the introduction of a new course in Animal Viruses. The enthusiastic response to this course in its first trial in 1960, and again in 1961, suggests that it will become a basic part of the summer tradition at the Laboratory.

Once again our scientists provided training opportunities for outstanding young undergraduates from colleges throughout the nation. This program, in its third year, is beginning to bear fruit—we receive reports on the career development of these students, and it is most gratifying to note their appearance as outstanding graduate students at leading graduate research centers. The demand for admission to our children's summer courses again exceeded our available facilities and this program is housed in temporary quarters.

Your attention is directed to the financial report which indicates the expenditure of \$525,000 during the past year. A considerable portion of these funds were used in our renovation and construction program, to match the contribution of the National Institutes of Health of the U. S. Public Health Service, in the James Laboratory construction. In order to complete this construction, our operating capital was invaded, and seriously depleted. Although we continue to receive extensive financial support from public agencies and large private foundations, your increased contribution, this year, is urgently needed to restore our badly depleted working capital.



Arthur W. Page

TO ARTHUR W. PAGE—A Tribute

*From the minutes of the annual meeting of the Board of Directors
of the Long Island Biological Association, September 18, 1960:*

Resolution

The members of the Long Island Biological Association, in their 37th annual meeting, desire to express their feelings of great loss in the death of one of their Founders, Arthur W. Page, and to place on record, their devotion for him and the work he did in development and growth of the Laboratory. He was a member of the Board of Directors, and of the Executive Committee from 1924 to 1958; Treasurer, 1924 to 1927; President, 1927 to 1940.

To enumerate the special activities in which he was intimately involved is not possible, for this would be to review the entire history of the Association. However, one of his actions was so entirely his own, and of such significance in its outcome, that special mention must be made. By arranging with the Carnegie Institution for a close coordination of the efforts of The Biological Laboratory and The Department of Genetics, a period of twenty highly fruitful years was inaugurated, of great benefit to both Laboratories, and contributing significantly to the advance of science.

In recognition of all these services, the Association directs that this resolution be entered on its records, and copies be sent to members of the family.



Dr. Walter B. James Laboratory
A year-round research facility
at the Biological Laboratory

Year-Round Research Program

Genetic Studies With *Drosophila Melanogaster*

Arthur Chovnick

During the past 15 years, major developments in the study of the hereditary material have been concerned with (1) the chemical structure of this material, (2) the mechanism(s) by which it intervenes in the behavior of the cell to control metabolic processes essential for growth, and (3) the mechanism of mutation.

Tools in Genetic Research

In all of these studies, microorganisms (chiefly bacteria and viruses) have been used as experimental organisms, primarily due to the tremendous advantages they provide in studying events which occur with low frequency. Thus, in studying genetic mutations, which occur as exceedingly rare events, the use of microorganisms has made it possible to sample bacterial populations as large as the human population of the United States, in a single petri dish within a time span of one day. Another tremendous advantage that microorganisms offer for such studies is the fact that the scientist deals with individual organisms which are single cells, and it is possible to study the genetic control of chemical events occurring in these cells quite directly. At this level of life, the events which lead to the complex structure of a higher organism such as man, a corn plant, or a fruit fly, have been removed and it is possible to study the operation of the genetic material at its primary action in controlling chemical events in individual cells.

The studies described in subsequent sections, dealing with research in bacterial genetics and microbial biochemistry, are examples of the manner in which our scientists take advantage of the special features of these organisms to broaden our understanding of most basic and important life processes.

The utilization of higher organisms as tools in basic genetic research has a long history, and these tools are important today for study of entirely different kinds of problems, equally important to man. For example, one area of exploration is concerned with the role of the hereditary material in the control of developmental processes. How does this material, with its primary role identified with cellular chemistry, operate to permit the development of di-

verse types of cells in the various tissues and organs of a complex multi-cellular organism? Of obvious pertinence to this question is an understanding of the manner in which genetic action at the level of cell chemistry is translated into control of the morphological, visible, characteristics of the individual.

Genes and Enzymes

The program of research in our laboratory is directed toward contributing to an understanding of these questions, and makes use of the fruit fly, *Drosophila melanogaster*. In this species, there are two regions of genetic material (genes) which are known to control, in some unknown manner, the production of a specific protein known as *xanthine dehydrogenase*. This protein is one of the many "enzymes" which serve to catalyze the many chemical reactions which characterize all life processes. Thus, all normal strains of this species, when examined, are found to possess considerable amounts of this protein. It is possible to extract from the fly large quantities of this material, and subject it to test tube experiments, which enable us to describe this molecule with respect to its chemical and physical properties. During the past year, with the use of X-rays, we have made a large number of mutations in the two genes which control the production of this enzyme. All of these mutations share one feature in common. Individuals that possess these mutations are defective in that they exhibit no activity for this enzyme. Extracts from such flies similarly are incapable of catalyzing any of the chemical reactions attributable to xanthine dehydrogenase. Of particular interest is the fact that it is possible to detect these genetic defects very early in development. Indeed, within 24 hours after the initiation of embryonic development by fertilization of the egg by a sperm cell it is possible to observe visible morphological differences between mutant and normal embryos. With this genetic system we have been engaged in a series of experiments to develop methods by which it would be possible to simulate the advantages of microorganisms for large scale sampling of rare events in a complex, multicellular organism.

During the past year these methods have been developed, and it is now possible to investigate the structural organization of a gene in the fruit fly using the many mutations which we have produced and utilizing selective procedures which permit tremendous

population sampling. The experimental analysis now is in progress, and it is quite apparent from our studies that the resolving power of genetic analysis in higher organisms utilizing selective procedures is quite comparable to that of microorganisms.

Another question under investigation is the manner in which these genes control the production of the protein enzyme, xanthine dehydrogenase, in the cells of the fruit fly. It is quite likely that at least one of the two genetic units under study controls enzyme synthesis by providing a code of information which instructs the cells in ordering the amino acid building blocks which comprise the protein molecule. If this notion is correct, then mutational alterations should provide altered information which then leads to the production of a structurally altered protein, very similar to the enzyme, but not capable of serving as active enzyme. Experiments designed to develop procedures for critically answering this question have been underway for some time, and shortly we hope to have the answer.

Bacterial Genetics

Paul Margolin

Studies of the structural and functional relationships within a very small section of the chromosome of the bacterium *Salmonella typhimurium* has continued. This region carries the genetic information which the cell utilizes for producing the enzymes required to synthesize the amino acid, leucine, an essential component of protein. This information presumably is a molecular code consisting of the purine and pyrimidine bases arrayed linearly in deoxyribonucleic acid molecules.

In order to investigate this molecular code of genetic information it is important to obtain some type of "map" of the region under study. One way to do this is to collect many bacterial cells which have independently lost the ability to synthesize leucine. These cells are called leucine auxotrophs because they will not grow unless we provide them with this amino acid. Each mutant has a mistake at a distinct and usually unique place in the genetic information code, which causes it to be unable to produce properly constructed enzymes for leucine synthesis.

The positions of the various mutants can then be mapped by genetic recombination experiments. These utilize the phenomene-

non called transduction in which a bacterial virus acts as a vector, transporting bacterial genetic material. Another phenomenon called abortive transduction has permitted the linear array of mutant sites to be divided into four functional complementation groups. It seemed likely that these represented the genes determining four enzymes required for leucine synthesis, although the biochemistry of leucine synthesis was poorly understood.

Conditions were found under which feeding of complementation group I mutants could be shown by mutants of groups II, III and IV. This has led Dr. Jungwirth in Dr. Umbarger's laboratory to the isolation and identification of the compound which is probably the first intermediate in leucine biosynthesis; β -carboxy- β -hydroxyisocaproate.

More than 100 new leucine requiring mutants have been collected using the penicillin screening technique. These are presently being analyzed for complementation group and will then be mapped by three point recombination tests. These mutants have varying origins. Some are spontaneous and varying numbers were induced by 2-aminopurine, 5-bromouracil, nitrous acid, and X-rays.

Another means for mapping has also been used for some of the mutants. The frequencies of recombination between the sites of several leucine mutants and another, closely linked genetic marker, arabinose 9, were determined. These frequencies primarily reflect distances. Evidence was obtained that the probability of incorporation by recombination of any specific region of the genetic material was strongly affected by the molecular structure of the region. Simultaneously it was possible to show that negative interference plays a role in recombination mediated by transduction. This means that when a "crossover" (recombination) event occurs there is a higher probability than that expected by chance alone that a second "crossover" will occur nearby on the "chromosome."

A large number of the mapped leucine mutants have been studied with mutagenic agents for their capacity to be induced to revert (back mutate) to non-requirement for leucine. Ultraviolet irradiation and the chemical, diethyl sulfate, were used initially. Only two of the 68 tested were found to never revert either spontaneously or under the influence of mutagens. Of the 66 which reverted spontaneously, only three were not inducible by mutagens.

Microbial Biochemistry

H. Edwin Umberger

Dr. F. H. Mukai, using the purine analogue, 2-aminopurine, has been studying the nature of the response to induction of back mutations. He has found that there are two types of leucine auxotrophs. Twenty-one out of 28 mutants of spontaneous origin and 15 out of 15 mutants originating by 2-aminopurine induction responded to reversion by 2-aminopurine. The number of cell divisions following treatment with the mutagen was controlled by varying the enrichment of the plating medium. Among the 21 inducible spontaneous mutants 9 showed an increase of frequency of reversions when additional cell divisions were permitted, whereas 12 showed no dependence on the number of cell divisions permitted. An hypothesis was developed which interpreted these two classes as representing responses of the two alternative purine-pyrimidine base pairs. On the other hand, 13 of 15 mutants of 2-aminopurine induced origin showed an increased frequency of reversions following additional divisions. These observations suggest that 2-aminopurine pairs preferentially with one of the two pyrimidine bases and that such studies may permit us to infer the molecular structure at each mutant site.

Earlier work in other laboratories has indicated that the mechanism of mutations by ultraviolet irradiation required the cooperation of the protein synthesizing machinery of the cell. Dr. Raphael Falk from Hebrew University in Jerusalem spent 3 months in our laboratories undertaking a preliminary study of the mechanism of X-ray induced mutation. Using chloramphenicol, which prevents protein synthesis, he was able to obtain preliminary evidence for the involvement of protein synthesis in the establishment of X-ray induced mutations.



Dr. Obaid Siddiqi
in the bacterial
genetics laboratory

The work initiated by the microbial biochemistry group at Cold Spring Harbor July 1, 1960 represented a continuation of a long term program centered primarily on the elucidation of pathways by which microorganisms (and perhaps higher plants) synthesize amino acids, the building blocks of proteins. Because this kind of activity is somewhat different from activities at the Biological Laboratory in the past, it might be appropriate to outline the principle which guides the experimental approach to such a problem. For such studies, microorganisms have been chosen which can grow in an aqueous solution of a few inorganic salts (potassium phosphate, magnesium and ammonium sulfates) and a simple carbon and energy, such as glucose. From these raw materials, organisms such as *Escherichia coli* and *Salmonella typhimurium* can make the complete array of amino acids, vitamins, carbohydrates and nitrogenous bases that are needed for the formation of the proteins, polysaccharides, nucleic acids and other complex structures in the living cell. Such an organism, obviously must perform each of the several steps that are required to convert the breakdown products of the available energy source to some particular cell component, for example, an amino acid represented in the following hypothetical scheme by the letter, Z.



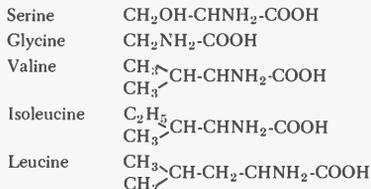
Each of the steps in such a pathway is catalyzed by a specific enzyme—an enzyme that may have no other function in the cell than to perform that single function.

It was not many years ago that, in analyzing such a sequence of reactions in microorganisms, one met that same difficulty that is almost always met by biochemists studying reactions in human and other animal tissues. That difficulty is in ascertaining that an isolated enzyme which can convert X to Y (reaction 3 above) in a test tube actually does so in the living organism. Even with the advent of radioactive isotopes, which were a tremendous aid in detecting interrelationships which might not have been possible with other methods, there was still the question of what steps are

obligatory in the formation of Z in the scheme shown above.

With the development of the field of microbial genetics, a field which owes so much to the research and teaching centered at Cold Spring Harbor, a new and powerful tool for the analysis of biosynthetic pathways became available. Thus, proof of the obligatory role of reaction 3 above in the formation of Z is obtained when a single step mutant requiring Z is isolated and found to differ from its wild type parent only in the capacity to catalyze this reaction.

This approach has been used by this group in studying synthesis of the following naturally occurring amino acids:



Thus far the main pathway of biosynthesis of serine and glycine (which are interconvertible) in bacteria is not known. Of the enzymes in several potential pathways that have been examined none has been shown to be the enzyme which is missing in bacterial mutants requiring serine or glycine.

Evidence that the two reactions, isocitrate \rightarrow glyoxylate + succinate and glyoxylate + glutamate \rightarrow glycine + α -ketoglutarate, constitute a minor pathway which leads to glycine and under certain conditions permits serine/glycine auxotrophs to grow without added serine or glycine. The existence of a minor pathway had been inferred earlier from the isotopic experiments of others. Studies to find the major pathway leading from glucose to serine are being pursued.

Dr. Leavitt has continued the study of the biosynthesis of valine and isoleucine. His work is centered on the study of a single enzyme system which catalyzes the first step in the valine pathway. As has been found so often for other pathways, this first enzyme has a control function in that it is inhibited by the endproduct of



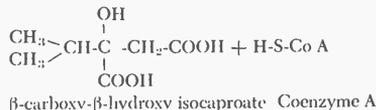
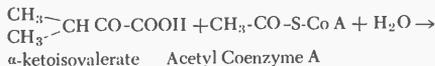
Dr. Christophe Jungwirth conducting Spectrophotometric determination

the pathway itself. Thus, the synthesis of valine should not proceed faster than its utilization, because, whenever valine is in an appreciable excess, it blocks further flow along the pathway by inhibiting the first enzyme in the pathway. Such mechanisms have been found to be simple though effective feedback mechanisms in other systems. In this case, there is a complication since the same enzyme catalyzes an essential (but not the first) step in isoleucine biosynthesis. Dr. Leavitt's work has revealed that if cells have such an effective control over the valine pathway, the isoleucine pathway is also blocked when excess valine is present. Cells of this type are therefore prevented by valine from growing but growth is restored by adding isoleucine. Dr. Leavitt is currently studying the mechanisms of development of resistance to this inhibitor.

The transfer of our activities to Cold Spring Harbor presented a unique opportunity for analysing the steps in a hitherto unknown biosynthetic pathway. This was possible because of the genetic work that Dr. Paul Margolin had done on a series of leucine requiring mutants of *S. typhimurium*. His genetic analysis had revealed that there were four distinct groups of mutants from which one would tentatively infer that at least four specific enzymatic steps were required for leucine biosynthesis (assuming each different group was blocked in the synthesis of a different enzyme).

In collaboration with Dr. Margolin, Dr. Christoph Jungwirth, a visitor to our laboratory from Vienna, has begun a biochemical analysis of these four groups of mutants.

Dr. Jungwirth has now discovered that three groups of Dr. Margolin's mutants accumulate a compound which can be used in place of leucine by the fourth group. He has isolated the compound and identified it as β -carboxy- β -hydroxyisocaproic acid. Furthermore, he has demonstrated the enzymatic synthesis of the compound by extracts of one of the mutants which accumulated it. This synthesis proceeds via the following reaction:



Although further work is necessary, it now appears that this reaction is the first specific step leading to the synthesis of leucine.

Cytogenetic Studies

Berwind P. Kaufmann

Working with cells of higher plants and animals, members of our group have approached problems of inheritance and variation through the analysis of changes occurring in chromosomes and other cellular components during normal processes of growth and development or after experimental modification.

In the development of a complex organism, cells of common origin and genetic constitution at times follow divergent pathways, leading to the establishment of clearly distinguishable specialized types. Considerable evidence is now available that irreversible changes may occur in both nucleus and cytosome during the course of cell differentiation and specialization, as a consequence of altered intergenic reactions or nucleocytoplasmic relations. Clues to the predisposing mechanisms may be found in the ensuing structural modifications, particularly if they are studied

at the level of fine structure by means of electron microscopy. Two approaches should be rewarding in this type of analysis: investigations of the differential mitosis and the distinctive properties of the two cells or nuclei resulting therefrom; and observation of the changes that take place within a given cell type as it assumes the structural and physiological characteristics of its specialized state.

Studies of both types were undertaken this year by Mr. Maruyama, in collaboration with Dr. Helen Gay of the Department of Genetics, Carnegie Institution of Washington. The first dealt with the pollen-grain mitosis in *Tradescantia*, whose products are the vegetative nucleus and the generative cell, both enclosed within the microspore coats. At telophase of the differential mitosis in the pollen grain of this plant, a cell wall is laid down between the morphologically identical generative and vegetative nuclei. Electron-microscopical discoveries regarding the nature of this cell wall demonstrate that a true barrier exists between the two nuclei from the time of their formation in the pollen grain. It seems highly possible that uneven cytokinesis, giving rise to cells with two radically different amounts and kinds of cytoplasm, may influence the differentiation of the sister nuclei, which subsequently become markedly different—the generative nucleus crescent shaped and the vegetative nucleus amoeboid. The cell wall may also regulate interaction between the fully differentiated cells of the mature pollen grain, and enable them to maintain separate functions. Earlier stages of pollen-grain formation are being studied to determine how the partitioning cell wall is laid down.

In the second study, developing stigmatic cells of *Tradescantia* were followed through seven stages of growth, from the smallest bud to the open flower. From the evidence obtained we have been able to piece together a tentative sequence of events in the morphological differentiation of the cytoplasm. The stigma cell undergoes periodic changes as it matures. These changes begin in the young cell with the formation of dense, elongate bodies, which by their location, near and parallel to the nuclear envelope and the endoplasmic reticulum, and by the structure of their limiting membranes, appear to be related to cytoplasmic membranes. At later stages of development, the bodies enlarge and fuse with one another, become less electron dense, and finally disappear as individual entities. Concurrently, a large vacuole of very low electron

density is formed. It is believed that these two events are correlated, and that materials of the dense bodies contribute to formation of the vacuole. The electron-microscopical observations have afforded evidence of morphological similarities between the two types of material. The vacuolar contents thus appear to be merely a more dispersed form of the material of the dense bodies, which originates within enlargements of the endoplasmic reticulum. Our current findings suggest that all these structures contain proteins; but the structural changes must now be analyzed in cytochemical terms, so that a valid interpretation of the synthetic processes and an understanding of their functional significance can be reached.

We have continued to investigate the mechanism of mutagenesis, in a particular effort to test the basic assumption that deoxyribonuclease (DNase) causes mutations and chromosomal breaks by directly disrupting phospho-ester linkages and thereby bringing about rearrangement of DNA nucleotides. In experiments begun last year we attempted to induce mutations in *Drosophila melanogaster* by injection of male flies with solutions of pancreatic DNase; and the preliminary results indicated that this enzyme is a mild but effective mutagenic agent. The progeny of DNase-injected males revealed sex-linked lethal mutations and chromosomal rearrangements to a significantly higher degree than the progeny of buffer-injected males. In collaboration with Mrs. Gillies, and Mrs. Jennie S. Buchanan of the Carnegie Institution, we have now extended the data by determining the frequencies of sex-linked lethals induced in germ cells of *Drosophila* males injected with either phosphate buffer alone, DNase dissolved in phosphate buffer, or an enzymatically inactive protein of about the same molecular weight as DNase, namely, bovine plasma albumin, also dissolved in phosphate buffer.

Again, the frequencies of lethals induced by DNase are significantly higher than those found among the progeny of buffer-injected or untreated males. An unexpected result, however, is the finding that bovine plasma albumin also has an appreciable mutagenic effect, not significantly different from that of DNase dissolved in buffer. Since bovine plasma albumin, which lacks nuclease activity, does have mutagenic activity, we must consider the possibility that the osmotic properties of a given solution can influence the materials of heredity.

These newer experiments thus sustain our earlier conclusion that DNase is an effective mutagenic agent, but raise serious questions about any *a priori* assumptions as to its mode of action. Work now in progress, to determine the ability of bovine plasma albumin to produce chromosomal rearrangements similar to those induced by DNase, may help to answer these questions.

Studies of the Horseshoe Crab

Alexander Sokoloff

Investigations begun in 1957 on the horseshoe crab *Xiphosura (Limulus) polyphemus* has been continued. During the summer of 1961 a census of the population of adult horseshoe crabs was carried out, and simultaneously several hundred crabs were marked (with a thumb-tack) for future identification.

Of great interest was the recovery of one of the animals marked during 1958. This finding contradicts the assertion made in some natural history books that the horseshoe crab molts throughout its lifetime. It suggests the possibility that the horseshoe crab ceases to molt once it has attained the mature (adult)



Recovery and measuring of marked horseshoe crabs

stage, and provides a partial explanation for our failure to find cast-off skins as large as the largest crabs washed on the beach after a storm in the fall of the year.

The fact that marked animals can be recovered after two or three years encourages the pursuit of answers to two other aspects of the natural history of the horseshoe crab: a. The duration of life of the adult and b. The rate of migration of these animals.

Comparative Genetics of Flour Beetles

Alexander Sokoloff

A research program on the genetics of beetles has been initiated. Beetles seem to be, from the standpoint of numbers of species, one of the most successful insect orders for, out of the more than 1,000,000 species of insects described, one species out of four is a species of beetle. And yet, very little is known about the genetics of beetles as a whole. In order to gain more knowledge of the genetics in beetles three species of flour beetles, *Tribolium castaneum*, *Tribolium confusum* and *Latheticus oryzae* have been chosen. In the three years that these beetles have been studied intensively a fairly large number of easily identifiable mutations have been found and the method of inheritance of the genes responsible for these mutations has been worked out. Since similar sex-linked mutations are now available in all three species of beetles, experiments are under way to locate the relative position of the various genes responsible on the sex-determining chromosome to answer the question: what has happened to the position of these genes in the evolution of this group of beetles? At the same time efforts are being made to discover other mutations which may be useful in population genetics problems or in teaching.

Research in Psychobiology

H. A. Abramson

Experiments during the year 1960-1961 have been divided into two parts: (A) Investigations leading to blocking the effect of d-lysergic acid diethylamide (LSD-25) on both animals and man, and (B) extending the effects of LSD-25 and its derivatives to large fish, especially carp, with a view toward game conservation. This work has been carried out in coordination with Dr.

Howard A. Loeb, Chief of the Fish Laboratory at Livingston Manor, New York. Dr. Loeb works under the auspices of the New York State Game Conservation Department.

Blocking LSD

(A) We have previously shown that brain extract as well as the extract of other tissues, including blood, more or less inhibit the action of LSD-25 on the Siamese fighting fish. It was also found in earlier experiments that in all likelihood the blocking agent was primarily an organic compound, because it was destroyed after four hours of boiling. These experiments are now in the process of repetition with special extracts of dried blood. If our preliminary experiments are confirmed, we may be on the path to discovering a technique of not only blocking the LSD-25 reaction in the Siamese fighting fish, but also discovering a new class of compound which may be of importance in treating mental illness in man.

Fish Conservation

(B) Dr. Loeb of the Fish Laboratory has stated that "None of 1,800 assorted compounds force-fed to carp has produced more than, random surfacing or any directed movements of the fish." Any class of compounds which produce surfacing and directed movements of carp, therefore, may be of importance in the control of fish populations, as well as in the study of the physiology of the fish themselves. We now know on the basis of our work last year that a group of compounds related to lysergic acid diethylamide (LSD-25) produces surfacing behavior of carp with the movement directed toward the surface when the drug is in the outside liquid. Feeding these drugs to fish is also effective.

It has been previously shown in work from this laboratory that very small quantities of derivatives of d-lysergic acid, like lysergic acid diethylamide (LSD-25) and lysergic acid ethylamide (LAE-32), have a surfacing effect on small Siamese fighting fish (0.3 to 5.0 gm). Less than 0.5 micrograms per milliliter of LSD-25 in the outside liquid causes most of the fish to go to the surface of the liquid and remain relatively immobile. One microgram per milliliter results in almost 100 per cent surfacing.

Preliminary experiments have shown that both LSD-25 and LAE-32 affect small goldfish in somewhat the same way they affect

Siamese fighting fish. Our work with goldfish merely confirmed what had been known at this laboratory for many years, since the goldfish was one of the first fish studied when our observations were made on the Siamese fighting fish.

Carp at least 2 pounds in weight were sent to this laboratory for study. The fish, all over 12 inches in length, were kept in a running spring close to the laboratory.

Experiments with these large carp disclosed that as little as one microgram per milliliter of LSD-25 in the outside liquid led to surfacing behavior of the fish well within 30 minutes. In fact, in large tanks this effect has been observed within 3 minutes. After 30 minutes all 3 fish in the tank containing LSD-25 were at the surface of the liquid in a nose up-tail down position. This surfacing of the carp lasts for hours and is also observed in ponds into which carp treated with LSD-25 have been placed. In preliminary experiments with Mr. Edward White of the New York State Fish Hatchery, Cold Spring Harbor, and with Mr. Loeb, neither eastern brook trout nor bass reacted in the same way that carp did. This reaction of carp to LSD is not restricted to this compound alone. Thus, LAE-32, the monoethylamide of d-lysergic acid also produces surfacing behavior. Feeding Psilocybin (obtained from the Mexican mushroom) to small goldfish also produces an effect of surfacing the way that LSD-25 does.

The mechanism of action of LSD-25 on the fish is unknown. As we have reported previously certain oxidase poisons like sodium azide act similarly to LSD-25 on the Siamese fighting fish. The surfacing behavior of the Siamese fighting fish may also be readily brought about by simple anoxia. It would appear that LSD-25 probably acts by poisoning one of the many oxidase systems which are found in living tissue, because the action of LSD-25 occurs whether the LSD-25 is injected intraperitoneally or placed in the outside liquid. As a matter of fact, the regression lines for the reaction-dose curves of LSD-25, whether in the outside liquid or injected, and Psilocybin when injected, are parallel.

Experiments in larger tanks and field trials are planned to study both the surfacing behavior and the edibility of fish exposed to compounds like LSD-25 and LAE-32, with a view toward their use not only in fish conservation programs but also in survival kits.

Harold A. Abramson, *Investigator*
Doris Blume, *Research Assistant*
Caroline Bradley, *Technician*
Arthur Chovnick, *Laboratory Director*
Stuart Cohen, *Technician*
Gladys Dean, *Research Assistant*
M. K. Datta, *Research Assistant*
Henriette Getner, *Research Assistant*
Cloria Gillies, *Technician*
R. Peter Kernaghan, *Research Assistant*
Ruth Kellogg, *Technician*
Marian Krauss, *Research Assistant*
Richard I. Leavitt, *Research Associate*
Paul Margolin, *Investigator*
Keizo Maruyama, *Research Assistant*
Kenneth McFall, *Research Assistant*
Bernardine Miller, *Research Assistant*
Frank H. Mukai, *Research Associate*
Barbara Prokop, *Research Assistant*
Abraham Schalet, *Investigator*
Doris Schoonmaker, *Technician*
Alexander Sokoloff, *Investigator*
Joy Talsma, *Research Assistant*
Vincent Tarantola, *Research Associate*
Albert E. Taylor, *Technician*
Matthew Taffel, *Technician*
H. Edwin Umbarger, *Investigator*

Research Staff

Chandi Charan Das, *Kukuriapada, Cuttack Orissa, India*
Raphael Falk, *Hebrew University, Jerusalem*
Helen Gay, *Carnegie Institution of Washington*
Christoph Jungwirth, *University of Vienna*
Berwind P. Kaufmann, *Carnegie Institution of Washington*
Ilse Schwink, *Max-Planck Institute fur Tierzucht, Mariensee, Germany*
Ohaid Siddiqi, *The University, Glasgow, Scotland*
Katherine B. Warren, *Adelphi College*

Collaborating Investigators

The Association is indeed pleased to recognize the generous support of various agencies which make our research possible. During the past year, research at the Laboratory was supported by the following research grants:

National Institutes of Health, United States Public Health Service, Division of General Medical Sciences:
RG-5336, RG-7178, RG-7464, RG-7675

National Institutes of Health, United States Public Health Service, National Cancer Institute:
CY-3773, C-4440, CF-5750

National Institutes of Health, United States Public Health Service:
G-7842, E-3501

National Science Foundation:
G5739, G6431, G17285

National Association for Retarded Children
Association for Aid of Crippled Children

Grants-In-Aid

XXVI Cold Spring Harbor Symposium "Cellular Regulatory Mechanisms"

The XXVI Cold Spring Harbor Symposium on Quantitative Biology was held from June 4th through June 12th, 1961. Approximately 400 scientists attended the meetings, including 25 from abroad. There was a large contingent present from the Pasteur Institute of Paris, where much of the pioneering work in this field was done.

The topic for this meeting proved to be a timely choice—timely because this field has made very significant advances in the past few years, and while some of the fundamental problems appear to have been solved, other new problems were defined and discussed during the Symposium.

The efforts of biochemists on the one hand and of geneticists on the other hand, over the past twenty or thirty years, had shown that all the chemical manifestations of life and the whole functioning of cells as chemical machines depends on special biological catalysts called enzymes. There exist some one to two thousand different reactions which a cell can perform simultaneously. Each of these reactions is catalyzed, that is to say, controlled by a single enzyme and it might be said that any property that a cell exhibits ultimately depends on the types of enzymes which it possesses. The question then of how enzymes themselves are synthesized by cells is one of primary importance. It was, in fact, very largely the subject of this conference.

Major contributors to the organization of the program were: Drs. Bernard D. Davis (Harvard University), Eugene W. Knox, (New England Deaconess Hospital), Jacques Monod (Pasteur Institute), Van R. Potter (University of Wisconsin), Gordon M. Tomkins (National Institutes of Health), and H. Edwin Umberger (Long Island Biological Association).

Symposium Support

The XXVI Cold Spring Harbor Symposium was supported by the following foundations and agencies:

The Rockefeller Foundation
National Institutes of Health, U. S. Public Health Service
National Science Foundation
United States Atomic Energy Commission

United States Air Force under Grant AF-AFOSR-61-73; monitored by the Air Force Office of Scientific Research of the Air Research and Development Command.

Genetics, Medicine, and the Future of Man

In conjunction with the Symposium, the Long Island Biological Association sponsored a public panel discussion concerning medical advances in diagnosis and treatment of hereditary diseases and the consequences and genetical implications for the future of man. Held at the Eastwoods School on Wednesday evening, June 7th, this meeting was attended by approximately 100 members of the community.

Panel Members

The biochemical aspect of the problem was discussed by Professor Bernard D. Davis of Harvard Medical School; the medical viewpoint was presented by Dr. W. Eugene Knox of the Cancer Research Institute of New England Deaconess Hospital and the genetical aspect was discussed by Dr. Arthur Chownick of the Long Island Biological Association.

Summer Courses

Over the past seventy years, our year-round staff, augmented by leading investigators from institutions throughout the world, have conducted a series of intensive courses in new research areas, which are not available in our universities and colleges. The subject matter of these courses have changed in time, but the basic philosophy underlying them has not.

These courses are designed to provide, in a short period of time, the latest methods and tools for research in the various areas to established research workers, post-doctoral level students, and advanced graduate students. Each year our laboratory manuals are revised to incorporate new procedures, and they reflect the trends in research in modern microbiology. In conjunction with these courses, a series of seminars are arranged which provide, in effect, a Symposium on latest research developments in the respective fields.

Following the introduction of a new course in Microbiology of Vertebrate Cells and Quantitative Animal Virology in 1960, the response was so enthusiastic that the course was continued in 1961,

together with the Courses on Bacterial Genetics and Bacterial Viruses. It is contemplated that the courses will continue along these lines for some time.

Competition for the limited number of places in our courses is quite keen, and our "students" are drawn from institutions throughout the world. Having recognized early the significance of a "molecular and quantitative approach" to biological problems, selection of our students has favored biochemists, physical chemists, physicists, and mathematicians who are interested in moving into biological research. We are quite proud of the role our courses have played in providing a vigorous corps of outstanding research workers which led to rapid development of these research fields.

Courses for the Summer of 1960

- 1) *Bacterial Genetics*: June 20 to July 8.
Staff—Maurice Fox, *Rockefeller Institute for Medical Research*
Paul Margolin, *Biological Laboratory, LIBA*
Norton Zinder, *Rockefeller Institute for Medical Research*
- 2) *Bacterial Viruses*: July 11 to August 5.
Staff—Robert S. Edgar, *California Institute of Technology*
Edward S. Lemox, *New York University School of Medicine*
- 3) *Animal Viruses*: August 8 to August 26.
Staff—Richard M. Franklin, *Rockefeller Institute for Medical Research*
Edward Simon, *Purdue University*

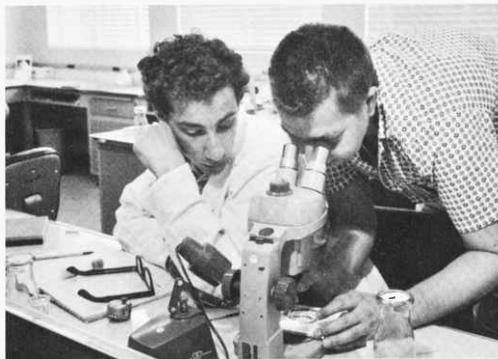
Courses for the Summer of 1961

- 1) *Bacterial Genetics*: June 19 to July 8.
Staff—Paul Margolin, *Biological Laboratory, LIBA*
Frank Mukai, *Biological Laboratory, LIBA*
E. Elizar, *Rockefeller Institute for Medical Research*
N. Zinder, *Rockefeller Institute for Medical Research*
- 2) *Bacterial Viruses*: July 10 to August 5.
Staff—Carsten Bresch, *University of Cologne, Germany*
Alan Campbell, *University of Rochester*
M. Levine, *University of Michigan, College of Medicine*
- 3) *Microbiology of Vertebrate Cells and Quantitative Animal Virology*:
August 7 to August 26.
Staff—Richard M. Franklin, *Rockefeller Institute for Medical Research*
Edward Simon, *Purdue University*

This program of research training is supported by a grant, CRT-5032(C2), from the National Cancer Institute, National Institutes of Health and by a grant from the National Foundation, CPERT 133.



Undergrad student
Frances Messik
counting bacterial colonies



Undergrad summer
researcher Alan Rein, and
Dr. Arthur Chovnick

Summer Guest Investigators

The Biological Laboratory continues to encourage guest investigators to spend their summers here. In addition to participating in teaching courses and seminars, these scientists conduct research alone or in collaboration with each other and with members of our year-round staff. Some of them accept college undergraduate students who conduct experiments under their supervision, as part of the NSF program of the Laboratory.

By bringing together leading investigators from distant institutions for periods of several months, the Laboratory effects a unique exchange of ideas and collaborative experiments which have, in the past, led to significant research advances. In the informal summer atmosphere at Cold Spring Harbor, the scientific activities are enhanced intellectually by the presence of this group.

Summer Investigators—1960

Dr. Robert S. Edgar	<i>California Institute of Technology, Pasadena</i>
Dr. M. Fox	<i>Rockefeller Institute for Medical Research</i>
Dr. Richard Franklin	<i>Rockefeller Institute for Medical Research</i>
Dr. S. Granick	<i>Rockefeller Institute for Medical Research</i>
Dr. Leonard D. Hamilton	<i>Sloan Kettering Institute for Cancer Research</i>
Dr. R. D. Hotchkiss	<i>Rockefeller Institute for Medical Research</i>
Dr. Norman Melchech	<i>St. Louis University, Missouri</i>
Dr. Edward Simon	<i>Purdue University, Lafayette, Indiana</i>
Dr. Felix Wasserman	<i>The Public Health Research Institute of N.Y.C.</i>

Summer Investigators—1961

Dr. Alan Bernheimer	<i>New York University School of Medicine</i>
Dr. Carsten Bresch	<i>University of Cologne, Germany</i>
Dr. Alan Campbell	<i>University of Rochester</i>
Dr. Ellis Englesberg	<i>University of Pittsburgh</i>
Dr. Richard Franklin	<i>Rockefeller Institute for Medical Research</i>
Dr. Samson Gross	<i>Duke University School of Medicine</i>
Dr. Rollin Hotchkiss	<i>Rockefeller Institute for Medical Research</i>
Dr. Jerard Hurwitz	<i>New York University School of Medicine</i>
Dr. Norman Melchech	<i>St. Louis University School of Medicine</i>
Dr. Darryl Pratt	<i>University of Florida</i>
Dr. Edward Simon	<i>Purdue University</i>

Conference for College Teachers of Genetics

The second session of the College Teachers Conference was conducted in 1960 from August 8th to August 27th. Sponsored by the National Science Foundation in a program directed toward

strengthening teachers' mastery of the new developments in science and mathematics, this conference was designed to provide a background of information concerning recent developments in the rapidly advancing field of genetics.

The conference director was Dr. Paul Margolin of the Biological Laboratory, and twenty college faculty members from throughout the United States participated in this program of lectures, discussions and laboratory exercises.

(This program was supported by a grant, NSF-G11367, from the National Science Foundation.)

Summer Research for College Students

Another aspect of the summer program at Cold Spring Harbor concerns the participation of college students at the undergraduate level in active research projects, under the supervision of established investigators.

Initiated in 1959, this program has received the enthusiastic support of staff and students. In 1960, nine and in 1961 ten, highly selected students from colleges throughout the nation spent the summer months on the grounds of the Biological Laboratory.

The specific projects of each student were the responsibility of the individual research supervisor to whom they were assigned. The program director conducted a weekly seminar for the undergraduates, restricted in attendance to the students. Without prior notice, the students were asked to discuss their research, methods, and the significance of the work. These meetings were marked by their informality and free exchange of ideas and information between the students.

Of particular import in the development of these students is another feature of the Laboratory's summer program, in which all students participated. During the course of the summer, a large number of prominent research workers from leading institutions throughout the world present seminars on their work. The undergraduate students profited greatly from these seminars.

In evaluating this program the director, Dr. Arthur Chovnick stated that the major, and most obvious accomplishment was the considerable intellectual growth on the part of the students. Such growth was reflected by 1) their greater understanding of fundamental principles of biology; 2) their increased appreciation of major problem areas currently under investigation; 3) their awareness of the required tools, both physical and intellectual, for

modern biological research and the pertinence of this information to their own future training; 4) their personal acquaintance with research, active research workers and centers, has led to modifications in plans for graduate study.

The following students were enrolled in the National Science Foundation sponsored program from June 19th to September 2nd, 1961:

- 1 Miss Marietta Casale, Sophomore, Indiana University; Study of chromosomes in human blood cells.
Supervisor: Dr. B. P. Kaufmann, Adjunct Staff Member
- 2 Miss Gail Harriet Choder, Junior, University of Pittsburgh; Glucose Effect in *Escherichia coli*.
Supervisor: Dr. E. Englesberg, Guest Investigator
- 3 Mr. Jeffrey Edward Flatacard, Junior, Johns Hopkins University; Effect of "Trenimon" on nucleic acid synthesis in mouse L-cells.
Supervisor: Dr. R. M. Franklin, Adjunct Staff Member
- 4 Mr. Ronald Barton Garren, Sophomore, Dartmouth College; Genetic studies of eye pigment formation in several beetle species.
Supervisor: Dr. A. Sokoloff, Staff Investigator
- 5 Mr. Alfred Lewis Goldberg, Sophomore, Harvard University; A possible synthetic lethal in *Drosophila melanogaster*.
Supervisor: Dr. A. Schalet, Staff Investigator
- 6 Miss Frances Mezik, Junior, Cornell University; Complementation studies of induced auxotrophs in *Salmonella typhimurium*.
Supervisor: Dr. P. Margolin, Staff Investigator
- 7 Miss Kirsten Olsen, Junior, Wells College; Studies on DNA in *Drosophila melanogaster*.
Supervisor: Dr. B. P. Kaufmann, Adjunct Staff Member
- 8 Mr. Alan Robert Rein, Senior, Reed College; Maternal Effects in *Drosophila melanogaster*.
Supervisor: Dr. A. Chornick, Laboratory Director
- 9 Mr. Jonathan Lincoln Rosner, Junior, Swarthmore College; Autoradiographic studies of RNA synthesis in mouse L-cells infected with Mengocivirus.
Supervisor: Dr. R. M. Franklin, Adjunct Staff Member
- 10 Mr. John Roger Roth, Senior, Harvard University; Studies of Chemical Mutagenesis in *Salmonella typhimurium*.
Supervisor: Dr. Frank Mukai, Staff Investigator

(Program supported by a grant, NSF-G12102 and 1961 program supported in a grant, NSF-G15869, from the National Science Foundation).

Workshop In Nature Study for Elementary and Secondary School Teachers

During the summer of 1956, the Laboratory instituted a nature-study workshop, designed primarily to familiarize elementary and secondary school teachers with the natural environment of the Long Island Area, including the animals and plants living there; and those aspects of the environment which affect these organisms.

The course consists of field trips to ponds, streams, seashore, woodlands, fields and other natural habitats, for purposes of collecting and first-hand study, with indoor laboratory work time

divided between lectures and practical work. The various activities in this course are planned to help teachers integrate nature study into the school curriculum and to stimulate scientific curiosity in youngsters.

Those who complete the two-week course receive two credits from the New York State Education Department.

During the summer of 1960, the course was conducted by Marvin Rosenberg, Ass't. Professor of Biology at the State University of New York, Long Island Center and Otto Heck, biology teacher at the Island Trees High School, Levittown. 24 teachers were enrolled in the 1960 workshop.

During the summer of 1961, the course was conducted by Otto Heck and Charles Braun, who is currently on the staff of the Department of Astronomy, Columbia University. 11 teachers were enrolled in the 1961 workshop.

Nature Study For Children

Due to the mounting interest of local parents and their children in the nature study courses at Cold Spring Harbor, the Laboratory has instituted two one-month sessions each summer.

The director of this program, Mr. Marvin Rosenberg, has observed a fivefold increase in attendance over the past five years and noted that local elementary school teachers have observed a heightened interest in science among children who have taken the summer courses at Cold Spring Harbor.

During the summer of 1960, 189 children from the ages of six to fourteen took the courses; instructors were Mr. Rosenberg and Mr. Heck. The headquarters during 1960 were at the Rectory of the Church of St. John's, adjacent to the New York State Fish Hatchery on Rt. 25A.

During the summer of 1961, 301 children were enrolled in the two courses; the instructors were Marvin Rosenberg, Otto Heck, Barbara Sheehan and Charles Braun. In addition to the instructors, each class had an assistant to help on the field trips and in laboratory work. The headquarters for 1961 were in one of the laboratory buildings called "The Animal House", on the grounds of the Biological Laboratory.

The Association gratefully acknowledges the contribution of the Huntington Federal Savings and Loan Association. Their contribution provided scholarships for 12 Huntington students in 1960 and 17 students in 1961.



The biochemistry laboratory in the second-floor addition to the W. B. James Laboratory



View of the renovated laboratory for *Drosophila* Genetics

Buildings and Grounds

Renovation and expansion of the Walter B. James Laboratory was completed and this year-round research facility was fully occupied in the Spring of 1961. The *Drosophila* group occupies the first floor and the biochemistry group and microbial genetics group occupy the second floor of this Laboratory.

Headquarters for the Nature Study Program

Due to the condemnation of the Wawepex Laboratory, headquarters for the Nature Study program for the past 20 years, the 1960 courses for children were housed in the Rectory of the Church of St. John's, adjacent to the New York State Fish Hatchery. The Association is grateful to the Reverend Bleecker for providing temporary headquarters, so this popular summer activ-



The new laboratory for bacterial genetics on the second floor of the W. B. James Building

ity could continue without interruption.

The 1961 courses were housed in the Animal House on the Laboratory grounds, and continued unabated.

Symposium Attendance

Due to the unprecedented demand for attendance at the 1961 Symposium, the housing facilities on the grounds were over-taxed and many of our guests were housed at various neighboring motels. In addition, the auditorium facilities were over-taxed and a satisfactory arrangement to accommodate the overflow was effected by installing a closed-circuit TV system in the adjoining building, Blackford Hall.

Financial Report

For the period May 1, 1960 - April 30, 1961

As of April 30, 1961 our unrestricted assets were as follows:

Cash	\$ 12,517.77	
Accounts receivable	19,807.94	
Inventory of books	11,349.85	
Deferred expenses	1,056.25	
Investments (market value \$21,854.76)		
Bonds \$14,806.00		
Stocks 3,664.07	18,470.07	
Land, buildings and equipment	\$556,768.09	
Total		\$619,969.97

Our liabilities were as follows:

Accounts payable and Taxes	\$14,494.11	
Grants and contracts, unexpended	64,428.35	78,922.46
leaving unrestricted funds amounting to		541,047.51
represented by:		
Endowment Fund (Dr. Wm. J. Matheson Bequest)	\$ 20,000.00	
Net worth	521,047.51	
Total		\$619,969.97

In addition we hold cash and investments in the amount of \$19,874.21 representing restricted funds as follows:

Mark H. Adams Memorial Fund	\$ 1,600.39	
Blackford Memorial Fund	5,000.00	
Charles Benedict Davenport Memorial Fund	6,680.28	
Charles Benedict Davenport, Junior, Fund	1,425.06	
Temple Prime Scholarship Fund	2,676.30	
Dorothy Frances Rice Fund	2,492.18	
Total	19,874.21	

For the year 1960-1961, our receipts were as follows:

Grants, contracts, research fees	\$408,672.30	
Members contributions	13,201.03	
Special contributions	3,360.00	
Interest and dividends	3,084.53	
Profit on sale of securities	7,075.30	
Operating receipts (rentals, dining hall, booksales, etc.)	82,905.85	
Total		\$518,299.01

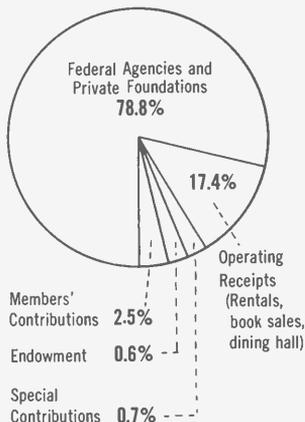
Our expenditures were as follows:

Research and educational program	\$259,659.62	
Administration	45,735.96	
Plant maintenance	48,191.46	
Dining hall	13,416.59	
Building renovation	157,506.75	
Total	\$524,510.38	

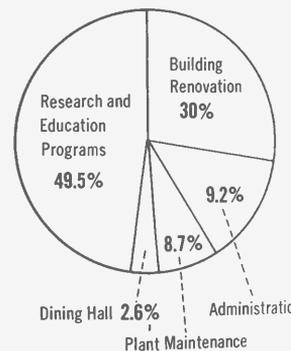
Total Expenditures over Income

\$ 6,211.37

Sources of Funds



Distribution of Funds



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Symposium Dinner Parties

During the XXVI Symposium in June, 1961, Mrs. Franz Schneider and Mrs. William Smoot organized a series of dinner parties for participating scientists. The Association gratefully acknowledges the hospitality of the following members of the community who served as hostesses:

Mrs. Robert W. Ayer
Mrs. Loren C. Berry
Mrs. Sidney Butler
Mrs. Howard Corning, Jr.
Mrs. Miner D. Cray, Jr.
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Mrs. Richard B. Takami
Mrs. Eugene S. Taliaferro
Mrs. Bruce Tuttle
Mrs. Harold L. Werner, Jr.
Mrs. A. M. White

Professional Meetings

The annual meeting of investigators in the field of bacterial virus research, attended by approximately 100 scientists, was held on the Laboratory grounds from August 28th to September 1st, 1961.

A meeting of research workers in *Drosophila* was held on the Laboratory grounds from December 27th to 29th, 1961. Approximately 25 workers from the eastern part of the country attended.

Special Events

Annual Meeting of the Long Island Biological Association

The 37th Annual Meeting of the Long Island Biological Association, held in the Lecture Hall, Cold Spring Harbor, on September 18th, 1960, was attended by over a hundred members and friends of the Association.

The major address of the meeting was presented by the nuclear physicist and biologist, Professor Leo Szilard. Introduced as an illustrious alumni of the Laboratory's summer courses, Dr. Szilard presented excerpts from his latest book "The Voice of the Dolphins", dealing with the problem posed by the bomb. Instrumental in the development of atomic energy, Dr. Szilard is concerned with international relations and the impact of the bomb upon them. In 1946 he turned to research in biology and his work on the genetic characteristics of viruses opened up important studies of mutations.

Plans for future expansion of the Biological Laboratory facilities were displayed at Blackford Hall and the new laboratories and research projects initiated at the Walter B. James Laboratory were shown and discussed by staff members.

Tea was then served in the dining room, under the supervision of Mrs. William S. Smoot and other members of the Women's Committee.



Collecting marine organisms for Nature Study Program

PHOTO CREDITS: N. Messik — Cover,
Pgs. 3, 6, 7, 9, 13, 16, 17;
New York Times
— Pg. 21; Fairchild Aerial
Views — Back cover.

EDITOR: Leonora Frisch

An aerial photograph of a large, dark lake surrounded by dense green forest. The lake has several sandy beaches and numerous small white boats scattered across its surface. A rectangular area on the right side of the lake is highlighted in a semi-transparent blue, showing a cluster of buildings and a parking area. In the upper left, there are some agricultural fields and a few houses. The overall scene is a mix of natural beauty and human development.

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