BIOLOGY
1952
AT THE CALIFORNIA INSTITUTE OF TECHNOLOGY

A Report for the Year 1951-1952
on the Research and other Activities of the
Division of Biology
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THE YEAR IN REVIEW

Many questions dealing with the fundamental why of living things have occupied the attention of the Institute's biologists during the past year.

For instance, why and how in the fertilization of an egg cell, a process so basically important to higher plants and animals, does the egg accept one and only one sperm cell, even though a hundred or more may sometimes arrive at its surface simultaneously?

How does a blood cell synthesize the vital oxygen-carrying red protein complex hemoglobin? Or to state the problem more generally, how do living creatures build up the thousands of kinds of giant protein molecules that are so important in their structures, their vital activities, their inheritance and their evolutionary futures?

From a chemical standpoint, what are genes, those remarkable protein-containing particles that are the smallest and simplest parts of living systems capable of biological reproduction and of the kind of mutational change that makes evolution possible? By what mechanisms are these units of life transmitted from one generation to the next and how do they function in directing growth, development and metabolic activities.

What brings about the sudden transformation of a higher plant from the vegetative state to the reproductive state of flowers and fruits?

What are the factors that limit to a discouraging 2% the efficiency of crop plants in transforming the energy of sunlight into the chemical energy locked up in the sugars and other molecules they synthesize?

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How do plants and animals transform the chemical energy of their food materials - sugars, fats and proteins - into the energy required for growth and other activities?

How are viruses related to higher forms of life, how do they reproduce, what is the mechanism by which they transmit their characteristics to their descendants, and how can we learn to control them more effectively?

These and hundreds of related questions have occupied the Institute's biologists during the past year. Progress is slow since each new advance must be based on a vast amount of patient, probing, systematic experimentation.

At the end of each year when the results have been brought together and evaluated it is always a satisfaction to see how much is known that was not known 12 months before.

This report summarizes the year's activity of the Division of Biology. It is intended for members of the Division, for other staff members of the Institute who wonder what biologists do, for members of the administration who may be concerned as to why it takes so many dollars to keep biologists at work, for Trustees who take the final responsibility for what is done at the Institute, and for the many friends and colleagues who believe in the work of the Division and who have encouraged it in many ways, including the very essential one of helping to provide the funds required to carry on.

In its research programs the Division of Biology has always worked closely with the Division of Chemistry and Chemical Engineering, a collaboration that in recent years has come to be designated "The Joint Chemistry-Biology Program".

This program has evolved because biology at Caltech is, to a large extent, the application of the principles of chemistry and physics to the study of living organisms. The relationship between the Divisions of Chemistry and Biology at Caltech not only assures the application by the biologist of the known in chemistry to studies of living organisms, but also draws the attention of the chemist to those new fields of chemistry which must be explored if progress is to be continued in biology.

Current activities of members of the Division of Chemistry of obvious interest to biologists include the following:

Professor Pauling and Professor Corey have made a most noteworthy contribution to our understanding of the molecular struc-
ture of proteins. Doctor Itano and others in Chemistry have made significant further progress in their studies of hereditary diseases in man which are characterized by altered hemoglobin molecules. Professor Campbell and his associates have been busily engaged in learning more about the chemistry of antigens and antibodies and their interrelation. Professor Niemann's group has continued to investigate enzymes from the standpoint of their structure and their action. Doctor Foster has contributed significantly to the understanding of plant growth processes. Professor Zechmeister and coworkers have studied fluorescent compounds with carcinogenic properties.

The two divisions have shared physical facilities, research budgets, and a good many ideas - in every instance to the advantage of all parties concerned.

Much of the work reported here and in the separate report of the Division of Chemistry bears broadly on the cancer problem. To a large extent this work has been made possible by the very substantial Laurabelle Arms Robinson Fund, two specific grants from the American Cancer Society and one grant from the Damon Runyon Fund. No separate report of cancer work as such has been made because so much of it, like the protein structure work of Pauling and Corey and the protein synthesis work of the Borsook group, is fundamental to all of biology as well as essential to any real understanding of the nature of the unregulated growth that is cancer.

**RESEARCH HIGHLIGHTS**

The bulk of this report will be found devoted to summary accounts of research in progress. Often these will be found difficult to follow in detail by persons not specializing in the particular fields of the work being reported. For this reason the work of the Division is here summarized in a general and less technical way.

Animal Biochemistry has concerned itself for a number of years with the problem of protein synthesis. The technique used has been that of allowing whole experimental animals, tissue slices, suspensions of whole cells, or cell-free homogenates to build up proteins from amino acids labeled in known positions with radioactive carbon. The proteins are then digested, the component amino acids separated, and the distribution of the labeled carbon atoms determined by standard tracer techniques.

During the past year a large part of the work has been devoted to a study of the incorporation of labeled amino acid into immature red
blood cells (reticulocytes) of the rabbit. The rate of incorporation is strongly influenced by four factors, namely, (1) certain amino acids, (2) carbohydrate metabolism factors, (3) a non-protein substance in plasma, and (4) a factor present in liver and spleen. There is an interesting possible application of this work in acclimatizing animals to upper atmospheres by increasing the hemoglobin content of their red blood cells. This possibility will be investigated by the group working in collaboration with Captain Ashton Graybiel of the Pensacola Naval School of Aviation Medicine.

The physiological and biochemical effects of the administration of betaine and glycocyamine to experimental animals have been investigated to provide background information for betaine-glycocyamine heart disease therapy.

Comparative Physiology. Studies of the central nervous system of the crayfish have been continued. The crayfish has been found to contain many fewer nervous elements than, say, the vertebrates. Yet the simpler crayfish system is fully capable of making adequate responses to its environment. The main difference between the nervous system of the crayfish and that of the vertebrate lies in the greater amount of interaction of the nervous components of the former. It has been found, for example, that a single giant fiber of the crayfish may make connections at six or more different synapses and it can be demonstrated that a single impulse in this giant fiber will initiate a complex series of responses to an environmental situation. Nervous economy is also in evidence elsewhere. In maps of the nerves going to the muscles of the claws of the various crustacea, it has been shown that there are very few axons involved in their motor activity. All the leg muscles of a hermit crab, for instance, may be inhibited by a single axon.

The economy principle has been further demonstrated with respect to sensory innervation. Some interesting muscle receptor organs in the tail of the crayfish, which discharge in response to stretch when the abdomen is flexed, are being studied. A small number of these receptors are sufficient to signal all the necessary information concerning the position of the crayfish's tail. Further work is aimed at defining the role of these receptors in reflex behavior. Because they are easily isolated they are well suited to a general study of the physiology of receptors.

In insects, the fact that there are few axons is interesting because it is associated with the extraordinary behavior of the flight musculature. It is well known that wing beat frequency may reach very high values which are not compatible with the times usually involved in the contraction and relaxation of muscle fibers. Prelimin-
ary work has confirmed the phenomena observed by other workers in various Hymenoptera and Lepidoptera. The sphinx moth has been particularly studied and some of the neuromuscular characteristics of the flight muscles are known. It appears here that the special arrangement of a few axons to the flight muscle allows a rapid, strong contraction during flight, which would not be possible with a more formal mechanism.

The interest in Vertebrate Physiology has centered mainly around the nervous system, although some work has also been done on the blood circulation. Problems of nerve conduction have been studied. The potassium losses of peripheral nerve, incurred by asphyxiation, have been determined. The reflex activity of the spinal cord has been investigated, with use made to a considerable extent of asphyxiation as a tool. The cerebral cortex, in particular the so-called spreading depression, which is induced by electrical stimulation and may lead to convulsive activity not unlike that characteristic of epileptic seizures, has received a good deal of attention. The electroencephalogram has been studied, especially its reaction to weak stimuli which tend to suppress the cortical activity. The work on circulatory apparatus has been concerned especially with pressures and flow in the vascular system.

In Bio-Organic Chemistry, further important contributions have added to our understanding of the origin of Los Angeles smog from the emission of large quantities of hydrocarbons and nitrogen oxides into the air. This year it was shown that ozone is formed during the photochemical oxidation of hydrocarbons and their oxidation products, resulting in severe rubber cracking.

In the field of Embryology, new insight has been obtained into the mechanism of fertilization in the eggs of animals. In the sea urchin, the animal most thoroughly investigated in this respect, the egg produces a glycoprotein, fertilizin. It has been found that fertilizin causes a lateral displacement of the midpiece, a significant part structurally of the sperm. This change may be of significance in the fertilization process.

A study of the fertilizin system in rabbits suggests that the same mechanism is also present in mammals.

The short life of sea urchin sperm diluted in sea water is probably tied up with the presence of trace metals. Their removal by the addition of chelating agents greatly prolongs their life.

In Corn Genetics and Cytology, work has continued on the cytological and genetical study of chromosome rearrangements in-
duced by X-rays and by atomic bomb radiation. Gene mutations induced in these same treatments are being studied genetically and biochemically.

Cytological examinations have been completed on 400 translocations isolated from Crossroads (Bikini) tests and 250 accumulated from miscellaneous sources. In addition, several hundred semi-sterile lines have been isolated from Eniwetok, gamma ray and other radiation tests, but as yet only a few have been studied genetically or cytologically.

Corn varieties grown by Southwest Indian tribes prove to be composites from three distinct sources: an ancient type, an "Eastern" complex, and a "Mexican" complex.

Additional work has been done on "blue fluorescent" seedling mutants of corn and a morphological basis has been discovered for the fact that sugary (sweet) corn is higher in its content of certain vitamins than is starchy (field) corn. It appears that niacin, for example, is largely confined to the aleurone layer, which is approximately twice as thick in the sugary type as in starchy. With the same concentration of niacin, this means twice the total amount in sugary.

Drosophila Genetics. Since its establishment 24 years ago, the Biology Division has been noted for its genetic work on the fruit fly Drosophila. This work has gone on actively during the year. An investigation from a taxonomic standpoint has been made of a genus of small flies related to Drosophila.

In recent years there has been a growing amount of evidence indicating that genes with closely related functions often lie physically close together in a chromosome. Such genes are called pseudoalleles and their functions may be markedly disturbed if they are separated by chromosome rearrangements. Several such complexes of genes, in particular the bithorax complex, have been studied in Drosophila during the past year. In this group, five genes have been discovered, the largest number so far detected in any such cluster. By varying the spatial relations of normal and mutant forms of these pseudoalleles, important clues have been found concerning the way in which individual genes control the development of the thorax and the abdomen.

The year in Neurospora genetics was marked by the discovery of a cytoplasmically inherited trait in Neurospora which appears promising for study from a biochemical point of view. Up to the present, the main emphasis has been on studies of nuclear-controlled inheritance. The cytoplasmic trait concerned, known as "poky" because of its slow growth, appears to possess a cytochrome-destroying enzyme.
not detected in wild-type Neurospora and to be characterized by negligible activities of the enzymes succinoxidase and cytochrome oxidase. These deficiencies are transmitted through the maternal parent by means of incipient fruiting bodies which are formed during the growing period of a single mating type. The asexual spores called conidia do not transmit the deficiencies.

In studies of mutants concerned with pyrimidine synthesis, apparent wild-type strains recovered from crosses of two mutants behave as mutants in further crosses to wild type. These "pseudo-wild" types may be forms which arise by non-disjunction of the chromosomes concerned and appear to be "disomic", i.e., to have one full set of chromosomes plus an extra one.

A number of gene-controlled enzyme systems in Neurospora have been studied during the year. Among such systems is the thermostable tyrosinase found in a strain of Neurospora which differs in a single gene from strains producing thermostable tyrosinase. A "black" mutant has been studied in which a tyrosinase inhibitor seems to be reduced in amount. Other enzymatic work has dealt with a D-amino acid oxidase in Neurospora; with lactase, an enzyme concerned with the utilization of the sugar lactose; and with tryptophane desmolase and an enzyme concerned with the cleavage of cystathionine.

Further studies have been made of the physical basis of genetic recombination in the colon bacillus, a problem made extremely difficult by the very low frequency with which such recombination takes place in a population of cells.

Several lines of work have been carried out in the field of Immunology. Following similar work on the tyrosinase of Glomerella, a serological study of the tyrosinase enzyme found in the Schaeffer black mutant and the temperature melanin mutant of Horowitz has been made. There has been further work on the serological relation of tobacco mosaic virus to the normal proteins of the tobacco plant. Red cell antigens of rats, rabbits and the opossum have been investigated. In collaboration with Doctor Clyde Stormont of the University of California at Davis, guinea pig antibodies effective against cattle red cell antigens have been studied. With immune sera, antigenic differences in types of Drosophila and races of the protozoan Euglena have been studied. Immune sera directed against different human hemoglobins normal with respect to the sickle cell character have been prepared for a study of isoalleles postulated by Doctor Itano of the Division of Chemistry.

In Marine Zoology, Professor MacGinitie has returned on a half-time basis to take charge of the work at the Kerckhoff Marine...
Laboratory at Corona del Mar. He has been chiefly occupied with completing a large report on the research begun while at Point Barrow, Alaska.

The Earhart Plant Research Laboratory has had an active year, and a variety of ecological and physiological problems has been studied. The physical plant underwent only minor changes, except for the construction of a fumigation hall, which will permit easier and safer handling of materials entering and leaving the building. The curtains between the light and dark rooms are gradually being replaced by sliding doors, which are a great improvement. The carbon filters installed last year have continued to protect the plants inside the laboratory against smog. An automatic air CO₂ analyzer has been put in operation. A closed-system air conditioning unit for growing plants in a radioactive CO₂ atmosphere has been constructed and put in use.

The work on sugar beets provides a good example of how the Earhart Laboratory can be useful in plant breeding. The plant breeder ordinarily measures the performance of varieties or strains in field trials carried on over a period of years, perhaps in several localities. If the investigator is fortunate in choosing a fair sample of years, climatically speaking, he may draw valid conclusions. But if he is less fortunate, he may draw quite misleading conclusions, for it is known that different varieties may differ widely in respect to adaptation to specific sets of climatic conditions. In working in a laboratory like Earhart, where conditions can be controlled experimentally, results are repeatable and tests can be made under the environmental conditions desired. In tests of eight sugar beet varieties under different conditions, their relative performances were not the same under all conditions. It has now been found feasible to determine the climatic factors responsible for optimum root and top growth and maximum sugar storage in the root.

It was through the use of sensitive indicator plants in the Earhart Laboratory that the plant-damaging components of Los Angeles smog were positively identified a year ago. This year, with the use of the oat coleoptile, there has been developed a smog test of higher sensitivity than any heretofore available. It has also been observed that cotyledons of certain plants seem to be much more sensitive to hydrocarbon oxidants of smog than are true leaves.

Investigations in Plant Physiology and Plant Biochemistry have centered on the study of plant growth and development. Special emphasis has been placed over a period of years on the study of the hormonal substances which are involved in the growth processes.
There has been evolved a comprehensive picture of plant growth as controlled and integrated by chemical messenger substances, the plant growth hormones. At the same time it has become increasingly clear that an understanding of the mechanisms by which the plant grows and through which the hormones exert their effects must ultimately be sought in the enzymatic machinery of the plant cell. The group is therefore involved in a detailed investigation of metabolic pathways and systems in the plant.

A major activity of the plant biochemistry group has been a systematic study of plant respiration and phosphorylation. There is beginning to emerge a clear picture of how oxidative respiration takes place in higher plants, how pyruvate is oxidized via the Krebs cycle by a mitochondrial system, how respiration is related to growth, salt accumulation, water uptake and other energy-requiring processes, and how the latter activities depend upon a coupled phosphorylation process in which inorganic phosphate is incorporated into the high energy phosphate linkages of ATP, which is then available for the various types of energy-requiring processes that go on in plants. The roles of many of the enzymes concerned have been determined. Through this work the Institute group has cleared the way for a new approach to many of the problems of plant growth and development.

Light effects on plants have been studied. The light control of flowering by photoperiod, the stimulation of leaf growth and the inhibition of stem growth, all involve pigments with strong absorptions in the red at 6600 Å. In the case of stem growth inhibition, light appears to decrease the ability of the stem to take up the hormone indoleacetic acid (IAA) and also somehow increases the activity of IAA oxidase which catalyzes the destruction of this growth hormone.

It has been shown that day length exerts its influence on the flowering of plants through effects on the auxin balance of the leaves. Short days favor a low effective auxin level. The flowering of short-day plants is inhibited by applied auxin, whereas the flowering of long-day plants is promoted by the same treatment. Plants induced to flower by proper light treatment were grafted onto hosts not so induced. The result shows that flowering is induced by some substance that moves across a graft union. There is evidence that the flowering hormone is the same in long-day and in short-day plants.

A number of visiting investigators have contributed materially to the program in plant physiology. Professor Wanner of the University of Zürich and Professor Fujiwara of Tohoku University have discovered hitherto unrecognized organic phosphates in plants and Fujiwara has established arginine as a plant phosphate acceptor. Professor
Yamada of the National Institute of Agricultural Research of Konosu, Japan, has shown that rice coleoptiles grow better under water than in air because oxidative destruction of auxin is reduced in emersed tissues.

The presence of a virus-like component in crown gall tumors of plants was established. This component is characteristic of the disease rather than of the host plant.

In connection with soil erosion control in local watersheds, a study of native plants is under way in an attempt to find new plants that will make good ground covers. It has been found that some existing plants can be made to provide better protection through the application of fertilizers, especially those rich in nitrogen.

In Virology, unusual progress has been made at the Institute during the year in the study of bacterial and animal viruses. An hypothesis has been formulated which accounts for many of the quantitative aspects of genetic recombination in bacterial viruses. According to the theory, a mixed infection of a bacterium with different genetically marked types represents not a single genetic cross but an experiment in population genetics.

A question which may be asked by a person learning about virus research for the first time is: "Why work on bacterial viruses when an equivalent understanding of the viruses that produce diseases in man would be of so much more importance from a medical point of view?" The answer is that the techniques by which animal viruses, including those which infect man, are studied are so difficult compared with those used in bacterial virology that knowledge of the latter can go forward enormously faster. It should be added that past experience in analogous situations leads us to believe that the basic principles of virology will be the same for all viruses, whether they inhabit a bacterial cell or man.

That the above reasoning is correct is well illustrated by recent work in Kerckhoff in which a successful transfer was made of the simple techniques of bacterial virology to two animal viruses. In bacterial virus studies, viruses are worked with quantitatively by mixing a few virus particles with a large number of sensitive host cells and plating the mixture on a standard bacterial culture plate of solid medium. The bacteria grow and opaque the plate, except where a virus particle lands. At such points there arises, owing to multiplication of the virus, a circle of dead bacterial cells which appears as a clear hole in the opaque background. This hole is called a plaque. By counting the plaques produced in a given mixture one knows accurately how
many virus particles were initially present. Furthermore, one can easily recover the progeny of a single virus particle by transferring a single plaque to a suitable bacterial culture.

By developing a method in which the opaque bacterial layer in the bacterial virus technique is replaced by a single layer of susceptible animal cells grown in a tissue culture medium and in which subsequent infection is brought about with a suspension of virus particles, a way has been devised to produce visible plaques of dead cells comparable to the bacterial virus plaque technique. This method, named for its originator as the Dulbecco technique, works beautifully for two animal viruses, namely, the one that causes Western Equine Encephalomyelitis and the one that is responsible for Newcastle's disease in chickens.

Plans are under way to see if the newly developed Dulbecco technique can be used for the study of poliomyelitis virus and other viruses responsible for human diseases. If it can, progress will be greatly speeded up, for small tissue culture plates can be substituted for the scores of monkeys and the crates of eggs now necessary in the use of the chick embryo technique.

**THE FUTURE**

For the development of a sound future program of the Division, two urgent needs have arisen. The first is a further development of the Plant Research Center at the Dolk-Clark-Earhart site to take care of growing experimental plants free from smog injury. Much of the work of Bonner, Galston, Lang and their associates is concerned with the chemical control of the growth and flowering of plants, and the smog damage frequently incurred at Orlando and at Dolk is a most serious obstacle. Experiments must often be discarded after months of work have gone into them. Greenhouse space next to the present Dolk laboratory, with a filter system that will free the intake air of smog for both Dolk and the new greenhouse, would solve this problem.

A second urgent need is additional space to take care of the virus work now being carried on in Kerckhoff. There are two reasons for this. First, because of the present crowded condition in Kerckhoff it is difficult to expand the animal virus program in the way justified by the gratifying progress made during the past year. Secondly, a segregated virus laboratory should be available, but cannot be provided in the present building in any economically sound way. Because of the crowding and lack of isolation, temporary arrangements have had to be made to do work on poliomyelitis at the new Huntington Memorial
Hospital Medical Research Laboratory. This is not only inconvenient but will be impossible after the activities of this laboratory are under way to an extent requiring the use of all the available space. This problem could be solved by the construction of the contemplated new wing to the biology laboratories.

The need for space for the virus laboratory is closely related to a need for expanded facilities for those activities of the Chemistry Division bearing on medical and biological problems. It is hoped that a plan can be devised and the funds found to solve simultaneously both aspects of the space problem.

RESEARCH

Two hundred and fifty persons were attached at one time or another to the staff of the Division of Biology during the current year. Of these, the Faculty members, the independent investigators, and the graduate students totaled 131. The remainder of the staff was made up of technical assistants and the office and shop employees. The accomplishments of the research staff members are recorded in some technical detail in the following pages.

Projects are arranged according to groups of workers classified under subject headings which correspond more or less closely to conventional academic departments. In certain instances, this procedure results in the inclusion of projects under headings not entirely appropriate to them. For example, Professor Sturtevant's work on Irises is included in the Drosophila Genetics section because his main research interest is in Drosophila.
ANIMAL BIOCHEMISTRY

Staff

Professors: Borsook, Haagen-Smit
Senior Research Fellows: Deasy, Dubnoff, G. Keighley
Research Fellows: Abrams, Lowy, Schweet, Windsor
Research Assistants: Aronson, Bartron, Gunderson, Jeffrey, M. Keighley
Graduate Student: Lester

Protein Synthesis

Investigators: Borsook, Haagen-Smit, Deasy, G. Keighley, Lowy
Support: American Cancer Society
United States Public Health Service
Atomic Energy Commission (operating through the Office of Naval Research)

This group has, during the past year, confined itself to studies on the incorporation of labeled amino acids into rabbit reticulocytes. It has been found that the incorporation is greatly accelerated by four groups of factors.

1. Four amino acids - histidine, valine, phenylalanine and leucine. It is interesting that it is precisely these four amino acids which have been found to be the most significant amino acids in blood regeneration in anemic rats (Sebrell).

2. Carbohydrate metabolism factors - glucose and either Co I, Co II or ATP. The last three cofactors are interchangeable and equally effective.

Neither the amino acids nor the carbohydrate factors are effective alone. Together they increase the rate of incorporation two to three times. When red cells are incubated for three hours or more at $38^\circ$ they lose most of their ability to incorporate labeled amino acids. This ability is almost completely restored by the addition to the reaction mixture, after three hours of pre-incubation, of the four amino acids named above with glucose and either Co I, Co II or ATP.

3. In addition to the two accelerating factors there is a substance in the plasma of all animals which greatly accelerates incorporation. The accelerating substance can be obtained in protein-free
filtrate and tests have shown it to be no known cofactor or familiar metabolite. It is of small molecular weight, stable at room temperature and stable over the pH range of one to 12.

4. Liver, spleen and yeast contain still another accelerating factor which is either more potent than the plasma factor or present in higher concentrations.

The group is now engaged in the isolation of the plasma and liver factors, and has attained about a hundredfold purification so far. Chromatographic evidence indicates that although the liver and plasma factors are similar they are not quite the same.

The stimulating factors (other than amino acids and carbohydrate metabolism factors) are present in the normal erythrocytes of all animals tested, as well as in rabbit reticulocytes. Normal erythrocytes cannot incorporate labeled amino acids. This inability cannot, therefore, be caused by the absence of cofactors and must be ascribed either to lack of the necessary enzyme system or a difference in the state of the protein.

Mr. Aronson, a visiting student from the Weizmann Institute in Israel, has been working on a filter paper chromatographic method for determining quantitatively the individual amino acids in plasma, using not more than one or two milliliters of plasma. In the course of this work it was found that the filter paper universally used for chromatography contains peptides and inorganic bases. The peptides have hitherto made it impossible to carry out accurate quantitative work by filter paper chromatography. He has worked out a method of removing these contaminants from the paper and by the addition of suitable inorganic bases of restoring the chromatographic properties of the paper. The work is not yet completed but promises to provide the quantitative method desired.

Effects of Glycocyamine and Betaine Administration on Animal Tissues

Investigators: Borsook, G. Keighley, Windsor
Support: International Minerals and Chemical Corporation

In order to learn the mode of action of glycocyamine and betaine (B & G), metabolites which are fed on an experimental basis to patients with heart disease, a series of animal experiments were initiated.

Tissue creatine levels were determined on voluntary muscle, heart muscle, adrenal glands, and brain tissue after long-term feeding
of B & G by stomach tube to experimental animals. Control animals were fed equicaloric quantities of dextrose. Experimental heart disease was produced by the parenteral administration of thyroxine in large doses, and creatine content of the above-mentioned tissues determined, with and without the feeding of B & G. In addition to the chemical analyses of those tissues, heart muscle and pituitary glands were fixed for histological and pathological examination.

The effects of B & G administration on hemoglobin formation were studied on laboratory animals whose erythrocytes had half the lifetime of the human red blood cells. This was extended to include animals with the same erythrocyte lifetime as that of humans.

Experiments are now being planned to learn the effects of B & G administration on the high-energy phosphate-esters of voluntary and heart muscles.

**Biochemical Role of Vitamin B₁₂**

Investigator: Dubnoff  
Support: United State Public Health Service

Further studies have been carried out on the E. coli mutant requiring either methionine or vitamin B₁₂. This requirement is replaceable in part by homocysteine. Two variants of this mutant have now been isolated. One requires only homocysteine or certain other reducing agents for maximal growth in nitrogen. Homocystine, the oxidized form of the methyl acceptor, is also somewhat active, provided para-aminobenzoic acid or the methyl donor dimethylthetin is added. The second variant does not grow on homocystine but does grow to some extent on homocysteine. Traces of vitamin B₁₂ or dimethylpropiothetin or para-aminobenzoic acid stimulate the growth on homocysteine.

These and other data suggest that vitamin B₁₂ acts in a reducing system since its replacement requires anaerobic conditions and reducing compounds. In its absence para-aminobenzoic acid synthesis is insufficient for methyl group formation. This requirement is specifically satisfied by dimethylpropiothetin.

**The Fate of Radioactive Amino Acids in Mammalian Tissues**

Investigator: Abrams  
Support: Eli Lilly and Company

There are many gaps in our knowledge of both the metabolic degradations of the amino acids and the manner in which they become part
of the tissue proteins. We have attempted, in the last year, to apply to this problem a general method which is somewhat different in principle from the usual approach, since no a priori assumptions as to the nature of the expected products are required. The method consists of resolving from a complex mixture by means of chromatography on columns of appropriate material (e.g., ion exchange resin, starch) all the soluble radioactive products produced by the action of the tissue enzymes on the radioactive amino acid in question. Subsequent identification of the radioactive products can then be expected to reveal the details of the degradative or synthetic pathways.

When the method was applied to histidine labeled in the carboxyl group with C\textsuperscript{14}, it was found that rabbit liver, in vivo, and guinea pig liver, in vitro, could convert histidine to glutamic acid at a fairly rapid rate. Furthermore the C\textsuperscript{14} label was found to be in the γ carboxyl group of the glutamic acid. This result provided the key to deciding which of certain previously suggested alternative pathways was probably correct, namely, the following: histidine → urocanic acid → isoglutamine → glutamic acid. In support of the scheme another highly radioactive component isolated from the same system gave glutamic acid after hydrolysis and its general properties coincided with those of isoglutamine.

Similar studies with radioactive leucine and lysine are in progress.

**Incorporation of Radioactive Lysine into Protein**

Investigators: Schweet, Borsook  
Support: United States Public Health Service

A soluble fraction of guinea pig liver extract was shown by Borsook and co-workers to incorporate C\textsuperscript{14}-labeled lysine into protein. This system seemed to offer certain unique opportunities for the study of protein synthesis.

The incorporation has the usual properties of enzyme-catalyzed reactions: the rate doubles with a 10-degree increase in temperature; heating at 50° inactivates the system; it is specific, since of a number of amino acids tested only L-lysine is incorporated; the active fractions have been purified fiftyfold by using protein precipitants; and an assay system has been developed which gives highly reproducible results. The amount of incorporation under the assay conditions is proportional to the amount of enzyme and the specific activity of the added lysine.

Another phase of the work has centered around the co-factor requirements. It has been confirmed that Ca ion is necessary for the re-
action. Ba and Mg ions are inactive, but do not inhibit. No other disso-
ciable co-factor has been demonstrated, but the presence of nucleic
acid in all the preparations may indicate that it plays some role in the
reaction.

Recently, some studies have been made of the radioactive pro-
tein which is the product of the reaction. The radioactivity cannot be
removed by the usual tests, such as ninhydrin, alkali treatment and
dialysis, heating with trichloracetic acid, etc. Peptic digests of the
protein have been fractionated on Dowex columns and the radioactivity
was found in a large number of fractions, but not in the free lysine
fraction.

**Synthesis of Labeled Amino Acids and Amino Acid Analogs**

Investigator: Lowy
Support: American Cancer Society
United States Public Health Service
Atomic Energy Commission (operating through
the Office of Naval Research)

In conjunction with the work of Borsook’s group on protein bio-
synthesis, C\textsuperscript{14} labeled L-histidine, L-leucine, L-glycine and L-lysine
were synthesized with the use of BaC\textsubscript{14}O\textsubscript{3} as starting material.

After hydrolysis of radioactive proteins produced in vitro by
rabbit reticulocytes incubated with each of the above amino acids, the
added amino acid could be recovered in good yield accounting for all of
the radioactivity. In the case of glycine, 85% was recovered as gly-
cine and 15% as serine.

By reacting such proteins with dinitrofluorobenzene (reagent
for free amino groups) and examining the hydrolysates, it was estab-
lished that the radioactive glycine, serine, leucine and lysine were
bound through their amino groups.

A new α-amino acid, cyclopropylaminoacetic acid, was synthe-
sized and its structure established by oxidation to cyclopropane carboxal-
dehyde. Because of its similarity to the amino acid valine it may have
significance as an anti-metabolite in some biological systems.

**In Vitro Incorporation of Amino Acids into Proteins**

Investigators: Borsook, Lester
Support: General Funds

To simplify the study of protein synthesis, an attempt is being
made to develop an in vitro system in which the incorporation of labeled
amino acids into proteins can be demonstrated.
Cells of *E. coli* treated with penicillin swell and gradually lyse. The lysate supernatant will incorporate $^{14}$C-labeled L-leucine into protein at one-eighth to one-fourth the rate of the whole lysed suspension. The activity could not be accounted for on the basis of the small number of viable cells present, although the presence of active abnormal, non-viable cells has not been excluded. Unfortunately, the activity is short-lived, lasting only three to four hours.

Lysozyme-treated cell suspensions of *Micrococcus lysodeikticus* in which complete lysis results exhibit activity up to 40% of the original activity of the untreated whole cells.

**ANIMAL PHYSIOLOGY**

**Staff**

Professors: Van Harreveld, Wiersma, Research Associate: Tietz
Research Fellows: Florey, Russell, Stamm
Graduate Students: Furshpan, Lindsley, Ripley, Wolfgram
Research Assistants: Estey, Scheurenberg

**Physiology and Pharmacology of Muscle Stretch Receptors in the Crayfish**

Investigators: Wiersma, Furshpan, Florey
Support: Hixon Fund, General Funds

Much interest has recently been aroused in the small units which are known as the spindle organs, within vertebrate muscle. Each unit consists of a group of specialized muscle fibers which receive both sensory and motor innervation. The sensory terminals respond to the stimulus of stretch. The motor innervation, by mediating contraction in these so-called intrafusal muscle fibers, instrument changes in tension upon the receptor region. The sensory discharge resulting from any given amount of stretch may thus be altered. The spindles are the peripheral mediators of "muscle sense" (the non-visual awareness of the position of the various body parts), and because of the motor innervation which serves to alter their sensitivity are well suited for the important role they play in the learning of delicate and skilled movements.

There has also appeared recently an anatomical description of muscle receptor organs in the abdomen of various decapod crustacea. A study of these stretch receptors has been undertaken in the crayfish
as a further attempt to define the neuronal basis of crayfish reflex behavior. The physiology of the isolated receptors has been examined prior to such a determination of the central-nervous pathways of the impulses coming from and going to them.

The crustacean spindle organs show remarkable similarities to those of the vertebrate, with a motor innervation which can affect the discharge in a manner similar to that described above. Two such receptor organs are found associated with the extensor musculature of each side of each abdominal segment. One adapts to continuous stretch very slowly, being capable of maintaining discharge up to an hour even though isolated from its blood supply. It is well suited to signaling continuous information of the degree of flexion of the abdomen. The other receptor adapts rather rapidly and has a high threshold of stretch, and probably functions in the vigorous swimming movements of the crayfish "escape" reflex.

Some recording of muscle potentials set up by stimulation of the receptor organ's motor nerves has been done and further corroboration of the Wiersma-van Harreveld theory of crustacean innervation has been obtained. Local potentials capable of summation were found, and the magnitude and electrical sign of these depended upon the position of the "active recording electrode.

An interesting aspect of these organs is their sensitivity to drugs. In low concentrations acetylcholine enhances the excitability of the organ. Eserine sensitizes the preparations to acetylcholine (ACh); atropine blocks the ACh effect.

Since atropine is unable to block the normal response to stretch, it is believed that ACh may not be involved in the normal process of impulse creation. Further studies along these lines may shed light on the fundamental process involved in impulse generation in neurons.

Spreading Depression of Cortical Activity

Investigators: Van Harreveld, Stamm
Support: Markham Fund, United States Public Health Service

Stimulation of the cerebral cortex may produce the arrest of the normal electrical cortical activity (electrocorticogram). This depression of cortical activity spreads very slowly over the cortex of the experimental animal. The phenomenon was found to be accompanied by a slow potential change and by a wave of vasoconstriction. The suggestion was made that the latter, by interference with the cortical blood supply, might be responsible for the depression of the electrocorticogram. Eliciting the spreading...
depression repeatedly causes a change in the phenomenon. First spikes appear during the depression. After some more stimulations the depression may be replaced by a full-fledged convulsive pattern which, like the spreading depression, is accompanied by a slow potential change. A possible explanation of this observation would be that the vasoconstriction observed in fresh preparations becomes exhausted by repetition and that now other features of the processes involved in the spreading depression can manifest themselves. One of these would be the production of a convulsive pattern. In the fresh preparation this convulsive pattern would be suppressed by the cortical oxygen lack caused by vasoconstriction.

If this hypothesis were correct, it should be possible to change spreading depression into a convulsive pattern in a fresh preparation by preventing the vasoconstriction. A number of ways of achieving this end were investigated. Three procedures which are well known to produce cortical vasodilatation and thus counteract the vasoconstriction typical of the spreading depression were found to be feasible. These were the topical application of the vasodilators acetylcholine and pilocarpin and the systemic administration of carbon dioxide in a concentration of 10 to 15%. In all instances, these treatments changed the spreading depression into a convulsive pattern. The changes produced by carbon dioxide are especially noteworthy, since they are readily reversible and since carbon dioxide in the concentrations used tends to depress the activity of the cerebral cortex.

**Effects of Cortical Lesions upon Hoarding Activity in Rats**

*Investigator: Stamm  
Support: Hixon Fund*

A good deal of interest has been demonstrated in localizing the physiological activities which determine so-called instinctive behavior in animals. It has been shown that hoarding activity can be elicited experimentally with rats. During a half-hour daily hoarding trial, a sliding door of the rat's home cage is opened, which gives the animal access to a runway at the end of which is a food bin filled with pellets. The number of pellets which the rat returns to its home cage is an index of its hoarding activity.

Since many types of instinctive behavior, such as copulation, are greatly reduced or eliminated by subjecting rats to cortical lesions, it was attempted to determine the influence of lesions upon the onset of hoarding behavior. Experimental rats were subjected to rather large lesions; 30 to 60% of the cortex was removed, and hoarding behavior was compared with that of control animals. It was found that these
lesions definitely reduced hoarding activity. Fewer of the experimental rats hoarded at all and those that did hoard collected fewer pellets than did the control animals. It was not possible, however, to isolate a "hoarding center" in the cortex and the extent of the lesions did not seem to influence greatly the amount of hoarding exhibited.

In a subsequent study the effect of cortical lesions upon already established hoarding behavior was investigated. Tentative results seem to indicate that destruction of the olfactory cortex is more effective in reducing hoarding behavior than the elimination of other cortical areas.

**Action Substance of Sensory Nerves**

**Investigator:** Florey  
**Support:** Hixon Fund

Although the chemical transmitter in most types of autonomic and spinal nerves has been discovered for the vertebrates, the chemical transmitter is still unknown in the case of sensory fibers. Since Hellauer and Umrath in 1948 reported detecting a chemical transmitter of sensory nerves, some investigators (Holton and Perry, Holton and Holton, Florey, Sterz and Häusler) have worked with this substance and obtained very contradictory results.

Experiments have now been carried out to clear up the situation and isolate the factor indicated by the work of Hellauer and Umrath. The factor has been found, but it shows unexpected properties. An investigation of these properties is under way.

**Stretch Receptors in the Teleostei**

**Investigator:** Russell  
**Support:** General Funds

It has been assumed by previous investigators that fish possess proprioceptors, although their presence has never been demonstrated successfully in the Teleostei. Earlier physiological studies have shown that in both the ray (Raja) and dogfish (Scyllium), stretch receptors do exist, although their morphology is unknown. The task of demonstrating their receptor organ in the bony fishes has been difficult because of limited viability in the present nerve-muscle preparations.

An extensive investigation of the anatomy of the pectoral girdle of the common goldfish (Carassius auratus) has resulted in a nerve-muscle preparation which will be used for a study of the stretch receptor organ.
Sensory Innervation of Blood Vessels

Investigators: A. Van Harreveld, Lindsley
Support: General Funds

There is evidence that part of the sensory fibers innervating the skin of the trunk pass through the sympathetic chain (van Harreveld and Smith). The suggestion has been made that these fibers serve the blood vessels of the skin. Blood vessels of the trunk were isolated and stimulated by the injection of strong NaI solutions. Rise in blood pressure was recorded, as the indicator of stimulation. These experiments were performed in preparations in which a relevant portion of the sympathetic chain was removed and in control preparations with intact sympathetic system. No significant differences were observed. These observations do not support the thesis that the sensory sympathetic skin innervation is a vascular innervation.

Nerve Maps of Crustaceans

Investigators: Wiersma and Ripley
Support: American Philosophical Society
University of Hawaii
General Funds

The motor and inhibitory innervation of a number of Hawaiian forms has been studied at the Coconut Island Marine Laboratory of the University of Hawaii. The results have led to a reconsideration of the functional importance of peripheral inhibition in the arthropods. Although the idea is still widespread that inhibition is an important factor in the functional behavior of antagonistic muscles, this viewpoint must be given up in the face of what we now know about the innervation pattern. It was found, for instance, that all leg muscles of a hermit crab, Dardanus asper, share a single inhibitory fiber and that this fiber constitutes the only form of inhibitory innervation for most of the leg muscles. Two muscles, sharing a common motor axon, would always contract at the same time, if no measures were taken. However, each receives, in addition, an extra inhibitory fiber. Since this same arrangement of motor innervation of these two muscles is found in all decapods, it is believed that the differences in the inhibitory patterns which the different tribes show are caused by differences in the manner in which they have solved this problem.

It has also been found in two animals that a single muscle may be moved in different directions by the "fast" and the "slow" motor fiber, whenever the joint permits rotation.
Lower crustacea have been investigated for the first time, and the results have shown clearly that in these forms, too, the innervation of muscles by a few nerve fibers occurs and fast and slow contractions can be distinguished.

**Insect Neuromuscular Mechanisms**

**Investigator:** Ripley  
**Support:** General Funds

In following up preliminary work on the sphinx moth, *Celerio lineata*, a preparation suitable for study of the innervation and characteristics of the transmission and contraction processes of the dorsal longitudinal muscles has been developed. This preparation allows simultaneous recording of the muscle tension and the nerve and muscle action potentials. The preparation is simple and is viable for several hours. Stimulation of the axons with frequencies up to about 30 per second causes discrete twitches of the muscle. Above this frequency, provided that the strength is very slightly in excess of the threshold, the contractions fail, owing to refractoriness somewhere in the transmissive process. This refractoriness must be of the order of 30 milliseconds in duration. Its locus has not been identified but it cannot occur in the muscle. With higher strength of stimulation at frequencies greater than 30 per second the muscle responds with tetani, which reach a maximum at about 100 per second.

On recording the muscular tension during spontaneous flight, it is observed that the natural frequency is between 30 and 40 per second and that the contractions are much greater than any recorded during artificial stimulation of the flight nerve. If pairs of artificial stimuli are employed in the nerve, however, the contraction approaches the tensions observed during spontaneous flight. This suggests that the four axons of the flight nerve are distributed in such a manner that there is innervation overlap at the muscle fibers and that a sequence of nerve impulses in the four axons gives what is essentially spaced stimulation of the muscle. The nerve action potentials during spontaneous flight are in agreement with this postulate and histological work is planned to obtain more definite evidence.

**Modes of Conduction in Myelinated Nerve Fibers**

**Investigator:** Wolfgram  
**Support:** General Funds

The thicker vertebrate nerve fibers are covered by a layer of a fatty substance. This myelin sheath is interrupted at regular intervals
It has been claimed that excitation jumps from node to node. The mechanism of this saltatory or jumping conduction was proposed as follows: the action potential of an excited node produces a current which flows through the axoplasma of the internodes and the node to be stimulated, and then through the external medium of the fiber back to the excited node. It is claimed that, by increase of the resistance of the extral path of this current, conduction can be stopped. By a complex procedure, it was possible to raise the resistance of the extral path to several times the resistance of the axoplasma between two nodes. This did not arrest conduction. These experiments are not quite conclusive in proving that a mode of conduction other than the saltatory exists, since the currents involved are so small that the capacitance of the apparatus becomes a non-negligible factor.

**BIO-ORGANIC CHEMISTRY**

**Staff**

Professor: Haagen-Smit  
Research Associate: Bradley  
Research Fellows: Goldsmith, Lowy, Zarudnaya  
Microanalyst: Swinehart  
Research Assistant: Fox  
Graduate Student: Viglierchio

**Study of Air Pollution in the Los Angeles Area**

Investigators: Haagen-Smit, Bradley, Fox  
Support: Los Angeles County Air Pollution Control District  
General Funds

The investigations on the air pollution problem in the Los Angeles area were continued. Crop damage and eye irritation experienced during typical smog periods are the result of gasoline oxidized by nitrogen oxides and sunlight, and also of the oxidation products of gasoline and ozone. In these reactions, aerosols are produced which are responsible for the decrease in visibility observed during a smog attack. Typical smog odor accompanies these oxidations. All the major nuisance effects of Los Angeles smog can be reproduced by the photochemical oxidation of cracked petroleum products, and their simultaneous appearance is strong evidence of their common origin. As a result of these studies, a program for the recovery of hydrocarbons has been initiated.

The study of the atmospheric oxidation of organic material has led to the discovery of the formation of ozone in oxidative processes, a
finding which is of fundamental importance for the understanding of the reaction mechanisms involved.

**Study of Essential Oils**

Investigators: Bradley, Haagen-Smit  
Support: General Funds

The turpentine from Pinus albicaulis was analyzed in detail. Two new sesquiterpenes and a diterpene were discovered and their structure studied. This investigation is part of the complete survey of the turpentines of the genus Pinus, which is being carried out with the cooperation of Dr. N. Mirov of the U. S. Forest Service.

A study of the essential oil of Bursera microphylla was made as a contribution to the knowledge of the chemical constituents of desert plants. The major constituent of the oil was shown to consist largely of α and β phellandrene.

**The Proteose Fraction of Milk**

Investigators: Goldsmith, Haagen-Smit  
Support: Elbridge and Mary Stuart Foundation

The proteose (high molecular weight peptide) fraction of milk was isolated, purified and subjected to chromatographic analysis, by using a series of solvents developed in the course of the work for this purpose. With starch and paper chromatography a number of peptide fractions were obtained and their amino acid contents determined chromatographically and by microbiological assay techniques.

Electrophoretic analyses were carried out on several of the fractions at various pH levels, and it was shown that the chromatographically homogeneous fractions were mixtures of at least two and three peptides. It is therefore evident that, in the case of peptides, chromatographic homogeneity is not a sufficient criterion of purity.

In an experiment designed to determine the nature of some of the volatile and odoriferous constituents of milk, material collected in liquid nitrogen traps by passing nitrogen through a well-stirred and heated sample of milk was subjected to mass spectrographic analysis. No indication of the presence of sulfur-containing material was found, but the presence of small amounts of acetone, heptanone-2, and either several hydrocarbons, or their oxygenated derivatives containing one less carbon atom, was established.
Study of Anthocyanin Biosynthesis in Maize

Investigator: Zarudnaya
Support: Gosney Research Fund, Atomic Energy Commission (operating through the Office of Naval Research)

The biosynthesis of anthocyanin was studied in vegetative tissues of Indian maize. Attention was concentrated on the action of light on anthocyanin formation. The approximate action spectrum for pigment production was determined in a genetic strain in which pigment is formed only in illuminated areas. Blue light (4000 - 4400 A) was found to be effective in inducing pigment formation. It was also found that the photochemical reaction was a relatively short one, and was followed by a much longer process which could take place in the dark. The dark reaction was found to be temperature sensitive, oxygen dependent, and could be inhibited by general enzymatic poisons. The occurrence of both reactions could be demonstrated in strains which produce anthocyanin without light, by means of variation of temperature and light conditions. These observations are in agreement with the hypothesis that light activates a prosthetic group of an enzyme, which then functions in the dark. In the strains which do not require light for pigment production, the prosthetic group is already present in a partially activated form.

Isolation and Characterization of Wound Hormones

Investigator: Viglierchio
Support: General Funds

Extracts of wounded plant cells have been shown to induce cell division when injected into healthy tissue. The active component has been isolated from beans. It was found that similar substances are present in lemon peel extracts. Isolation of the physiologically active material is in progress.

EMBRYOLOGY

Staff

Professor: Tyler
Visiting Professors: Bishop, Borei
Graduate Student: Jaffe
Research Assistants: Merritt, Norris
Properties of Fertilizins and Antifertilizins of Eggs and Sperm of Marine Invertebrates

Investigator: Tyler  
Support: General Funds, Rockefeller Foundation, Guggenheim Foundation.

As part of a long-range program of investigation of the factors involved in the initiation of development of animal eggs, further studies have been made of the chemical nature and mode of action of fertilizins (derived from the surface coat of the eggs) and antifertilizins (from sperm) of marine invertebrates. In the course of this work with sea urchins a rather striking morphological reaction of the spermatozoa to the action of fertilizin has been recently discovered with the aid of the electron microscope. When a solution of fertilizin is added to a suspension of spermatozoa, the latter agglutinate temporarily. Subsequent to this agglutination the spermatozoa remain as motile as the untreated controls and appear normal under ordinary microscopic examination, but are incapable of re-agglutination and have lost their fertilizing ability. Examination of the treated sperm through the electron microscope shows that a particular structure, termed the midpiece, has been displaced. Normally this body is attached to the base of the head where the tail emerges. In the treated sperm it has separated itself from the base of the head and retains a small area of attachment laterally or reattaches itself in a more forward position, thus forming a lateral protuberance on the head of the sperm. The midpiece of the sperm is known to be composed almost entirely of certain cytoplasmic bodies, the mitochondria, which in recent years have been the subject of many investigations concerning their role in cellular metabolism and cytoplasmic inheritance. In fertilization in many species of animals, the midpiece and tail do not enter the egg which, if it requires mitochondria for special functions, has its own supply. The present observations indicate that the fertilizin present on the surface of the egg, by causing a separation of the midpiece, may be responsible for the exclusion of this structure from entry into the egg.

Another aspect of the agglutination of spermatozoa by fertilizin that is the subject of further investigation is the phenomenon of spontaneous reversal. A parallel to this is found in the agglutination of red blood cells of various vertebrates by certain viruses, such as influenza virus. Here, too, a spontaneous reversal occurs, and, as in the case of the spermatozoa, the red blood cells cannot be re-agglutinated. Investigations in the field of virology have presented evidence to show that the effect is due to an enzyme, carried by the virus, that destroys the specific virus receptors on the red cells. However, there is not as yet a convincing demonstration of such an
enzyme. In the case of spontaneous reversal of the agglutination of spermatozoa by fertilizin, the evidence is against an enzymatic action of this substance. Discovery of the mechanism of reversal in this case may, then, contribute to our understanding of the manner of infection of cells by viruses, and this aspect along with its relation to the problems of fertilization is considered in the further investigations that are planned along this line.

The fertilizin of sea-urchins are now known to belong to the class of large-molecular substances, the glycoproteins or mucopolysaccharides. Collateral interest attaches to their study by virtue of the fact that a number of other substances of biological importance, such as the blood group substances, certain naturally occurring virus-inhibitors (and probably also virus receptors on the susceptible cells), certain pituitary hormones and many immunologically active substances, also belong to this chemical group. The chemical studies on fertilizin have suggested the possibility that such substances are constructed of interlinked sugar and amino acid residues rather than of separable protein and polysaccharide moieties, as is generally supposed.

Antifertilizin was previously shown to be a protein and to be located on the surface of the nuclear region of the head of the sperm. The possibility that it may be a nucleoprotein has been examined and is excluded by the evidence. Active, purified preparations have been obtained which give no tests for nucleic acid. Hydrolysis of the molecule yields an assortment of at least 10 amino acids which are lacking in the di-amino types. This is consistent with other evidence that the substance is an acidic protein (isoelectric at about pH 3) rather than a basic protein such as would be obtained from nucleoproteins.

Investigations have been initiated concerning the possibility that fertilization in mammals involves the interaction of substances analogous to the fertilizins and antifertilizins known in invertebrates. Owing to the limited number of eggs obtainable in mammals the usual techniques of preparing extracts are not readily applicable. However, it has been possible by a relatively simple technique to demonstrate an agglutinative response of sperm in the presence of eggs. Rabbit eggs obtained by hormone-induced ovulation were mainly used for these studies. When the eggs are mounted in a
washed and diluted suspension of sperm (obtained by the artificial vagina technique) in a sealed slide preparation, a marked agglutination occurs in the vicinity of each egg. The reaction decreases with increased distance from the egg and, depending upon the preparation, is usually absent at about 10 egg diameters. The reaction occurs with eggs of various ages following ovulation and even with ovarian eggs. Thus, it is given by eggs at stages having the follicle cell coat, the corona radiata, or simply the zona pellucida, but not after formation of the albumen layer. The reaction is given by eggs that have been treated with trypsin but is absent or reduced when the eggs have been exposed to periodate. Concerning the specificity of the reaction, rabbit eggs do not act on bull and mouse sperm and only weakly on human sperm. Mouse eggs, which give a moderate reaction with mouse sperm, do not act on bull and human sperm and only weakly on rabbit sperm. Extracts of sperm of various species give an inhibition of the homologous reaction, but the experiments have not been sufficiently extensive to warrant a final decision on the extent of specificity of the inhibition. Egg extracts, with some agglutinating activity, have been also prepared. Whether or not a fertilizin-antifertilizin mechanism is involved in these reactions cannot as yet be conclusively stated but the evidence favors this interpretation.

A study has also been started of the immunological properties of the sperm of rabbits and other mammals. It has proved possible to induce antibody formation by iso-immunization with seminal plasma as well as with washed sperm. The reactions are complicated by the fact that normal rabbit serum is capable, in most instances, of agglutinating homologous sperm. Various kinds of heterologous reactions have also been obtained, such as the agglutination of rabbit sperm by human sperm extracts. Rabbit sperm agglutinate, too, in the presence of homologous uterine fluid. Further experiments are planned to elucidate the significance of these various reactions and their relation to fertilization.

Studies on the Life-Span of Spermatozoa

Investigator: Tyler
Support: Guggenheim Foundation

It has been shown earlier that the duration of motility and fertilizable life of spermatozoa of various invertebrates can be considerably extended by the addition of any one of a number of amino acids to the suspension medium. The amino acid was shown not to be utilized in the metabolism of sea urchin spermatozoa. The amino acid does prevent the initial great increase in respiratory rate that occurs when the spermatozoa are diluted, and then maintains respiration
at a low constant level after that of the controls has dropped practically to zero. It was also found that the normal early death of these cells when suspended in sea water is not due to starvation. In the presence of amino acid the spermatozoa are able to utilize more completely their endogenous substrate. The presence of added amino acid also permits the spermatozoa to survive anaerobically for a considerable period whereas normally in the absence of oxygen they quickly become motionless and die. It is known that amino acids form sparingly ionized complexes with ions of heavy metals. This fact, along with the evidence of non-utilization of the added amino acid, suggested the possibility that they act by binding certain heavy metal ions, ordinarily present in the dilution medium in amounts which are barely detectable chemically but sufficient to cause a rapid progressive deterioration of the spermatozoa. Further experiments with known chelating agents support this. Thus the following agents, at proper concentration and pH, have given extension of life span comparable in many instances with what is obtained by use of the amino acids: diethyldithiocarbamic acid, α-benzoinoxime (Cupron), 8-hydroxyquinoline (Oxine) and ethylenediaminetetraacetic acid (Versene). From the present evidence, and from what is known of their specific metabolic effects, it appears likely that Cu²⁺ and Zn²⁺ are the principal ions involved in this trace-metal action on spermatozoa.

Metabolic Changes upon Fertilization in Eggs of Sea Urchins

Investigator: Borei
Support: Rockefeller Foundation

During a two-months' stay at the Institute, Borei investigated the respiratory changes that occur upon fertilization in eggs of the sea urchin, Lytechinus pictus. For this purpose he employed the Cartesian Diver Microrespirometer, a sensitive manometric instrument devised by Dr. K. Linderstrøm-Lang, of the Carlsberg Laboratories, Copenhagen, who at one time (1931-1932) worked as a Rockefeller Foundation Fellow at the Institute.

There is a high respiratory rate in the unfertilized egg immediately after its removal from the ovary. Thereafter the rate gradually decreases. The egg can be fertilized at any point of this declining curve. After fertilization the respiration increases, and after some time reaches a characteristic level, which is independent of the respiratory rate before fertilization. Facts about the functional capacity of the respiratory system and about the changes in RQ indicate that the respiratory chain is operating after fertilization on other substrates than before. It is likely that these differences result from alterations in the dehydrogenase setup and in the colloidal cytoplasmic structure.
Measurements on fertilized eggs in the respirometer indicate that there is a time lag of about 10 minutes before the eggs respond to fertilization with an increased oxygen uptake. Acid formation, on the other hand, is already fully developed two minutes after fertilization, and has practically ceased at the time when the oxygen uptake begins to increase. It is suggested that the acid formation is connected with the cortical changes, especially the elevation of the fertilization membrane, and that it is independent of the changes in respiratory activity. The marked increase in oxygen consumption may be attributed to changes at fertilization in dehydrogenase activity and alterations in the colloidal organization of the egg.

Gamete Liberation and Early Development in Pelvetia and Hesperophycus

Investigator: Jaffe
Support: General Funds

These investigations concern the spawning stimulus and initial differentiation of the eggs of the brown marine alga, Pelvetia, and the possible significance of a \( \text{CO}_2 \) gradient in differentiation. It has been found that Pelvetia is stimulated to spawn by putting it in darkness after a period of strong illumination. Because this phenomenon had not been previously reported for any other alga and because of its analogy with the initiation of flowering in short-day higher plants, this light-dark effect was further investigated. Two findings of interest are that the spawning stimulus can apparently be transmitted in the alga at low temperatures only and that a period of only a few minutes' darkness can be a fully effective spawning stimulus. By slightly acidifying the sea water (to pH 6), the escape of the gametes of Hesperophycus (closely related to Pelvetia) from their capsules can be greatly delayed. The gametes are not injured by this treatment. Hence, this slight acidification is an excellent aid in obtaining unfertilized eggs in such monoecious species. Moreover, this acid block to gamete escape suggests a mechanism whereby the gametes are kept in their capsules in vivo until the latter are shed.

A relatively simple technique has been developed to support the eggs in order that \( \text{CO}_2 \) measurements can be made on different parts of the algal egg. Experiments are under way to determine whether or not regional differences in \( \text{CO}_2 \) production occur.
GENETICS: CORN

Staff:

Professor: Anderson
Research Associate: Longley
Senior Research Fellow: H. Teas
Research Fellows: E. Dale, Robertson
Research Assistants: Belmont, Bostwick
Graduate Student: Patterson
Superintendents: J. Dale, Hoover.

Genetic Effects of Radiation in Maize

Investigators: Anderson, Longley, Teas
Support: Atomic Energy Commission operating through Office of Naval Research

The investigations dealing with hereditary effects of radiation have been continued. Chromosome rearrangements are produced when chromosomes are broken and the broken ends reunite in different order so that parts of chromosomes become exchanged. If parts of non-homologous chromosomes are exchanged, a translocation is formed. If parts of the same or homologous chromosome are exchanged, an inversion is formed. Other, more complex, rearrangements may be formed but are usually not transmitted. Both translocations and inversions are characterized by partially sterile pollen owing to unbalanced chromosome complements in some of the products of meiosis. In translocations this is caused by adjacent chromosome segregation; in inversions, by crossing over within the inversion.

A total of 250 translocations has been accumulated from sources other than the Bikini and Eniwetok tests. These have all been examined cytologically and in most cases the chromosome identification and the location of breaks have been confirmed by linkage tests, or other data. A few additional ones are not yet completed.

About 400 translocations were isolated from the Crossroads (Bikini) tests. These have likewise been examined cytologically but linkage and other tests are not so far along. Nearly all of these are being maintained in stock.

The placement of genes on linkage maps is being advanced by use of pollen sterility, cytogenetic determinations, and duplicate, deficient segregates, as well as by standard gene-mapping techniques.
All chromosomes have been mapped in outline, with more extensive mapping of chromosomes 1, 2, 4, 6 and 9. Detailed mapping of the short arms of chromosomes 2 and 9 has been accomplished.

In biochemical studies of kernel development, the use of closely linked genes which can be classified early in ontogeny has been successfully employed. In the case of translocation 4-6a, the technique has been used to distinguish between immature sugary and normal seed by means of the white endosperm character.

**Preferential Segregation in Translocations**

Investigator: Longley  
Support: United States Department of Agriculture

Preferential segregation of the abnormal form of chromosome 10 (ab10) to the basal cell of the linear tetrad during megasporogenesis of maize is a phenomenon that is not completely understood.

In studies the past season preferential segregation has been tested by marking the translocated piece of 10 with dominant R and introducing with the ab10 the closely-linked recessive r allele. Progenies of reciprocal outcrosses to R-tester stocks were scored to determine the types of gametes transmitted. A similar series with normal 10 was grown for comparison.

The data from four A-type translocations involving chromosome 10 show that when only normal 10 is present the amount of crossing over between R and the break point increases from 8% to 26% as the point shifts from .6 to .1 on the long arm. The ratio of R to r alleles transmitted through the egg is 1 to 1 in both the crossover and the non-crossover classes for all four translocations. When ab10 replaces normal 10, the ratio of R to r is also 1 to 1 in the noncrossover classes but the ratio between the two crossover classes is 1 to 6.8 in all four translocations.

These tests indicate that preferential segregation at megasporogenesis is restricted to sporocytes in which crossing over has occurred between the translocated piece of 10 and ab10 homologue.

The data from translocation B-10 make it possible to note the effect on segregation of ab 10 when that chromosome is paired in the proximal region with 10B and in the distal region with B10. The segregation at megasporogenesis of ab 10 and 10B was as normal as the segregation of 10 and 10B, indicating that crossing over in the proximal one-third of the long arm of 10 does not interfere with regular segregation.
All distortions from the expected ratios of transmitted gametes produced by TB-10 a progenies were owing either (1) To loss of gametes with duplications or deficiencies for part of chromosome 10 or (2) to preferential segregation of the \( B^{ab\,10} \) and the normal 10 or the ab 10 and \( B^{10} \) homologues. The data show that when ab 10 crosses over with \( B^{10} \) the eggs that receive normal 10 are only 22% as numerous as those that receive \( B^{ab\,10} \), and that when 10 crosses over with \( B^{ab\,10} \) the eggs that receive \( B^{10} \) are only 10.5% as numerous as those that receive ab 10. Thus, the chromosome which receives the abnormal piece as a result of crossing over goes preferentially to the basal cell of the linear tetrad.

From these observations of the A- and B- type translocations it is concluded that the abnormal form of chromosome 10 initiates preferential segregation at megasporogenesis only when crossing over has occurred between it and its normal homologue in the region adjacent to the added abnormal piece.

**B Vitamins in Starchy and Sugary Corn**

Investigator: H. Teas  
Support: Atomic Energy Commission operating through Office of Naval Research.

Sugary corn endosperms have long been known to contain about one and one-half times as much niacin as starchy ones. When kernels of each kind were carefully dissected and the component parts assayed, it was found that the aleurone (outer cell layer of the endosperm), although it had about the same niacin concentration, was present in greater amount in the sugary type. Thus a thicker aleurone layer, a "morphological" effect of the gene, accounts for more than 90% of the "extra" niacin in sugary endosperm.

Determination of B vitamins on 24 sugary and starchy corn endosperm samples has shown that thiamine, riboflavin, biotin, choline, inositol, pyridoxine, niacin, and pantothenic acid were very significantly higher in sugary than starchy. It remains to be seen whether, like niacin, all of these differences originate in the thicker aleurone layer of the sugary kernels.

**Blue Fluorescent Mutants**

Investigator: H. Teas  
Support: Atomic Energy Commission Operating through Office of Naval Research
Greenhouse tests showed that three new blue fluorescent mutants of radiation origin are alleles. They differ from the original Bf-1 genetically and chromatographically. The Bf-1 gene was located in chromosome 5 by translocation linkage tests. Another new blue fluorescent mutant contains substances chromatographically similar to those of Bf-1, but since the latest mutant is expressed only as a dominant, tests for allelism are difficult to carry out.

A Dominant Necrotic Mutant in Maize

Investigator: E. Dale  
Support: General Funds

In contrast to the numerous recessive seedling mutations induced by exposure of maize seeds to the Bikini bomb, only one dominant mutation has been derived from the same material. This mutation, called "dominant necrotic," causes partial necrosis of the early seedling leaves. The first three or four leaves are usually the most strongly affected. An affected leaf shows from one to four transverse necrotic areas, which sometimes form complete necrotic bands, the pattern of the necrosis being rather similar to that of the zebra type of chlorophyll deficiency. Considerable loss of leaf tissue results not only by the death of the necrosed areas but also by the cutting off of healthy tissues distal to the necrotic bands. In spite of this handicap even severely affected seedlings survive and plants which have reached adult height usually show no necrosis.

Selfed necrotic plants have given quite variable proportions of necrotic and normal seedlings and no homozygous necrotic individuals have yet been found. This suggests that the gene for dominant necrotic has a recessive lethal effect.

Hopi Indian Corn

Investigator: Anderson (in collaboration with William L. Brown, Pioneer Hi-Bred Corn Company, Johnston, Iowa, and Roy Tuchawena, Jr., Moenkopi, Arizona) 
Support: General Funds

Previous investigators have found that most of the corn varieties grown by Southwest Indian tribes appear to be composites from three distinct sources:

1. An ancient type resembling the corn found in the ruins of the Basket Maker society which preceded the Pueblo culture. These are small, somewhat cigar-shaped ears with small, closely
packed, nearly isodiametric kernels.

2. An "Eastern" complex resembling the northern flint types grown in the northern half of the United States of the Rocky Mountains, which in turn resemble high altitude Guatemalan types. These are long, slender, few-rowed ears with heavy shank and enlarged base, the kernels broad and crescent-shaped.

3. A "Mexican" complex resembling the corn of the central Mexican plateau area, but reaching the Southwest from its northern periphery. These are many-rowed, large-diameter ears with deep, narrow, dented kernels.

The native Indian varieties are rapidly being lost by admixture of corn from outside sources. Of the Southwest Indian tribes, the Hopi are among the most conservative and probably come nearest to retaining their original varieties in relatively pure form. From studies and collections made in the Hopi Indian Reservation, a detailed description has been prepared of the two most characteristic Hopi varieties, the Hopi Blue and the Hopi White. The dye-corn variety "Kokoma" is also described, but from rather meager material, as this variety is grown only in small amounts and has become badly contaminated. The Kokoma corn in its purest form is morphologically much like the ancient Basket Maker corn from which it is probably a descendant. The Hopi Blue is perhaps the most characteristic Hopi variety and occupies a position intermediate between Kokoma and the Hopi White. In the Hopi White, which resembles more closely the Keresan and Tanoan Pueblo corn, the "Mexican" and Eastern traits predominate.

Studies on Viviparous Mutants

Investigator: Robertson
Support: Atomic Energy Commission operating through Office of Naval Research

Studies of the viviparous mutants of maize have included genes \( \text{vp-2}, \text{vp-5}, \text{vp-7} \) and \( \text{vp-9} \). \( \text{Vp-2} \) and \( \text{vp-5} \) have been tentatively located. Further linkage tests with the \( \text{vp} \) mutants are going on.

Self-pollination of plants heterozygous for viviparous-1 and homozygous for aleurone color genes has shown that the \( \text{vp-1} \) gene, when homozygous, interferes with the formation of color in the aleurone layer. Viviparous seeds from such ears are either colorless or possess only a slight tinge of color. Crosses have been made to determine if the \( \text{vp-1} \) gene has any effect on plant color.

Tests of the possible role of auxin (IAA) in maintaining dormancy of the developing seeds were made. Viviparous-5
and normal embryos from the same self-pollinated ear were cultured on media containing 0γ, 5γ, 10γ, and 20γ of IAA per ml. Although the amount of germination observed was proportional to the IAA concentration, there was no concentration at which germination was prevented entirely in either the normal or mutant embryos. In the higher concentrations (10γ and 20γ) the mutant embryos were relatively more inhibited than the normals. These data would indicate that IAA is not the normal inhibitor of premature germination.

Studies of Chromosome Rearrangements

Investigator: Patterson
Support: General Funds

Chromosome rearrangements are of particular value in maize because they may be followed both genetically and cytologically. They may thus be used to correlate genetic and cytological maps of the chromosomes. They have also provided a valuable means of marking chromosome regions devoid of known genes, of locating unplaced genes, and of artificially linking genes on different chromosomes.

During the past year linkage studies have established the genetic positions of 16 translocations in chromosome 9 with reference to the genes C, sh1, and wx and of 14 translocation in chromosome 2 with reference to the genes Ig1, gl2, B, sk1, and v4.

Other studies have demonstrated the usefulness of duplicate-deficient gametes in cytogenetic investigations. Adjacent disjunction from a heterozygous translocation complex yields spores duplicated for one arm of the "cross" configuration and deficient for the opposite arm. A functional duplicate-deficient egg when fertilized by a sperm of normal chromosome constitution gives rise to a plant having one chromosome segment in triplicate and another segment represented singly. When appropriately test-crossed, a duplicate-deficient plant gives modified trisomic ratios for genes carried in the triplicated segment and a hemizygous test for genes in the deficient segment.

Functioning of duplicate-deficient eggs has been highly variable and appears to be strongly influenced by environmental conditions. In these studies there have been no confirmed instances in which duplicate-deficient pollen grains have functioned.
GENETICS: DROSOPHILA

Staff:

Professor: Sturtevant
Research Associate: Morgan
Associate Professor: Lewis
Senior Research Fellow: Novitski
Research Assistants: Gencarella, Bleecker
Graduate Students: Hinton, Judd, Lindsley

Taxonomic Studies of Drosophila Species and Related Groups of Small Flies

Investigator: Sturtevant
Support: General Funds

The various species of Drosophila have been much used for the study of comparative genetics and the genetical analysis of the results of evolution. As a background for this work it has seemed desirable to carry out a study of the classification, distribution, and natural history of the existing species. This has in turn led to similar studies on related groups of small flies which have been largely neglected by entomologists. During the year much time has been spent on this work, making use in part of extensive collections of material borrowed from the United States National Museum, Cornell University, University of Kansas, and Iowa State College.

Genetics of Iris

Investigator: Sturtevant
Support: General Funds

Many plants, both wild and cultivated, are tetraploid - i.e., have four sets of chromosomes instead of the two sets characteristic of most higher animals and plants. There are a series of problems connected with this condition, having to do with chromosome mechanics, developmental physiology, evolution, and practical breeding. Studies are being carried out on the cultivated Iris, where both diploid and tetraploid forms occur. From a geneticist's point of view the material has some serious disadvantages - notably the fact that a generation requires two years. There are, however, some compensations - such as the facts that individuals may be kept indefinitely, that crosses between individuals with very different chromosome numbers are possible, that the history of the cultivated strains is fairly well known, and that an extremely great and extensive diversity, genetically, is thus
available for analysis. During the year, two brief papers on Iris genetics have been published. There are a great many amateurs interested in breeding Iris, and they have a large store of enthusiasm and of information; it is part of the project to encourage and help these amateurs and to try to make their efforts yield more results.

**Diploid Intersexuality in Drosophila**

Investigator: Morgan  
Support: General Funds

The comparative study of diploid and triploid intersexes of *Drosophila melanogaster* and other species of Drosophila has been continued. This study is concerned with the relation of differences between the two kinds of intersexes to their different chromosomal constitutions.

**Gene Action and Position Effect**

Investigator: Lewis  
Support: Atomic Energy Commission operating through the Office of Naval Research

The initial steps by which a gene is able to produce a specific physiological effect on the organism remain largely a mystery. The so-called position effect of genes has shown that the first interactions between the products of gene activity may sometimes occur within the nucleus and in such a way that the closer the genes concerned are to one another the more efficient will be their operation. One such cluster of interrelated genes in Drosophila is under intensive study. Three separable genes in the cluster had been previously known. Recently two new members have been identified, bringing the total number of genes of "pseudoalleles" in the cluster to five - the greatest number yet known for such clusters. Each of the five genes appears to have its own specific function in the development of the organism but these functions are somehow closely interrelated. The function of the cluster as a whole appears to be the carrying out of a chain of chemical reactions at the site of the genes in the chromosome and with as many steps, possibly, as there are component genes.

One of the genes in the above cluster results in the death of the organism when it is present in two doses. In order to learn more about its physiological effects, a method has recently been devised for producing individuals in which part of the body remains normal while another part carries two doses of the lethal gene. A few survivors of this type have already been obtained and the physiological effects observed for the lethal gene suggest that it controls one of the first of the postulated series of reactions carried out by the cluster.
Double Ring Chromosomes

Investigator: Novitski
Support: Atomic Energy Commission operating through the Office of Naval Research

The series of steps outlined in the 1950-51 report for the production of a double X ring chromosome in Drosophila has been completed. It is hoped to finish the genetic analysis of this unusual chromosome in the near future.

Spermatogonial Exchange Involving Long X Chromosome Inversion

Investigator: Lindsley
Support: Atomic Energy Commission Predoctoral Fellowship administered by the National Research Council General Funds

Among the progeny of males carrying the inverted X chromosome In(l)sc8, individuals are found which have lost the terminal euchromatic segment of the inversion. This has been interpreted in the past (Sidorov) as the result of exchange between the distal heterochromatin of the inversion and the short arm of the Y chromosome, and, indeed, the reciprocal recombinant type may be recovered, i.e., a Y with its short arm replaced by the terminal euchromatin of in(l)sc8. The fact that such derivatives occur in clusters and represent recombination which has occurred in males indicates that the events in question are mitotic rather than meiotic in origin.

An analysis of 44 such recombinants recovered among 273,721 offspring of males carrying the distal heterochromatin of In(l)sc8 reveals that previous notions represent an oversimplification of the actual case.

The new observations have been interpreted to indicate that the distal heterochromatin of In(l)sc8 may pair mitotically and undergo exchange with any other sex-linked heterochromatic segment, with the possible exception of YL. Such pairing may occur in either direction as if there were no linear differentiation of pairing specificities in the heterochromatic segments, or as if a reverse repeat system were operating. Inversion of ordinary pairing relationships followed by exchange may result in the formation of dicentric bridges. The evidence indicates that such bridges may break at anaphase.

The fate of a freshly broken chromosome end in the soma of Drosophila is rather obscure. That stable breakage products are
recovered testifies to the fact that in at least a portion of cases these broken ends heal. No evidence has been found favoring any sort of fusion cycle subsequent to bridge breakage, but the experimental arrangement favored detection of only a chromatid type of cycle.

**Ring Chromosome Elimination**

Investigator: Hinton
Support: Atomic Energy Commission Predoctoral Fellowship, administered by Oak Ridge Institute of Nuclear Studies; General Funds

Catcheside's ring, a closed-X chromosome with an X-ray-induced inversion, is unstable. The chromosome is eliminated during mitotic divisions, producing up to 30% gynandromorphs among the female progeny. Studies of this unique chromosome have shown that the instability factor resides in neither of the large autosomes. Successive outcrosses result in ring stability and attempts to reconstitute instability by backcrossing to unstable lines have been negative. Gynandromorph production is paralleled by the occurrence of patroclinus males, and the frequency of both is a function of the mother's age. While the ring is lethal in the male, infrequent crossovers in the region of the forked locus have resulted in male-viable, but stable, rings. In two exceptional cases, unstable crossover rings have been passed through the male.

By employing ring elimination as a tool, the expressions of two small deficiencies for the tip of the X chromosome have been studied in mosaic individuals. This technique will be extended to a comparative study of sex-linked lethal mutations.

**A Position Effect at the White Locus**

Investigator: Judd
Support: General Funds

The translocation T(1,4)w^VD_3, involving the X and the fourth chromosomes, shows Notch and a mottled effect for the genes white, split, facet, and diminutive. The present investigation was undertaken to determine whether the mottling of white was an effect owing to the presence of the rearrangement or whether some other change had occurred at the white locus which was independent of the translocation. Crosses were set up so that a new allele of white could be substituted by crossing over for the allele originally in the translocated chromosome. The complementary crossover type would put this original allele into a normal chromosome. The latter crossover type has not been recovered, while the former has. The results
indicate that the mottling is associated directly with the chromosomal rearrangement. Further tests, however, are under way to determine whether mottling is shown when a (+) allele of white of still different origin is placed in the translocated chromosome.

GENETICS: MICROBIAL

Staff:

Professors: Beadle, Emerson (on leave of absence)
Associate Professors: Horowitz, H. K. Mitchell
Graduate Students: Ames, Dubes, G. Ellman, Fischer, Good, Greene, Hogness, Holloway, Jennings*, Metzenberg, Reissig
Research Assistants: P. Ellman, D. Haskins, Kilim, Surber

*Chemistry major

Cytoplasmic Inheritance in Neurospora

The following four abstracts are concerned with investigations on the genetical and biochemical aspects of some examples of maternal inheritance in Neurospora. This phenomenon is not new to biology but it has not been found previously in Neurospora and it is clear from the work that has been done that the mold provides excellent material for investigations of this kind of inheritance and its influence on cellular biochemistry. Earlier work with other organisms suggests that cytoplasmic inheritance may be very important in the processes of differentiation and development and it is hoped that these studies with Neurospora will contribute to an eventual understanding of these complex biological processes.

Non-Mendelian Inheritance of Slow Growth

Investigators: M. B. and H. K. Mitchell
Support: Rockefeller Foundation Fund, Atomic Energy Commission operating through Office of Naval Research

A slow-growth character, designated as poky, arose spontaneously in a standard wild strain. By means of crosses between poky and normal strains it was observed that the poky character
was transmitted to all the offspring if the incipient fruiting bodies which functioned in the cross were formed by a poky strain and fertilized by vegetative spores from a normal strain. This behavior was constant throughout the three generations which have been studied. If, on the other hand, incipient fruiting bodies were formed by a normal strain and fertilized by spores from a poky strain, no poky offspring appeared, nor was the poky character recovered in the next generation when incipient fruiting bodies were formed by a not-poky descendant of poky.

The inheritance of the poky character parallels that, in higher plants, of characters which are transmitted through the egg but not through the pollen. It is of interest to know what other traits in Neurospora are inherited in this fashion, and an attempt is being made to find other characters so inherited. So far, poky has been found again from a different wild strain from which was also obtained a strain designated semi-poky since its growth rate is roughly half that of normal strains and twice as great as that of poky, and since it is also inherited in the same way. That semi-poky and poky strains resemble each other in their biochemical differences from wild type has been shown by investigators who are studying the physiology of these strains.

Studies on the Succinic Acid Oxidase System of Wild-Type and Poky Neurospora and the Isolation of Cytochrome c from Poky


Support: Williams-Waterman Fund
Atomic Energy Commission operating through Office of Naval Research
Gosney Fund
Rockefeller Foundation Fund

Preparations of particulate material have been made from mycelia of wild-type and poky Neurospora of various ages (one, three, six and seven days for wild type; three, four, five, six, seven and nine days for poky), and the succinic dehydrogenase, succinoxidase, and cytochrome oxidase activities of these preparations have been measured. Succinic dehydrogenase activities were measured by the Thunberg technique and succinoxidase and cytochrome oxidase activities were measured manometrically. Relatively high succinic dehydrogenase, succinoxidase, and cytochrome oxidase activities were displayed by all preparations from wild type. From the poky cultures, on the other hand, preparations which had all three types of activity were obtained only from
mycelium which was 5 days old or older. Thus three- and four-day poky cultures yielded preparations in which no succinoxidase or cytochrome oxidase activity was detected, although all the poky preparations were at least as active as the wild-type preparations in succinic dehydrogenase. It seems probable that these differences between wild type and poky are related in some way to the differences in growth between the two kinds of mold.

The use of trichloracetic acid to extract cytochrome c, a practice which has been successfully employed with heart muscle, was found ineffective in extracting cytochrome c from poky mycelium. Several other methods were tried, the best of which was the extraction of the well-ground mycelium with a half-saturated solution of ammonium sulfate at a pH of about 8. The cytochrome c thus extracted was precipitated by saturating the extract with ammonium sulfate, following which the precipitate was redissolved in water and then reprecipitated by saturating with ammonium sulfate. The clear, red solution of this reprecipitated material was then dialyzed against 0.5% potassium chloride, and the cytochrome c concentration of the dialyzed solution was measured spectrophotometrically. Approximately 150 mg. of cytochrome c was obtained from 380 gm. (wet weight) of mycelium.

Respiration and the Cytochrome System in Wild-Type and Poky Strains of Neurospora.

Investigators: Tissieres, F. Haskins, H. K. Mitchell
Support: Gosney Fund
Williams-Waterman Fund
Atomic Energy Commission operating through
Office of Naval Research
Rockefeller Foundation

The cytochrome system of wild-type Neurospora is composed of the typical components a, b and c, easily visible under the spectroscope, and the properties of the individual components are similar to those of the cytochromes of animal tissues.

The cytochrome system of poky shows several peculiarities: a young culture contains about 10 times more cytochrome c than the wild type, that is 1.5% of the dry weight of the mold; cytochromes b and a are reduced to minute traces. This is consistent with the fact that a cell-free preparation of the mutant does not have succinoxidase or cytochrome oxidase activity.

While the respiration of intact mycelium of the wild type (\(QO_2\) = 60-70) is inhibited 70% by the usual respiratory inhibitors, cyanide
or azide (0.001-M), the respiration of the mutant mycelium is completely insensitive to cyanide or azide concentrations as high as 0.03-M.

The facts that (1) the cytochrome system is practically devoid of the components a and b, (2) there is no succinoxidase and cytochrome oxidase activity, and (3) the respiration is insensitive to usual respiratory inhibitors, indicate that the cytochrome system does not function in the usual way in the mutant and that a respiratory system of a special type, so far unknown, is concerned. The mutant was found to contain six times more flavin than the wild-type Neurospora. Practically all this flavin is present as flavin-adenine dinucleotide and it may be that an FAD enzyme system is responsible for the respiration of the mutant. It is of particular interest that the mutant has a relatively high respiration rate (QO₂ = 30) as the flavin respiratory systems so far reported in the literature have a rather low activity.

Another heme enzyme, catalase, was also investigated and its activity in the mutant was found to be more than 10 times that of the wild type. This result is consistent with the finding that the mutant contains large amounts of flavin, as flavin enzymes are known to produce peroxide, a substrate of catalase.

Some Biochemical Differences and Likenesses between Wild-Type and Poky Strains of Neurospora


Support: Atomic Energy Commission operating through Office of Naval Research
Rockefeller Foundation Fund
Williams-Waterman Fund
Gosney Fund

It has been found that poky strains contain an enzyme system that causes the destruction of cytochromes a, b and c. Other heme proteins such as catalase and hemoglobin are not acted upon appreciably by the enzyme preparation. This enzyme system has not been found in wild-type strains.

It has been shown that poky strains are significantly deficient in ribonucleic acids. Investigations are under way to determine which cellular fractions of nucleic acid are particularly affected.

Significant differences in sugar metabolism between poky and wild strains have been found and are under investigation.
Acid and alkaline phosphatases, tryptophane desmolase, amylase, invertase, uricase and a number of other enzymes have been studied in wild and poky strains. It is probable that the two types of mold do not differ significantly with respect to the activities of these enzymes.

**Neurospora Tyrosinase**

Investigators: Horowitz, M. Fling  
Support: Atomic Energy Commission operating through Office of Naval Research  
Nutrition Foundation

Investigation of the tyrosinase activity of various strains of Neurospora has shown that the tyrosinase activity of some strains is thermolabile while that of other strains is relatively thermostable. Extracts of strains of the former type are half-inactivated in four minutes at 59° C., whereas extracts of strains of the latter type are half-inactivated in 40 minutes at the same temperature. Crosses between the two types of strains have been made and the progeny analyzed. Analysis of 51 ascis from such crosses indicates that thermostability and thermolability are inherited as if determined by alleles of a single gene. The locus is at least 30 map units from the centromere. Its linkage group is unknown.

Under study is the question of whether the difference in tyrosinase thermostability represents a difference in the structure of the tyrosinase molecules produced by the two kinds of strains, or is caused by differences in the other constituents of the cell. At the present writing, a 13-fold purification of the thermolabile enzyme and a 46-fold purification of the thermostable enzyme (based on protein nitrogen) have been accomplished. No change in the rate of thermal inactivation of the thermolabile enzyme resulted from the 13-fold purification. In the case of the thermostable tyrosinase, a significant increase in thermostability was observed in the purified preparation. This and other evidence suggest that the tyrosinases produced by the two kinds of strains are structurally different.

**A Black Mutant of Neurospora**

Investigators: Schaeffer, Jennings  
Support: Rockefeller Fellowship  
General Funds

A mutant of Neurospora which develops a black pigmentation during growth has been found. Analysis of 20 ascis from a cross of the mutant to normal indicates that the mutant differs from normal
in a gene lying close to the centromere of the mating-type chromosome.

Biochemical study of the mutant has shown that the black pigment is a melanin, and the mutant exhibits an abnormally high tyrosinase activity. At the same time there is a decrease in the concentration of a natural inhibitor of tyrosinase which is found in Neurospora cultures grown on standard minimal medium. It is therefore probable that the high tyrosinase activity is the result of decreased synthesis of the inhibitor.

It has been found that exposure of the mutant to visible light during growth, especially blue light, depresses melanin formation. This is associated with a depression of the tyrosinase activity. This photoeffect is not mediated by an inhibitor, but is probably owing either to photoinactivation of the enzyme or photoinhibition of tyrosinase synthesis.

Genetic Recombination in Escherichia coli, K12

Investigator: Vogt
Support: Gosney Fund

Two hypotheses have been put forward to account for the phenomenon of genetic recombination in E. coli, strain K12.

(1) The recombinant types found in a mixed culture of two mutant lines may be interpreted as segregants from a hypothetical diploid zygote which arises by fusion of two parental cells.

(2) The transfer of genetic material from one strain to the other strain may occur by means of transforming agents which are released into the medium by one or both parental strains.

Conclusive evidence for the first hypothesis can come only from a microscopic demonstration of the postulated cell fusion preceding the formation of the zygote. Due to the low frequency of recombinants (one per $10^6$ parental cells), the chances of observing this cellular fusion are very small. Part of the present work centered, therefore, around a search for conditions which increase recombinant formation. Decisive factors in influencing the yield of recombinants are the age of the cells at the time of mixing and the nutritional environment during different phases of recombinant formation.

A second line of study was concerned with the effect of streptomycin and penicillin on the process of recombinant formation.
Recombinants may still be formed if a mutant strain has been pre-treated with streptomycin or penicillin; the yield in recombinants decreases rapidly, however, with increased time of exposure to the agents. The experiments indicate that cells of certain strains which have lost their capacity to form colonies on complete medium (normal criterion for viability) may still participate in genetic recombination. The experiments, however, do not necessarily imply that genetic material has been extruded from "killed" cells into the medium and been subsequently incorporated by the cells of the untreated mutant line (second hypothesis).

Chemical Studies on the Eye Pigments of Drosophila

Investigators: Forrest, H. K. Mitchell
Support: United States Public Health Service Fellowship
Rockefeller Foundation Funds

The work of Hadorn and Mitchell has shown that Drosophila contains a number of fluorescent pigments, some of which are accumulated specifically in certain mutant strains. As a possible method of approach to the determination of the chemical structure of the red eye pigment, it was decided to concentrate on isolating and identifying a yellow pigment which is present in the sepia mutant in large excess over the wild type. Large numbers of these flies (over two kilos) have therefore been raised and the pigment extracted from the macerated bodies with aqueous alcohol. A method for its further purification has been found in the formation of a silver salt from which the pigment can be regenerated, followed by large-scale chromatography on powdered cellulose columns. In this way, some quantity of pure material has been isolated and experiments are proceeding on the determination of its structure and its relation to the other eye pigments. Preliminary indications are that this pigment belongs to the pteridine class and may thus be closely related to the red pigment which has been claimed by several authors to contain the pteridine nucleus.

Biosynthesis of Riboflavin

Investigator: Forrest
Support: United States Public Health Service Fellowship

A fungus, _Eremothecium ashbyii_, which accumulates large amounts of riboflavin, has been studied with the idea that riboflavin-requiring mutants might be obtainable and that these might accumulate similar large amounts of some intermediate in riboflavin biosynthesis. Unfortunately this fungus is still classed as an imperfect one, so that no genetic studies will be possible. Several promising mutants
have been obtained by ultraviolet irradiation of a spore suspension.

**Threonine Biosynthesis in Neurospora**

**Investigator:** Garner  
**Support:** American Cancer Society Fellowship  
General Funds

The reaction scheme in the diagram below is suggested as a means of relating the four Neurospora mutants which will grow in the presence of threonine.

![Diagram](image)

This scheme is merely intended to indicate the probable relationships between the four mutants; it does not contain all the reactions involved nor indicate whether they are reversible.

The possibility of synthesis of ω-aminobutyric acid by either breakdown of threonine or by an alternate pathway allows for an explanation of most of the data regarding these mutants. The success of the scheme requires an irreversible or at least a rate-limited reverse reaction between threonine and compounds later in the sequence. These later compounds are readily used by strains 44105 and 44104 but only spare the threonine requirement of 35423 or 51504.

Strains 44104 and 44105 are closely related biochemically by their growth requirements, their behavior in double mutants containing a gene of the methionineless series, and by the fact that both will use canavanine or some product of canavanine metabolism. The two strains differ mainly in that 44104 will not use homoserine. This may mean only that exogenously supplied homoserine sets up a serious competition in strain 44104. Possibly related to this is the fact that 44105 generally requires less of a supplement than does 44104.

It has been suggested that \( X_2 \) may be the \( \beta-\gamma \) unsaturated amino acid. The available data do not indicate the nature of the step...
marked X₁. By way of speculation, the homoserine → threonine pathway may be a major one, with the other of lesser significance or at least of limited magnitude. Such an assumption could explain the fact that 44105 and 44104 grow faster on minimal agar than does 35423 or 51504 and would even allow for a common four-carbon precursor for both sets of reactions.

**Investigations on the Breakdown of Tryptophane by Neurospora**

Investigators: F. Haskins, H. K. Mitchell
Support: Williams-Waterman Fund

Previous work has shown that when Neurospora is incubated in a medium containing L-tryptophane, the tryptophane disappears rapidly from the medium and a blue fluorescent compound, anthranilic acid, appears. In recent experiments, an attempt has been made to learn something about the steps between tryptophane and anthranilic acid. It has been found that, after a suspension of mold is heated briefly at a temperature of 50°C, it is still capable of causing tryptophane to disappear from the medium, but that the production of anthranilic acid is reduced almost to zero. By appropriate treatment of the mold it is possible to recover, apparently unchanged, most of the tryptophane which had been lost from the medium, but no intermediate compounds have as yet been detected. That this uptake of L-tryptophane involves some specific mechanism is indicated by several experimental observations, the most convincing of which is that D-tryptophane is not taken up. It appears most probable that the initial step in the breakdown of L-tryptophane by Neurospora involves the uptake of the amino acid from the medium by a specific enzymatic mechanism.

**Mechanisms of D-Amino Acid Utilization in Neurospora**

Investigator: Holden
Support: United States Public Health Service Fellowship

The mechanism of utilization of D-amino acids for growth by amino acid mutants of Neurospora is unknown. The presence of a D-amino acid oxidase in this organism suggests that utilization may occur by oxidation to the α-keto acids with subsequent amination to the L-amino acids, possibly by transaminases. Growth experiments with appropriate amino acid mutants and studies on transaminases and the D-amino acid oxidase have been conducted in an effort to establish the mechanism of inversion of valine, leucine, histidine and lysine.
In an effort to provide in vivo evidence for the occurrence of $\text{B}_6$-catalyzed reactions during amino acid inversion, $\text{B}_6$-amino acid double mutants have been studied to determine whether the $\text{B}_6$ requirement for growth is increased when D-amino acids are used to satisfy the amino acid requirements. Such experiments indicate that the utilization of D-valine follows the postulated pathway of oxidation and transamination. The involvement of $\text{B}_6$-catalyzed reactions in leucine inversion, however, has not been critically evaluated because very small changes in $\text{B}_6$ requirements are not detectable in media currently in use. Recently, a reduction of the $\text{B}_6$ requirement by 10 to 100 fold has been achieved by supplementing a modified Fries medium with thiamin and amino acids, thus affording a medium for these experiments which is more sensitive to changes in the $\text{B}_6$ requirement.

The participation of transaminases in the inversion of histidine and lysine is unlikely, but is not yet conclusively dismissed. In a recent study Fincham failed to detect transamination of those amino acids in Neurospora, and a reevaluation of this problem, still in a preliminary stage, indicates that the reactions are very slow if they occur at all ($\sim 2\%$ reaction in three hours). The inability of some lysine mutants to use D-lysine appears not to be a result of low D-amino acid oxidase activity. An unexpected finding was that extracts of D-lysine utilizers readily oxidized L-amino acids whereas comparable extracts of non-utilizers showed no L-amino acid oxidase activity. It is not yet known whether this observation has any relation to the capacity of strains to use D-lysine. Growth data, however, strongly suggest that the inversion of lysine involves the terminal steps of lysine synthesis, and a similar mechanism may be in operation for histidine inversion.

To evaluate the relation of this compound to lysine inversion and synthesis, an attempt is being made to prepare the α-keto acid of lysine, in collaboration with Schweet and Lowy. Although the compound has never been isolated or synthesized, its proposed properties have occasionally been relied on to explain the anomalous metabolism of lysine in animals.

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**Lactase Formation and Lactose Utilization in Neurospora**

*Investigator: Landman*

*Support: United States Public Health Service Fellowship*

The work on Neurospora lactase was initiated with the aim of explaining, in enzymatic terms, the failure of several Neurospora strains to grow well on lactose. A comparison of the hydrolytic lactases from a wild type and a lactose-negative strain revealed no
difference in properties between enzymes. Comparison of lactase formation rates on various substrates during growth and in resting mycelium ("adaptation") eventually led to the conclusion that the lactose-negative strains grow poorly on lactose because they respond inadequately to the stimulatory action of lactose.

When rate studies of lactase formation were extended to several other lactose-utilizing strains of Neurospora, it was found, however, that the correlation between lactase production on lactose and lactose utilization no longer obtained. The discrepancies may perhaps be explained by assuming the existence of a non-hydrolytic mechanism for lactose cleavage which acts concurrently with the hydrolytic mechanism. This possibility is being investigated at present.

The adaptive formation of hydrolytic lactase has been investigated in some detail. It has been found that lactase formation may be obtained in response to lactose, galactose, xylose, arabinose and melibiose. In contrast to results obtained with growing mycelium, the pentose 1-arabinose is by far the most effective lactase-inducing substance in resting cultures. Measurable stimulation of lactase formation has been obtained with 1y of arabinose per mL. It has also been found that, at a given concentration level, sucrose suppresses the enzyme-inducing effect of lactose and xylose whereas arabinose continues to elicit lactase formation. This result suggests that the concentration level of an enzyme may sometimes be determined by competition between enzyme-inducing substances.

Genetic work with the lactose-negative mutants has been continued and simple segregations of lactose-negative characters have been obtained. Numerous modifiers which obscured results in earlier crosses have been largely eliminated.

Gene Interactions among Nutritional Mutants of Neurospora

Investigators: M. B. Mitchell, H. K. Mitchell
Support: Rockefeller Foundation Fund
Atomic Energy Commission operating through Office of Naval Research

Continuation of studies of recombinants of the pyrimidine mutant 37301 and its suppressor with proline, ornithine, citrulline and arginine mutants (described by Srb and others) has shown that three mutants which require ornithine, citrulline or arginine will not respond to ornithine if the suppressor gene, s, is present. In other words, they appear to be converted by s into mutants which have absolute requirements for citrulline or arginine. The mutant
gene, 37301, which introduces a requirement for pyrimidine, has a similar effect upon a citrulline- or arginine-requiring mutant in that this mutant, when the 37301 gene is present, no longer responds to citrulline. These effects suggest, as have the results of other workers, that although mutation of one gene, or of a group of closely linked genes, may introduce an additional nutritional requirement, other modifying genes may determine which reaction in the biosynthetic pathway concerned appears to be "genetically blocked." It had been observed previously that s not only suppresses the pyrimidine requirement of 37301, but also the requirement of two mutants which use proline, ornithine, citrulline or arginine. These and other observations indicate an interrelation in the biosynthesis of pyrimidine, proline and arginine, but because of the effects of the s and 37301 genes on ornithine and citrulline utilization it does not seem worth while to attempt to explain the nature of this interrelation in terms of schemes in which the sites of action of the various mutations are assigned to specific steps in biosynthetic pathways.

Pseudo-Wild Types from Pyrimidine Mutants

Investigators: M. B. Mitchell, H. K. Mitchell
Support: Rockefeller Foundation Funds
Atomic Energy Commission operating through Office of Naval Research

The term pseudo-wild type has been applied to strains which are phenotypically wild type but which when crossed to standard wild strains give 50% mutant progeny. Such strains have been obtained from crosses of certain pyrimidine mutants to other closely linked mutants as well as from crosses between very closely linked or possibly allelic pyrimidine mutants. In some cases it has been shown that both parent mutants are recovered from the outcross of a pseudo-wild strain. There is no indication, from crosses of pseudo-wild types to standard wild type or to a mutant closely linked to the parent mutants, that the wild phenotype of a pseudo-wild type is transmitted to any of its progeny. A tentative explanation of the behavior of these strains is as follows: that the wild phenotype is caused by the presence of disomic nuclei which arise during meiosis as a result of nondisjunction of the chromosome pair carrying the pyrimidine mutant genes; that during subsequent mitotic divisions disomic nuclei sometimes give rise to fully haploid nuclei, possibly as a result of somatic nondisjunction, and the pseudo-wild strain thus becomes a heterocaryon containing both haploid and disomic nuclei; that only the haploid and not the disomic nuclei function in crosses, so that the parent mutants, but not the wild phenotype, are recovered from outcrosses of pseudo-wild types. The cytological evidence necessary
to support this explanation is lacking and may be difficult to obtain, but attempts to obtain further genetic evidence are in progress.

**Pseudo-Wild Types in Neurospora**

Investigator: Pittenger
Support: Atomic Energy Commission Postdoctoral Fellowship

Wild-type progeny resulting from crosses between strains carrying different mutations in the same linkage group are usually assumed to be the product of crossing over between the two loci in question. In studies of certain groups of closely linked genes, phenotypic wild types have been found in the progeny of mutant x mutant crosses which are not the result of crossing over. Crosses between these apparent wild types and standard wild strains gave approximately 50% mutant progeny. Both of the original parental mutants were recovered from among the outcrossed mutant progeny. These pseudo-wild types are presumably analogous to those obtained from pyrimidine mutants by Mary Mitchell.

Preliminary tests indicate that the n+1 nuclei rarely, if ever, are transmitted through a cross. Presumably the disomic condition breaks down in some way during somatic division to form haploid nuclei. Apparently only these haploid nuclei function in fertilization.

At the present time, pseudo-wild types have been found involving three different linkage groups and the number of such pseudo-wild types recovered from a given linkage group suggests that the frequency of non-disjunction differs widely among the various chromosomes.

In connection with these studies, double mutants have been recovered involving closely linked genes with similar biochemical requirements, suggesting the existence of several complex genetic loci in Neurospora in addition to those reported in other organisms. At least two closely linked mutants which require isoleucine and valine for growth have been found. There is approximately 4% crossing over between these two genes. Two pyridoxine-requiring mutants, one pH dependent and the other pH independent, have been found to be two distinct loci and are less than one crossover unit apart.

**Histidine Biosynthesis**

Support: Atomic Energy Commission Predoctoral Fellowship
General Funds

A series of imidazoles accumulated by Neurospora and Penicillium histidineless mutants have been examined. Three of these imidazoles have
been crystallized and characterized and three others identified by chromatography. Procedures for synthesizing these various compounds have been developed and two of these have been synthesized to date.

The nature of the compounds isolated indicates that histidine is synthesized in Neurospora fairly directly from a sugar phosphate. Neurospora is not permeable to the presumed intermediates isolated and therefore the only way of showing they are intermediates is by enzymatic interconversion in cell-free systems. Experiments along this line have been started.

**A Neurospora Mutant Requiring an Unknown Substance for Growth**

**Investigators:** Mauron, H. K. Mitchell  
**Support:** Merck and Company Fund

A mutant strain of Neurospora that grows well in peptone-containing medium was found to grow; although poorly, in the presence of adenine plus arginine. The addition of several other amino acids stimulates growth but no known combination of known substances is as effective as peptone or a substance that has been highly purified from peptone.

**Fluoro-Substituted Phenylalanines as Growth Inhibitors**

**Investigators:** Surber, P. Ellman, H. K. Mitchell  
**Support:** United States Public Health Service Fund

In a study of the inhibition of wild-type Neurospora by fluorophenylalanine, a strain appeared which was adapted to a concentration which ordinarily causes complete inhibition of growth. This adaptation persisted when crossed with another wild type. Segregants of this cross which were adapted to fluorophenylalanine were also found to have a requirement for asparagine. Attempts were made to isolate a substance from an asparagine medium in which the mutant had been grown which would reverse the inhibition of wild type. Experiments have shown that such substances are present but that their action is nonspecific. Thus, the resistance of the mutant to fluorophenylalanine inhibition is evidently due to relatively high concentrations of several compounds (probably amino acids) that are also normal constituents of nonresistant strains.

**Fluoro-amino Acids**

**Investigators:** P. Ellman, Surber, H. K. Mitchell
Investigation of the metabolism of fluoro-amino acids in animal and mold tissues necessitated the development of a micro-method for the determination of fluorine in organic substances. A fluorometric method for fluoride ion (10-100 µg range) has been worked out. The procedure is applicable to organic fluoride derivatives but some details of technique remain to be developed.

**Neurospora Mutants with Unknown Requirements**

**Investigator:** Dubes  
**Support:** Atomic Energy Commission Predoctoral Fellowship  
**General Funds**

Sixteen strains of *Neurospora crassa* which had been classified previously as strains with unknown growth requirements have been investigated biochemically and genetically. The growth requirements of all of these have now been chemically defined. At least three of the 16 original strains are double mutants. The single mutants clarified in this work can now be classified as follows: one aromaticless, one pyrimidineless, one arginineless, two asparagineless, one slow-grower, one homoserineless, one methionineless, one temperature-sensitive leucineless, two succinicless, two serineless (formateless), one adenine-plus-methionine-less, and one p-aminobenzoicless. In general, these mutants have unique growth characteristics which prevented their characterization by earlier tests.

**Phosphorus-Containing Compounds in Neurospora**

**Investigator:** G. Ellman  
**Support:** Nutrition Foundation-  
**McCallum Foundation Fellowship**

**General Funds**

A paper chromatographic method for the separation and identification of the water-soluble phosphorus-containing compounds was devised. The method was applied to extracts of *Neurospora* (wild type 5256A). The presence of the following compounds was indicated: the adenosine phosphates, 3-phosphoglycerate, glucose-1-phosphate, glucose-6-phosphate, and fructose-6-phosphate. At least one major unknown was also encountered.

The lipid-soluble phosphorus-containing compounds were also investigated. The isolated phospholipids were converted to the yellow N-dinitrophenyl derivatives which were chromatographed on silicic
acid-celite columns. The fractions which contained phosphorus were hydrolyzed with acid, and the components were investigated further. Titration of the fatty acids revealed that some of the phospholipids did not have the classical phospholipid structure. The phosphate esters were identified by paper chromatography; most of the phospholipids contained glycerophosphate. The dinitrophenyl amines were isolated, and the serine, ethanolamine and L-amino-2-methyl-2-propanol derivatives were identified. The latter amine has not before been reported to occur naturally.

**Enzymatic Cleavage of Cystathionine by Neurospora**

*Investigator: Fischer*

*Support: General Funds*

Crude and partially purified extracts of Neurospora have been obtained which are active in cleaving L-cystathionine to yield homocysteine.

\[
\text{cystathionine} \quad \xrightarrow{A} \quad \text{homocysteine} \quad \xrightarrow{B} \quad \text{methionine}
\]

The latter has been identified by chromatography in two solvents and by reduction to homocysteine followed by enzymatic methylation to methionine, with the use of an enzyme from rat liver for the methylation (step C in the above scheme). The enzyme cleaving cystathionine has been obtained from two wild types and from a mutant blocked at step A in the scheme, but not from a mutant which had previously been shown to be blocked at step B. These results indicate that this enzyme is responsible for catalysing step B in vivo and that the nutritional requirement for homocysteine (or methionine) exhibited by the mentioned mutant is the result of a deficiency of the cystathionine cleaving enzyme. Since homocysteine is spontaneously oxidized to homocystine in air, it is not surprising that the latter is recovered from the reaction. Further details of the reaction are under investigation, as is also the question of whether the deficiency of enzyme activity in the mutant is ascribable to inhibition of the enzyme, or to failure of the mutant to synthesize it.
**Lysine Biosynthesis in Neurospora**

Investigator: Good  
Support: General Funds

Earlier investigations which showed that ε-hydroxynorleucine can act as an intermediate in the biosynthesis of lysine have been extended by synthesis of α-aminoadipic acid - ε-amide and α-aminoadipic acid-ε-semialdehyde diethylacetal. The amide supports growth of mutants that will use α-aminoadipic acid, but will not support growth of other mutants. The aldehyde cyclizes too rapidly to permit adequate biological testing. On the other hand, the corresponding five-carbon homolog of the semialdehyde was synthesized and found to be active as a precursor of proline and ornithine.

**Purine Oxidizing Enzymes in Neurospora**

Investigator: Greene  
Support: Atomic Energy Commission Predoctoral Fellowship  
General Funds

An investigation of the mechanism of uric acid oxidation catalysed by uricase of Neurospora is under way. The properties of the enzyme system and ways of purification of the system in order to remove sources of ambiguity in mechanism studies are being studied.

**Tryptophane Desmolase in Neurospora**

Investigator: Hogness  
Support: United States Public Health Service Fellowship

Tryptophane desmolase with its coenzyme, pyridoxal phosphate, is the enzyme system which catalyzes the synthesis of tryptophane from indole and serine in Neurospora. It has not been possible to demonstrate this activity in the C-83 mutant which requires tryptophane for growth. In an examination of the enzyme activity of cultures obtained from 20 asci of the cross 8a (wild type) X C-83A, no activity could be found in cultures which required tryptophane for growth. It thus seems reasonable to postulate that the lack of tryptophane desmolase activity and the requirements for tryptophane are the result of the same gene modification in C-83, the former being the cause of the latter. In all but one of the above asci the activity of the wild progeny was the same as that of the wild parent. In one ascus, however, while the two C-83 spore pairs and one wild spore pair had the normal characteristics, the other wild spore pair produced cultures which showed no enzyme activity when grown in the presence of tryptophane. Further tests on this wild type revealed that that activity
would be found in early stages of growth, but that the activity dropped rapidly to zero as growth increased. The point on the growth curve at which this rapid drop started was found to be dependent on the tryptophane concentration in the medium, being earlier the higher the concentration. It has not been established whether this wild type was a result of segregation of a closely linked modifier in C-83, or a new mutation.

It had previously been found by Mitchell that the histidine mutant C-84 has a tryptophane desmolase activity characteristically three times that of the wild strain, 8a. An examination of asci from the cross C-84A X 8a for enzyme activity revealed that, while the C-84 locus or one closely linked to it has a modifying influence on the enzyme activity, at least two other modifiers must also be present.

Metabolism of Aromatic Compounds in Neurospora

Investigator: Metzenberg
Support: General Funds

A mutant which will utilize hydroxykynurenine, hydroxyanthranilic acid or niacin has been shown to accumulate a-N-acetylkynurenine under certain culture conditions. The mutant had previously been shown to accumulate kynurenic acid.

Some work has been done on the question of whether some of the naturally occurring seven-carbon sugars can serve as direct precursors of aromatic compounds. No conclusive results have yet been obtained.

Threonine Metabolism in Neurospora

Investigator: Reissig
Support: General Funds

The behavior of L-threonine deaminase of Neurospora and its activation by pyridoxal phosphate have been investigated. The activation could be attributed to a hitherto unrecognized coenzymatic capacity of pyridoxal phosphate, or a complex threonine deaminase system which might include a step requiring pyridoxal phosphate as a coenzyme. The latter would be the case if the deamination of threonine involves a transamination. This alternative was rendered extremely unlikely (1) by a study of reaction balances (ammonia and alpha-ketobutyric acid are produced in equimolar quantities from threonine), (2) by the low activity of the desaminases of glutamic and alpha-aminobutyric acid, and (3) because addition of alpha-ketoglutaric acid fails to affect the course of the reaction. It was thus concluded that pyridoxal phosphate participates specifically in the deamination of threonine.

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Heterocaryosis in Neurospora

Investigator: Holloway
Support: Nutrition Foundation- McCallum Foundation Fellowship

It is known from the work of Beadle and Coonradt that certain biochemical mutants form heterocaryons when inoculated in pairs on minimal medium. In the current investigations it has been found that the ability to form such heterocaryons is controlled by genes independent of the biochemical mutants involved. These genes also appear to control the type of heterocaryotic growth produced. There appear to be six types of heterocaryons in the system under test (pantothenicless and lysineless), as follows:

1. Rapid starting (24 hours), wild-type growth rate (4.4 mm/hr.).
2. Delayed starting (60 hours), wild-type growth rate.
3. Rapid starting, slower growth rate (3.0 mm/hr.) gradually coming to a stop.
4. Delayed starting, slower growth rate.
5. Rapid starting, then stopping abruptly.
6. Delayed starting, then stopping abruptly.

There also are combinations which fail to form any growth whatsoever. It has not been conclusively proved to date that types 5 and 6 are heterocaryons.

The analysis of this system has been made by crosses involving the pantothenicless partner (5531) and testing progeny with standard lysine tester strains (4545A and 4545a). The results are explained on the basis that a number of genes, of the order of five or six, are concerned in the formation of a heterocaryon. In at least one case, the difference between fast (4.4 mm/hr.) and slow (3.0 mm/hr.) growth appears to be controlled by one gene.

IMMUNOGENETICS

Staff

Associate Professor: Owen
Research Fellow: Goodman
Graduate Students: Boroughs, Gershowitz, Rappaport, Ripley
Research Assistant: M. Hull
Various Serological and Immunogenetic Studies

Investigators: Owen and collaborators
Support: Rockefeller Foundation Fund

During the past year, investigations have been initiated or con­tinued in the serology and immunogenetics of various plant and animal materials. The categories of study include:

Serology of Tyrosinases. Primarily as a result of the provision by Schaeffer of Neurospora extracts highly active in tyrosinase, good antisera have for the first time been prepared against Neurospora tyrosinase. The antibodies precipitate and partially inactivate the enzyme, and offer some preliminary evidence of differences among strains of Neurospora. Serological tests of an idea of Schaeffer's that the in vitro inactivation of tyrosinase during its action might result from the adsorption of the enzyme to the melanin granules formed were negative; that is, neither as an antigen in immunization nor as a test antigen was there evidence that Neurospora tyrosinase was present in serologically distinguishable form on the melanin formed by its action.

Serology of Plant Viruses and Host Plant Relationships. Antibody reagents specific for the mild and severe strains of tobacco mosaic virus (Singer, Bald, Wildman, and Owen, Science, November, 1951) have been provided to the U.C.L.A. group for use in the plant virus program there. Our earlier observations of the existence of material serologically related to the virus, in healthy plant cytoplasm, has been confirmed. But the significance of this observation, in terms of the origins of virus material, and the reasons for its failure to be regularly and uniformly demonstrable, remain debatable. There are evidences that different preparations of the plant protein differ in ways that are, so far, beyond experimental control, but that may well be critical to this line of work.

Rat Red-Blood-Cell Immunogenetics. Rats in the breeding colony of Richard W. Lipmann, Institute for Medical Research, Cedars of Lebanon Hospital, have provided material of some general interest. The size and duration of this colony, and the system of matings employed, are such as to suggest that a rather high degree of genetic uniformity should prevail within it. Nevertheless, blood tests demonstrated that the colony has remained heterozygous for a pair of cellular antigens, controlled by alleles. The frequencies of these alleles are maintained at about .32 and .68, respectively. The distribution of genotypes among adults in the colony deviates from the random in ways that suggest unconscious selection favoring the
heterozygote, but discriminating more consistently against the homozygote for the "C" antigen than against the "D". It is hoped that physiological studies may eventually identify the characteristics conferring advantage or disadvantage on individuals in this situation.

**Rabbit Red-Blood-Cell Immunogenetics.** Mainly to provide reagents to correlate rabbit sperm and erythrocyte antigens, a brief study was made of individual immunogenetic differences in rabbit red blood cells. Two fair antibody reagents were developed from an isoimmune serum produced by a rabbit originally sensitized by Hinton. The antigens identified are independent in their occurrence in the colony. Another reagent, probably dependent on a normal isoantibody, identifies a third, rare antigenic characteristic.

**Opossum Red-Blood-Cell Serology.** The availability of three captive, native opossums led to a brief study of their red cells. Opossums appear to possess an antigen related to the human "B" antigen, since their cells absorb out β antibodies from the sera of people of blood groups O and A. There are also other antibodies, different from α and β, present in normal human serum, that react with opossum red blood cells. Opossum cells do not appear to be capable of reacting with human α antibodies, and no individual differences in the three opossums at hand were evident on the basis of either normal or immune sera. The opossums are well adapted to laboratory maintenance, and easily bled in quantity without injury to them, by cardiac puncture under ether anesthesia.

**Serology of Cattle and Guinea Pigs.** Individual guinea pigs were found to differ with regard to their normal antibodies against cattle cells; and cattle in turn differ in respect to the antigenic characteristics identified by these normal guinea pig antibodies. This has provided a basis for continued collaboration with Doctor Clyde Stormont, of the University of California at Davis. His laboratory is studying the cattle differences, using serum from guinea pigs bred at Caltech, while we are investigating the guinea pig differences, using cattle cells defined at Davis. Neither study has progressed to the point at which a firm conclusion may be stated as yet.

During the past year, we have also undertaken the first steps of an investigation of the relationships of antigenic sites, controlled by members of a complex multiple allelic series, on the bovine erythrocyte. This study is also inconclusive at present, as is the somewhat related study of bovine sperm and seminal fluid.

**Drosophila Serology.** In the hope that an in vivo action of antibodies on Drosophila larvae could be adapted to the clear-cut designation of individual differences, antisera were prepared against
larvae of a number of different stocks. Although fair precipitins for larval extracts were produced, the larvae themselves proved remarkably resistant to the antisera, and no tools for the identification of individual, immunogenetic differences were achieved.

**Serology of Euglena.** Reactions of antisera to cultures of Euglena derived from single-cell isolates have been considerably improved by the use of developing antibody technique. After the Euglena are sensitized by antibody, the addition of a chicken-anti-rabbit globulin antiserum in high dilution magnifies the agglutination of the Euglena considerably. Although the reactions obtained in this way are strong and clear-cut, it has so far been impossible to identify consistent immunogenetic differences among the Euglena cultures at hand.

**Serology of Human Hemoglobin.** Material provided by Dr. Harvey Itano, of the Division of Chemistry, is under study to see whether the hemoglobins produced by the normal isoalleles of the allele for sickle cell hemoglobin, postulated by Itano, are serologically distinguishable. Two fairly good antisera have been produced in chickens, but the problem of distinguishing the normal hemoglobins in heterozygous blood has not yet been attacked.

**Serological Systems Utilizing Chicken Antisera**

**Investigator:** Goodman  
**Support:** United States Public Health Service Fellowship

Chickens have been used as antibody producers against certain types of proteins which, owing either to their poor antigenicity or their solubility characteristics, have not been previously studied by the quantitative precipitin technique. Two desoxyribonucleoprotein complexes were investigated. One was prepared from commercial wheat germ and the other from rat liver. In both cases the extraction procedure of Mirsky was employed.

One series of injections was sufficient to produce precipitins in chickens against the wheat germ nucleoprotein. Subsequent injection series caused the production of more potent antisera. The precipitin reactions were always performed in media of high salinity (1 to 2 M NaCl) in which the nucleoprotein is soluble and in which the chicken precipitin is known to yield its maximal reaction. The character of these precipitin reactions clearly established that the complexes extractable from the wheat germ particles by 1 M NaCl are composed of more than one antigen. On the other hand, this antiserum was specific to substances within these particles, for there was no sizable cross-reaction of this antiserum with the
protein fraction that was soluble in low salinity (0.15 M NaCl, pH 7). Conversely, antiserum produced to the initially soluble proteins did not, after absorption with the particles, react significantly with the nucleoprotein preparations, though still reacting strongly with the proteins soluble in low salinity. The possibility exists that the particles, obtained by grinding the wheat germ in sand, contain in addition to the desoxyribonucleoprotein complex a type of protein which is also insoluble in low NaCl concentration but soluble in high. Attempts will be made to test this possibility.

The rat liver desoxyribonucleoprotein complex was a poor antigen in chickens, and at best weak antiserum was produced. This antiserum appeared to be directed to only a minor component of the complex, for significant precipitation was obtained only at the higher antigen concentrations. This antiserum also cross-reacted with the protein fraction of rat liver which is soluble in 0.15M NaCl. A histone fraction was prepared from the nucleoprotein, and at its higher concentrations it reacted to some extent with the antiserum. Further investigations must await the production of stronger antiserum.

Much more success has been had in producing antiserum to hemoglobin whose protein moiety has histone-like characteristics. Potent antisera, containing 1.5 to 2.5 mg. of antibody per ml., have been produced in chickens against both normal human carboxyhemoglobin and sickle cell carboxyhemoglobin. The reactivity of these antisera is now being investigated. As expected, a diluent of high NaCl concentration (8 to 12%) is needed to obtain maximal antibody precipitation. Small but real differences in antigenic specificity appear to exist between these two types of adult hemoglobin. With most of these antisera, slightly more antibody (5 to 20%) has been precipitated by the homologous hemoglobin (the one to which the antiserum was produced). One anti-normal hemoglobin serum was partially absorbed with sickle cell hemoglobin. The absorbed serum then reacted three times more strongly with normal hemoglobin than with sickle cell hemoglobin. Several antisera to normal adult hemoglobin have also been reacted against human foetal hemoglobin. The cross-reaction was much smaller than that obtained with sickle cell hemoglobin, and it can be concluded that large differences in antigenic specificity exist between foetal and adult hemoglobin.

Antigenic Relationships among Tissue and Circulating Proteins

Investigator: Rappaport
Support: General Funds

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Most data support the hypothesis that serum albumin is made in the liver. However, a number of questions have remained unanswered. Some of these are: 1. Is the circulating protein present as such within the hepatic cell? 2. Is the serum protein continually made anew from amino acids, or can large aggregates of polypeptide serve as precursors? 3. If precursors exist, do they bear any antigenic relationship to the circulating proteins?

These problems were approached in the following manner. Four cell fractions were prepared from perfused and thoroughly washed chicken livers and kidneys in M/15 phosphate buffer, pH 7.8. These fractions were then investigated by using rabbit antiserum prepared against purified chicken serum albumin (Sa).

Anti-Sa reacted with the supernatant fraction from both liver and kidney extracts. Quantitative analysis of the precipitin reactions suggested they were homologous ones. Partial and complete absorption studies confirmed that the reactions were caused by Sa. The data also revealed that only 0.53% of the total N in the liver supernatant and 0.15% of the kidney supernatant could be considered Sa.

The supernatants were also studied electrophoretically. It was calculated from area determinations that 3 to 5% of the total area for the liver proteins migrated with the mobility of serum albumin. The same was found for the kidney. From the precipitin data it is suggested that over 90% of these components are proteins other than Sa.

Although wide ranges of antigen and antibody concentrations have been examined, no cross-reactions between anti-Sa and the organ proteins were observed. The nuclear, mitochondrial, and microsomal fractions from livers, but not from kidneys, fix complement with anti-Sa. At present this reaction is poorly understood. Although complete absorption of the antiserum with Sa removes the complement-fixing antibodies, repeated absorptions of antiserum with mitochondria do not reduce the precipitin titer. This along with some other data makes the original observation of complement fixation appear doubtful.

After particle absorption the antiserum becomes highly anti-complementary. Further complement-fixation studies are impossible to perform. Work is in progress to clarify this situation.

The difficulties involved in removing all of the serum proteins from such highly vascular organs as the liver and kidney suggest that the trace amount of Sa found is a result of contamination. The possibility remains, however, that these small concentrations are normal to the cells.
Since no true cross-reactions were observed between anti-Sa and the organ extracts, it would appear that substances antigenically related to the serum protein are not present in the organ extracts. If it turns out that the reaction between anti-Sa and liver particles is nonspecific, then the idea that there is anything antigenically related to serum albumin within the liver cell must be discarded.

These data would emphasize the importance of the cell membrane. The final specificity of serum albumin, and perhaps all serum proteins, may be conferred at the cell membrane as it passes into the circulation.

**Immunogenetics of Pigeons**

Investigator: Gershowitz  
Support: General Funds

Previous workers have shown that several species of Columba differ in the types of species-specific red blood cell antigens they possess. By the use of rabbit anti-pigeon erythrocyte sera and a single pigeon iso-immune serum, the present study has shown that within the species there are also individual differences in the antigenic structure of pigeon red blood cells. One antigen seems to be inherited as a simple dominant (presence of the characteristic dominant over its absence). The modes of inheritance of five others have not yet been definitely ascertained. Preliminary experiments have indicated that there is no sharp line of differentiation among cells of different birds in their ability to act as positives or negatives toward a given reagent. The system is complex, and appears to include multiple serologically cross-reacting components. Present and future work is designed: (1) to identify the genetic control of these antigens and (2) to establish the nature of the cross-reactions involved in the study of these complex reagents.

**Establishment of Hemapoietic Grafts in Rat Embryos**

Investigator: Ripley  
Support: General Funds

It is known that in cattle twinning often results in placental anastomosis so that there is reciprocal exchange of foetal blood. This exchange results in interactions in hormonal and antigenic characters depending upon the sex and genotype of the twins. If the twins are genetically unlike for the blood group antigens it is found at birth and thereafter that the blood cell population is a mixture of the two phenotypes - one derived from each twin. Apparently the exchange of erythroblasts or blood islet cells occurs early enough in the ontogeny so that the exchanged cells become established and do
not evoke the production of antibodies. To investigate this problem further, attempts have been made to establish a similar condition in rats by a surgical technique.

A closely-bred strain of rats segregating for the blood factors C and D was obtained from Dr. Richard W. Lippman. Homozygous matings were made in the two sets simultaneously. Erythroblasts from foetal liver of the 12th to 14th day of gestation are injected into the circulation of foeti of the opposite blood type, with a microinjection apparatus. As yet no results are available to indicate whether the introduced erythroblasts have become established.

Serology of Plant Enzymes

Investigator: Boroughs
Support: General Funds

The broad specificity of acid phosphatase activity in the cytoplasm of green leaves suggests the possibility that the cytoplasm may contain a complex of phosphatases with different substrate specificities. On the basis of pH optima, however, it has not been possible to distinguish more than one such type of enzyme. It was thought that by preparing an antiserum to a purified phosphatase and examining the antigen-antibody system some evidence for the separation of specific phosphatase enzymes might be obtained.

Sugar beet leaf cytoplasm treated with heat and acid to remove most of the nonenzymatic proteins was used as an antigen in rabbits. A precipitin reaction down to a dilution of 1:64 of the antigen was observed. The Michaelis constant for the antigen and the supernatant was about $2.5 \times 10^{-3}$ M, with the use of phenyl phosphate and sodium glycerophosphate as substrates. Very little activity was present in the precipitate. With nitrophenylphosphate as substrate, however, the Michaelis constant of the supernatant was $3 \times 10^{-4}$ M, whereas that of the antigen and the washed precipitate was $10^{-3}$ M. The precipitate also showed peroxidase activity. No cross-reaction occurred when the antibody to sugar beet leaves was tested with whole cytoplasm from spinach, Turkish tobacco, or orange leaves.

The differential partitioning of phosphatase activity between supernatant and precipitate, with respect to different substrates, suggests an antibody fractionation of a phosphatase complex. It is not yet possible, however, to state unequivocally that enzyme separation has been accomplished.
MARINE ZOOLOGY - KERCKHOFF MARINE LABORATORY

Staff:
Associate Professor: MacGinitie
Superintendents: Chalmers, Smith

Marine Ecological Factors of Arctic

Investigator: MacGinitie
Support: General Funds

Work has been continued on the preparation of a report on research carried out at the Arctic Research Laboratory at Point Barrow, Alaska. In addition to determining the marine ecological factors of the region, over 700 species of animals were collected. The work was done under the auspices of the Office of Naval Research, Johns Hopkins University and the Institute.

PLANT PHYSIOLOGY - EARHART PLANT RESEARCH LABORATORY

Staff:
Professor: Went
Associate Professor: Galston
Research Fellows: Ashby, Garman, de Rafols, Hellmers, Hull, Soriano, Ulrich, Verkerk, Zarudnaya
Graduate Students: Johnstone, Rogers, Niemann
Research Assistants: Corbett, Juhren, Morgan, Ohki, Weinbaum
Superintendents: Keyes, Cunningham
Forestry Research Aide: Kelleher

Light Utilization by Tomato Plants

Investigator: Went
Support: General Funds

Much work has been carried out on the efficiency of light utilization by tomato plants. It was found that, at or below light intensities at which the photosynthetic process is saturated, the utilization of light is largely controlled by (1) the previous conditions in which the plants were growing and (2) the conditions following exposure to light. The first of these two limitations was found to depend on the sugar content of the plants: if high owing to previous
light saturation, or when low owing to prolonged darkness, photosynthesis is much less efficient. The latter condition can be relieved by spraying sugar on the plants prior to light exposure.

Both growth and night temperature conditions largely control the amount of photosynthesis. It was concluded that only under conditions of optimal utilization of carbohydrates can an optimal rate of photosynthesis be attained. Thus physiological factors are of great importance in controlling photosynthetic CO₂ reduction.

**Photoperiodic Behavior of Strawberries**

Investigator: Went  
Support: General Funds

The photoperiodic behavior of strawberries was further investigated. As found earlier, this is strongly influenced by temperature: At 17° and 20° C. the Marshall strawberry is strictly a short-day plant; at 10° C. and 6° C. it flowers at all day lengths, even in continuous light. It was found that this behavior is the result of an increasing sensitivity for light at higher temperatures: Thus at 10° C., flowering can no longer be inhibited by 1000 foot candles of light, whereas at 20° C. it is inhibited by lengthening an eight-hour day with less than 200 foot candles of light. The temperature analysis also brought evidence for a dual control of flowering by light. At 17° C. the number of photoinductive cycles necessary for floral initiation is smallest, increasing towards higher and lower temperatures.

**Studies on Veratrum**

Investigator: Went  
Support: Riker Fund

When the results of laboratory experiments with Veratrum viride were tested in field experiments, it was found that predictions as to optimal growth in the field were completely borne out. Since the dormancy requirement of mature plants of V. fimbriatum was so much shorter than V. viride, fimbriatum seedlings were used to study dormancy and growth of this species, under various storage and growing temperatures and light and rain conditions. As in mature plants a two-month storage period was sufficient. The best storage temperatures were from 4° to 6°. At 0° many plants rotted.

V. viride plants have been used to find a good propagation method. Needles were pushed through the buds to destroy the growing point and encourage the formation of new buds. With this
method only a small amount of tissue was damaged. Additional plants received vitamin solutions and rooting hormones. Plants received in 1949 are now growing for the fourth season and have increased in number. Other \textit{V. viride} plants are being treated with radioactive CO$_2$.

**Differentiation and Growth of Reproductive Organs in Maize**

Investigators: Zarudnaya, Galston  
Support: Gosney Fund  
Rockefeller Foundation Fund  
Atomic Energy Commission operating through Office of Naval Research

The mechanism of differentiation of the reproductive organs of plants is at present poorly understood. The maize plant offers several interesting possibilities for studies in this problem. It is known, for instance, that under some environmental conditions, the male organ (the tassel) may begin to function as a female organ, and produce seeds. Several genes are known which greatly alter the pattern of reproductive development: the tassel-seed gene transforms the tassel to a seed-producing organ, and the silkless gene causes the formation of anthers on the ear, thus partially converting the female organ into a male organ.

There were some a priori reasons for believing that temperature and internal level of auxin may influence sexual differentiation patterns. These experiments, carried out in the Earhart Plant Research Laboratory, furnished additional data on this question. High temperatures were found to promote full development of the seeds, but to inhibit development of the tassel, whereas lower temperatures permitted the production of luxuriant tassels, but poorly developed seeds. It was also found that daily sprays of an auxin, indoleacetic acid, inhibit growth of the male organs without affecting ear development. On the other hand, daily sprays of an anti-auxin, triiodobenzoic acid, have a pronounced inhibitory effect on the growth of the ear. Thus, the effective level of auxin in the plant appears to be one of the factors controlling the expression of sexual characters.

In one particular genotype, silkless, temperature was found to have a profound effect on the response of the plant to applied auxin. Silkless plants grown at low temperatures responded to daily auxin sprays with greatly increased growth. No such effect was noted with silkless plants grown at high temperatures, or with normal plants grown at any temperature. Thus, in silkless corn, the effects of temperature upon growth appear to be mediated, at least in part, through the auxin economy of the plant.
An unexpected result of these experiments was the ageotropic behavior and greatly stimulated growth of the prop roots in plants sprayed with triiodobenzoic acid. Experiments now in progress with excised root tips show that this anti-auxin also stimulates the growth of corn roots in vitro. It is hoped that the use of anti-auxin in the medium may make possible the indefinite culture of corn roots, a result which would solve one of the classical problems of plant physiology.

**Plant Control of Soil Erosion**

Investigators: Hellmers, Ashby, Kelleher  
Support: Forest Service, United States Department of Agriculture

This group from the California Forest and Range Experiment Station is engaged in a research program to improve the cover of vegetation on the mountainous area of the Los Angeles River watershed. Studies being conducted at present concern the germination, growth, and nutrition of plants suitable for stabilization of steep slopes. Results of these studies will be applied in the field to improve the vegetation on critical erosion areas. This program is aimed at the reduction of silting in reservoirs and of flood damages in the populated valley areas.

1. Climatic requirements and tolerances of chaparral zone species. In the Earhart Laboratory work is under way to determine climatic requirements and tolerances of grasses and shrubs found in the chaparral zone of the San Gabriel Mountains. In addition, a number of exotic species promising for erosion control are being studied. Results to date show that the species fall into two classes: first, those that are adapted for growth at winter temperatures in southern California and that also grow well at early summer temperatures; second those that are not adapted to winter temperatures but grow well only at warmer temperatures. The majority of species are stimulated to earlier flowering by long photoperiods.

2. Nutritional status of Los Angeles River watershed soils and possibilities of cover improvement by improved nutrition. Current work in the Orlando Greenhouse involves studies of the nutritional status of native plant species on residual soil types indigenous to the Los Angeles River watershed. The species tested to date are Pinus coulteri, Quercus chrysolepis, Pseudotsuga macrocarpa, Ceanothus crassifolius, and Spartium junceum. These were grown on soils derived from Wilson Diorite, Lowe Granodiorite, and Anorthosite parent material and differentially fertilized with nitrogen, phosphorus, potassium, calcium, and a mixture of microelements.
consisting of manganese, boron, zinc, copper, and molybdenum. Results showed nitrogen to be consistently deficient and phosphorus alone or in combination with nitrogen to be deficient in some instances. The addition of potassium or calcium did not result in any statistically significant response to growth. The microelements, in the concentration used, proved to be toxic. Greenhouse studies are also under way to determine any color or morphological characteristics that develop in native chaparral woody species due to a nutritional deficiency. These deficiency symptoms will then be used as a diagnostic tool in the field.

Field applications of nitrogen, phosphorus, and a combination of these elements on sites dominated by Adenostoma fasciculatum and Arctostaphylos glandulosa resulted in a significant growth response on Lowe Granodiorite and Anorthosite soils. Field tests are being conducted to determine the most effective level of nitrogen application for maximum growth of the species existing in the area. Proper soil fertilization shows promise as a method for developing better vegetative cover on critically eroding areas above reservoirs and above communities exposed to flood damages.

Ecology of Desert Plants

Investigator: Soriano
Support: Guggenheim Foundation Fellowship

A very interesting series of experiments on the germination of desert plants has been concluded. It was found (1) that the amount and the intensity of precipitation control germination in many desert plants, with a very pronounced optimum at 15 to 25 mm. of rain distributed over a 10 to 24 hour period, (2) that the conditions during germination strongly influence the further development of the plant, (3) that storage conditions before germination also influence the later development. Thus we come to the conclusion, at least for desert plants, that many new facts concerning germination must be learned before this process is understood. Concerning point two, it was found that Erucaria boveana grew taller and had more and larger leaves as the amount of precipitation increased, with an optimum at 100 mm.; at 200 mm. all these effects decreased again.

Light effects on germination were very pronounced in Eschscholtzia and Amaranthus fimbriatus. Although continuous light inhibits germination, light exposure on the second day for a few minutes only increases germination.
Effect of Smog on Plants

Investigator: Hull
Support: Air Pollution Control District of Los Angeles

The physiological conditions under which smog damage to plants is most or least pronounced has been analyzed in considerable detail. In general a lack of water increases the resistance of plants to smog. The most interesting fact which has been established was that the mysterious daily fluctuations in sensitivity of oat seedlings to auxin, which have been observed for the past 15 years in darkrooms completely isolated from the outer world, are caused by gaseous impurities in the air, which can be filtered out by activated charcoal and which parallel smog incidence. In this way it was established that 10 to 15 years ago air pollution existed in the Los Angeles area. With a semi-quantitative method, the Avena test, available to measure smog damage, the hourly intensity of smog can be established.

Numerous filtering agents have been tried in an effort to eliminate the toxic materials from the atmosphere. The most efficient substance is activated carbon. Glass wool impregnated with solutions of reducing agents such as stannous chloride or ferrous sulfate were effective in neutralizing the oxidants in the smog, and gave complete protection to the cotyledons but not to the leaves. The effect on plants was similar to that caused by fumigation with sulfur dioxide. This suggests that the damaging effect of the latter gas is primarily the result of a reducing action rather than some other specific effect.

One other filter which was almost as effective as carbon in removing pollutants from the air was a mixture of zinc and iron filings. These metals, upon becoming oxidized, appear to neutralize the toxic oxidants of the smog.

Growth and Development of Sugar Beets

Investigator: Ulrich
Support: Beet Sugar Development Foundation through the University of California

In one experiment, eight sugar beet varieties, two open-pollinated, four inbred, and two hybrid, were grown at a high level of nutrition in six climates. The controlled climates had either a natural day length of 10.6 to 14.3 hours or eight hours. The temperatures, which were approximately optimum for beet growth, were $20^\circ\text{C.}$ or $26^\circ\text{C.}$ from 8 a.m. to 4 p.m. and $14^\circ\text{C.}, 20^\circ\text{C.}$, or $26^\circ\text{C.}$.
from 4 p.m. to 8 a.m. In this experiment it was found that the relative performance of each variety was not the same in each climate. For example, the better performance of G. W. 304 compared with U.S. 22/3 occurred mainly in the cool climates. Similarly, hybrid vigor was observed only in the cool but not in the warm climates. In fact, the hybrid H148 produced less sucrose than either of its parents when grown at 26°C for an eight-hour day. Accordingly, these results illustrate the importance of growing plants in controlled climates in crop improvement studies.

In this same experiment the sucrose produced by most of the varieties studied was nearly trebled by increasing the photoperiod from eight hours to natural day length. As expected, the beets grown within the narrow range of temperatures used did not differ greatly in sucrose production. Only by decreasing the day and night temperatures simultaneously for the eight-hour day were the sucrose yields of the plants increased. The beet root weights, sucrose percentages, top weights, weights of living blades, and numbers of living blades generally paralleled the sucrose weights. Surprisingly enough, the total numbers of leaves produced in each climate, except the warmest, were the same. Thus the life expectancy of leaves of plants grown in climates with long days or with cool days or nights is much greater than in warm climates with short days.

In another series of experiments it was found that in the normal development of the sugar beet plant top growth takes precedence over storage root development until the concentration of sucrose within the beet root has increased to a favorable level. Once this favorable level (4% to 8%) has been attained by the storage root, the sucrose synthesized in excess of respiratory and top growth requirements is utilized primarily for increasing the size of the storage root. During this period of storage root development the sucrose concentration within the beet root does not change appreciably when the plants are grown with an ample supply of nutrients in a constant but suitable climate. This suggests that beets have no self-regulatory mechanism for sucrose accumulation, but are dependent upon external stimuli directly or indirectly associated with a change in climate, particularly a lowering of night temperature coupled with a nitrogen deficiency. These events could account for the increase in sucrose concentration as observed in field-grown beets harvested in the fall of the year.

**Effect of Air Conditions on Plant Growth**

*Investigator:* Verkerk
*Support:* American Society of Heating and Ventilating Engineers
*General Funds*

With the use of two greenhouse tomato varieties often used in the Netherlands, it was found that if the light is strong enough, as it
is in greenhouses during summer in the Netherlands and in Pasadena all the year round, the best temperatures for growth are about 23° C. during daytime and 17° C. during the night. Faster growth but rather thin-stemmed plants result with higher night temperatures such as 22° C. A good but slow development occurred with a day temperature of 23° C. and a night temperature as low as 12° C.

In latitudes higher than that of Pasadena the decreased light in winter necessitates the use of lower temperatures. A day temperature of 17° C. and a slightly lower night temperature will be much better in that case. There must be a balance between light and temperature so that the lower the light intensity, the lower the temperature must be to obtain normal but of course slow plant development.

Air movement is good for pollination of flowers, but a strong wind is bad. Plants in Earhart died with the strongest air movement (1000 feet per minute). A moderate wind (200 feet per minute), as far as can be told now, has the same effect as lowering the temperature; the more wind, the more this lowering effect is observed. If it is very warm in a greenhouse, air movement between the plants will undoubtedly be beneficial.

Ecology of Rubber-Bearing Plant Taraxacum Kok-saghyz

Investigator: de Rafols
Support: Del Amo Foundation Fellowship
General Funds

Growth and rubber formation by the rubber plant Taraxacum Kok-saghyz was examined under controlled conditions.

In studies of the growth in constant temperature under artificial light (eight-hour photoperiod) it was found that optimal development took place at 17° C. with 750 foot candles of light intensity. Plants at 26° C. and 30° C. did not survive more than two months. At 17° C. the leaf shape was less lobed. The size of the roots was maximal at 20° C.

The response of the plant to the intensity of light was measured at 20° C. day temperature and at 14° C. night temperature, with eight hours' photoperiod. At less than 500 foot candles, the growth of the plants was very slow and several plants died.

Other experiments were carried out with different periods of light at 14° C., with an intensity of 750 foot candles. The optimal and most luxuriant growth was obtained with 16 hours' light.
The influence of temperature was tested with natural light (eight-hour photoperiod) and also in darkness. The root system has its optimum development at $23^\circ C$. The leaves were more developed at $20^\circ C$. and the first flowering period appeared at $17^\circ C$. four months after sowing.

Other experiments were carried out by varying the night temperatures and keeping the day temperatures constant, and vice versa. At $20^\circ C$. day temperature and $11^\circ C$. night temperature the flowering period appeared 45 days after sowing.

Generally low temperatures at nighttime give more sturdy plants, which is correlated with the amount of rubber synthesized. More influence was observed at $11^\circ C$. night temperature after the growth of the sixth leaf.

Light is an important factor for both vegetative growth and the synthesis of rubber. The general development of the plant is in all cases better with low temperatures and longer exposure to daylight.

Paper chromatograms of sugars of this plant show that in the leaves, stems and flowers only glucose, fructose and sucrose are found. In the roots, no glucose was detected. However, fructose, sucrose, inulin and four unidentified spots (belonging to the ketose group) were detected.

In the terpene analysis it was possible to separate four terpenes.

**Mechanism of Salt Absorption**

Investigators: Nieman, Johnstone
Support: University of Southern California Faculty Research Fund

Investigations of recent years indicate that the active absorption of salts by living plants is an energy-consuming process and that the required energy is primarily derived from respiration. This study is concerned with the mechanism of salt absorption by pea seedlings and the relation of this process to root respiration.

The results indicate that there are at least two different phases in the absorption of KCl from a dilute solution. The first phase is completed within one hour after the roots have been placed in the solution. During this period salt absorption is relatively rapid but the rate of root respiration remains essentially constant.
The second phase is marked by a slow, steady, absorption accompanied by an increase in root respiration. The rate of absorption bears a direct but not a stoichiometric relation to the increase in respiration.

The concentration of KCl solution has a pronounced effect on absorption during the first phase. The amount of salt absorbed increases steadily as the solution concentration is increased from 0.001 to 0.01 molar. At a concentration of 0.005 molar or higher, KCl is absorbed and retained by the roots. At lower concentrations, the roots first absorb KCl and then subsequently return it to the solution. The second phase of absorption is much less sensitive to the solution concentration, but here too a positive concentration absorption relation exists.

It is proposed that the first phase of absorption involves an ion-exchange mechanism, presumably a surface phenomenon, which is not directly dependent upon metabolic activity. During the second phase, surface-adsorbed ions are transported to the interior of the absorbing cells. It is this process which is dependent upon energy supplied by respiration.

**Controlled Plant Growth of Tomato**

**Investigators:** Moore, Johnstone  
**Support:** University of Southern California Faculty Research Fund

San Jose Canner tomato plants were cultivated in varied nutrient solutions under three different day temperatures and night temperatures of 30°-23° C.; 26°-20° C., 23°-17° C.

The results of vegetative activity, except for leaf color, show a positive correlation with temperature. Leaf drop is inversely proportional to flowering and fruit setting and directly proportional to the controlled temperatures. Plants at the two lower temperatures in all solutions produce the largest number of flowers and fruits, while nitrogen-high solutions yield the largest number of perfect fruits. Blossom-end rot occurs in all fruits of the nitrogen-high and potassium-high solutions.

**Fire and Germination Studies**

**Investigators:** Went, M. Juhren (in collaboration with G. Juhren  
**Support:** General Funds

Several ecological problems were studied during the year. The effects of fire on the germination of chaparral plants were studied.
In another investigation it was found that seeds of barley and other plants contain inhibitors for the germination of weeds. This may explain the effects of so-called smother crops, which, when sown thickly, will keep weeds down. An investigation of the optimal growing conditions of several grasses in their early development was concluded.

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**PLANT PHYSIOLOGY AND BIOCHEMISTRY - KERCKHOFF, DOLK AND ORLANDO LABORATORIES**

Staff:

Professor: Bonner  
Visiting Professor: Wanner  
Associate Professor: Galston  
Senior Research Fellows: Laties, Axelrod  
Research Fellows: Bandurski, Campbell, Camus, Fujiwara, Lang, Links, Yamada  
Graduate Students: Boroughs, Crosby*, Eggman, Foster*, Goldacre, Helmkamp, Jansen, Kurtz, Liverman, McRae, Sachs, Saltman  
Research Assistants: Baker, Durrell, Michelson, Millerd  
Superintendent, Orlando Greenhouse: C. Newman

* Chemistry Major

**Factors Which Determine the Activity of Plant Mitochondria**

Investigator: Laties  
Support: Rockefeller Foundation, National Science Foundation

Intracellular particles, known as mitochondria, have been shown to be the site of respiratory pyruvate oxidation. In the plant, as in other organisms, the oxidation of pyruvate proceeds by the tricarboxylic acid cycle. With cauliflower buds as a source of material, factors influencing the respiratory activity of mitochondria have been studied.

It is known that the activity of mitochondria is lessened when the particles are suspended in hypotonic solutions. This damage is not entirely physical, nor a result of swelling, since it has now been found that injury to mitochondria can be almost entirely precluded if dilute solutions of neutral salts are substituted for water as the
hypotonic swelling solution. The osmotic contribution of the salt being inconsequential, it appears that hypotonicity per se is not the cause of the damage, but is a preliminary condition or damage brought about by salt-free environments.

Mitochondria of cauliflower buds have in vitro an appreciable requirement for exogenous ADP or DPN. It is of interest that the requirement may be satisfied by either of these two compounds. This observation is most easily explained on the basis that the fundamental requirement may be for an adenylate and that cauliflower mitochondria contain the enzyme which splits DPN to adenylic acid.

It has been found that the uncoupling agent, dinitrophenol (DNP), is effective in the presence of low concentrations of adenylate in increasing the rate of respiratory oxidation by cauliflower mitochondria.

Experiments involving the respiratory inhibitor malonate have shown that when a Krebs cycle acid such as citrate α-ketoglutarate is used in high concentration as a substrate for mitochondria a disproportionately large contribution of the total oxidation is made by the first oxidation step. Thus, with the oxidation of citrate at high concentrations, malonate inhibition is not observed. This means that little of the citrate is oxidized as far as succinate. It is suggested that oxidations of the tricarboxylic acid cycle are ultimately dependent upon a common electron or other mediating systems. This imposes an upper limit upon the rate of substrate oxidation in the presence of high concentrations of any one particular substrate. Oxidation of this substrate will contribute a disproportionately large share to the total.

Oxidation of Pyruvate by Plant Mitochondria

Investigator: Millerd
Support: Frasch Foundation

It has been established that the oxidation of pyruvate to carbon dioxide and water is achieved in plants by an enzyme system which is organized into a particulate cytoplasmic complex. The particles which bear this complex have been isolated from seedlings of the mung bean and appear to oxidize pyruvate through the reactions of the Krebs cycle. These particles not only correspond in size to the mitochondria but in addition stain vitally with Janus Green B and hence may be considered to be identical with mitochondria. The oxidation of pyruvate is dependent upon the concomitant oxidation of a catalytic amount of one of the di- or tricarboxylic acids, and in this function the various acids are approximately equivalent. Preparational
procedures for the isolation of such particles have been defined. Among the most important requirements for full activity of the isolated particles is the presence of phosphate in the grinding medium. It has been shown that the capacity of the isolated particles for electron transport is sufficient to account for all of the respiration of the intact tissue.

**Oxidative Phosphorylation in Plant Systems**

Investigators: Millerd, Bonner  
Support: Frasch Foundation

The coupling of phosphorylation to aerobic respiration has been achieved with mitochondrial preparations from a variety of plants. In general the procedure used has been to allow the mitochondrial preparation to oxidize substrate in the presence of inorganic phosphate, adenylate and D-glucose. The uptake and incorporation of inorganic phosphate into organic form, which is coupled to substrate oxidation, results in the formation of adenosine triphosphate (ATP). The phosphate thus incorporated into ATP may however be immediately transferred to glucose with the formation of glucose-6-phosphate. The latter reaction is mediated by hexokinase, which occurs bound as a constituent of the mitochondria. The progress of phosphorylation may therefore be simply followed by determination of residual inorganic phosphate.

The complete system for demonstration of oxidative phosphorylation by mung bean mitochondria contains the substrate to be oxidized, 0.02M a-ketoglutarate, and 0.01 M inorganic phosphate, 0.001 M adenylate as adenylic acid, and 0.001 M MgSO₄. In addition, 0.01 M NaF is added to inhibit ATPase activity; and 0.1 M D-glucose, to act as terminal acceptor for phosphate incorporated into ATP; and 0.2 M sucrose, to maintain appropriate osmolar relationships.

The plant system under study transfers but one molecule of inorganic phosphate to ATP for each electron pair removed from the substrate. This is true for each and every oxidative step of the Krebs cycle. Efficiency of the plant system is thus lower than that of animal mitochondrial systems.

The data available establish that oxidative phosphorylation is a process mediated by plant mitochondria of varied origin (avocado, mung bean, cauliflower, pea seedling, Jerusalem artichoke). In all of the instances which have been studied thus far, the ratio of atoms of phosphorus esterified to atoms of oxygen consumed has remained in the neighborhood of one.
Finally, in the plant as in other systems the coupling of substrate oxidation to phosphorylation can be severed by uncoupling agents, such as 2,4-dinitrophenol.

The knowledge of energetic coupling in plants which has been obtained during the course of these investigations has given us a broad new insight into the basic biochemistry of the plant and a whole new approach to the study of plant growth and development.

Regulation of the Respiratory Rate in Plant Systems

Investigators: Bonner, Millerd, Bandurski
Support: Frasch Foundation

It has been common plant physiological knowledge for some years that increases in respiratory rate accompany such physiological processes as growth, salt accumulation and active uptake of water. Now there is evidence which suggests that these increases in respiratory rate are a result of and are brought forth by the work which the tissue is called upon to do. In addition, it is possible to outline in some detail the mechanisms by which respiratory rate is geared to and regulated by the work requirement of the tissue. This understanding has been made possible by the fact that now it is possible to prepare isolated plant mitochondrial systems which are capable of carrying out respiratory oxidations in vitro. The rate at which substrate is oxidized by isolated plant mitochondria is dependent not only upon the presence of substrate and other usual factors but also upon the presence of a phosphate acceptor such as adenylate. In the presence of adenylate, substrate is oxidized, and inorganic phosphate is simultaneously taken up and incorporated into ATP. For several of the plant systems which have been investigated during the past year, dependence of substrate oxidation upon the presence of a phosphate acceptor is nearly complete. Respiratory rates may be increased fivefold or more in the presence of such an acceptor. It can further be shown that the compulsory coupling between substrate oxidation and phosphorylation is severed in the presence of 2,4-dinitrophenol. 2,4-Dinitrophenol may be used as a reagent for ascertaining the extent to which the rate of normal tissue respiration is limited in vivo by phosphate-accepting systems. It may be concluded that the rate of respiration in plant tissues is regulated by the capacity of the phosphorylative or adenylic system and that this in turn is regulated by the rate at which the phosphate acceptor is renewed to utilization of ATP. The rate at which active ATP-using work is carried is, in the last analysis, primarily responsible for regulation of respiration rate.
The Nonglycolytic Metabolism of Glucose-6-phosphate

Investigators: Bandurski, Axelrod, Links
Support: Frasch Foundation, General Funds
United States Department of Agriculture
National Science Foundation

We have found that in certain plant tissues glycolysis occurs not only along the classical Embden-Meyerhof pathway, but also along an alternative route. This alternative route for the metabolism of glucose-6-phosphate is the Warburg-Lipmann-Dickens (WLD) scheme. The first step is the oxidation of glucose-6-phosphate to 6-phosphogluconic acid by glucose-6-phosphate dehydrogenase, a reaction similar to that known for yeast and erythrocytes. A second enzyme, 6-phosphogluconic dehydrogenase, then catalyzes the oxidative decarboxylation of 6-phosphogluconic acid to a pentose phosphate. This enzyme has now been shown to be present in a number of higher plants. It has been partially purified and has been characterized with respect to its sensitivity to inhibitors, substrate affinity and specificity. The first pentose formed is apparently ribulose-5-phosphate. Owing to the presence of a pentose isomerase, the keto pentose, ribulose, is in equilibrium with the aldo pentose, ribose-5-phosphate.

Incubation of ribose-5-phosphate with an enzyme obtained from spinach leaves results in disappearance of the pentose phosphate. Approximately one sedoheptulose phosphate and one triose phosphate are formed per two pentose disappearing. The mechanism of this reaction is as yet unknown. Some evidence has however been obtained for the existence of a substance which accumulates as the pentose disappears and later disappears as heptose is formed.

If TPN is added to an incubation mixture containing ribose-5-phosphate and the spinach enzyme, the TPN is reduced. Simultaneously with the reduction of TPN there is liberation of inorganic phosphate and a disappearance of triose phosphate. It thus appears that the reduction of TPN is the result of the presence of a TPN-linked triose dehydrogenase. Pyruvic acid has been isolated from the reaction mixture as the 2,4-dinitrophenylhydrazone derivative.

That pyruvic acid is formed by the alternative system for the metabolism of hexose phosphate has implications not only for respiration but also for the path of carbon in photosynthesis.


Enzymatic Phosphate Transfer

Investigators: Saltman and Campbell
Support: DuPont Predoctoral Fellowship
Frasch Foundation

The dominant role of energy-rich phosphate compounds in the metabolism of animal and yeast cells has long been recognized. It has only been recently, however, that attention has been turned to elucidation of the formation and utilization of the energy-rich phosphate found in higher plants. One phase of this investigation has been the identification and characterization of the enzymes of glycolysis. During the past year, two enzymes involved in the transfer of energy-rich phosphates have been studied and characterized in plants for the first time.

Hexokinase, the enzyme which catalyzes the formation of glucose-6-phosphate from glucose and ATP, has been found to be distributed widely in plants. Much enzymatic activity is associated with the mitochondria. Although a further fraction is present in the plant as a soluble enzyme, it is possible that this is in general formed by degradation of the mitochondrial enzyme during preparation. Plant hexokinase resembles yeast rather than muscle hexokinase in its properties and substrate specificity.

Adenylate kinase (myo-kinase) has also been discovered and studied in plant tissues. This enzyme catalyzes the reaction of two molecules of ADP to form one molecule each of ATP and AMP. Direct demonstration has been accomplished by the determination of increased phosphorylation of glucose obtained when a plant extract is added to a reaction mixture containing glucose, ATP and purified yeast hexokinase. This enzyme has been characterized with respect to its sensitivity to inhibition and to pH and partially purified.

Arginine Phosphate and Arginine Kinase in Plant Systems

Investigators: Fujiwara, Saltman, Bandurski
Support: DuPont Fund
Frasch Foundation
General Funds

Although the mechanism for the formation of energy-rich phosphate compounds in higher plants is now relatively well understood, the manner by which this energy is held in reserve has presented an interesting problem. That any great amount of the energy-rich phosphate can remain in the form of ATP is unlikely, both because of the lability of this compound and because of the low total adenylate concentration in plant tissues.
In experiments on oxidative phosphorylation by plant mitochondria, it was found that arginine could act as the final phosphate acceptor equally as well as glucose. This pointed to the existence in the plant of the enzyme arginine kinase, which catalyzes the reaction

\[
\text{Arginine} + \text{ATP} \rightarrow \text{Arginine phosphate} + \text{ADP}.
\]

The presence of arginine kinase in the plant has now been demonstrated by two independent methods. These are measurement of the disappearance of arginine in a reaction mixture containing arginine, ATP and enzyme, and manometric determination of \( \text{CO}_2 \) evolved during the course of the reaction in bicarbonate buffer. The products of the reaction have been chromatographed and it has been shown that they include a phosphate coupled to an amino acid, which may be arginine phosphate.

The study of this reaction in the plant is of interest in the light of the mechanism for the accumulation of energy-rich phosphate in invertebrate muscle. During invertebrate muscular contraction arginine phosphate directly transfers its energy-rich phosphate to ADP. It will obviously be of interest to identify more precisely the role of arginine phosphate and arginine kinase in the metabolism of the higher plants.

**Purification and Characterization of a Phosphatase**

**Investigator:** Boroughs

**Support:** Frasch Foundation

The phosphatase of green leaf cytoplasm has been studied. It was found to have a pH optimum of about 5.5, which is characteristic of other plant acid phosphatases. The enzyme is inhibited by cyanide, fluoride, zinc, and chelating agents, and could be purified by dialyzing against acid buffers, the phosphatase activity stopping in the neighborhood of pH 2. The return of such a preparation to a more neutral pH range does not restore activity, but the addition of certain metals reactivates the enzyme to as much as three-fourths of the original activity.

These facts are contrary to the opinion formerly held that acid phosphatases are nonmetallic enzymes and that they are incapable of being reactivated. Copper, cobalt, manganese, magnesium, and iron are capable of this reactivation in decreasing order of effectiveness. Copper at \( 10^{-5} \) M, for example, is as good as cobalt at \( 10^{-3} \) M.
The specific activity of the enzyme present in spinach cytoplasm was followed after dialysis against citrate buffers of various degrees of acidity, and a spectrographic analysis of the metals was concomitantly followed. Cobalt was not present in the ash, and the amount of magnesium was found to be unrelated to the enzyme activity, but in the case of copper, manganese, and iron, the enzymatic activity was directly related to the amount of metal present. Since the enzyme is not specifically reactivated by copper, it is not possible to state that it is a copper enzyme, but it is assuredly a metal enzyme whose properties are now shown to be more closely related to the better known alkaline phosphatases than was formerly suspected.

**Carbohydrate Translocation in Seedlings**

Investigator: Wanner  
Support: Rockefeller Foundation Fellowship  
General Funds

In connection with studies on carbohydrate translocation in progress at the botanical laboratories of the University of Zürich, the occurrence and the transport of sugars in corn seedlings were investigated with the method of paper chromatography. The solvents developed by Bandurski and Axelrod were applied to extracts from corn embryos and endosperms. It was found that acid extracts from endosperm contain free orthophosphate and glucose-1-phosphate. In the embryos only traces of free orthophosphate and no glucose-1-phosphate were found. Nearly all phosphate, free and bound, migrating into the embryo from the endosperm, was trapped in an organic phosphate-containing compound of unknown nature. Chromatography of the embryo-extract in acid solvents gave one intense spot of this compound. In alkaline solvents the substance seems to be split into two compounds which both contain phosphate. The one with the larger Rf value shows a strong absorption of UV light of short wave length. By eluting this substance from the paper and measuring its UV absorption an absorption curve was obtained with two maxima at wave lengths below 250 mmü.

**Flower Formation as a Result of Chemical Treatment**

Investigators: Liverman and Lang  
Support: Lalor Foundation  
Frasch Foundation  
Lucy Mason Clark Fellowship  
General Funds

During the past year, we have achieved both a better understanding and a measure of chemical control over the flowering of
short- and long-day plants. It is clear that the photoperiodic response of short-day plants may be separated into a number of component or partial processes including the following: (1) a high-intensity light process, (2) a dark process, (3) a low-intensity light process by which (2) may be suppressed. Earlier studies have shown the relation of the high-intensity light process to photosynthesis. For the high-intensity light requirement in the flowering process there may be substituted sugars, such as sucrose, galactose, fructose, which are fed through the leaves of the experimental plant. It has further been shown that the dark process in Xanthium and in Chenopodium is inhibitable by applied auxin. This has been interpreted as meaning that auxin may have an important effect upon the process of the synthesis of floral hormone in the leaves and that the dark period may be important only in that it insures attainment of some sufficiently low, critical threshold auxin level.

Experiments on the low-intensity light process have shown that suppression of flowering by low-intensity light interruption of the dark period can in turn be suppressed by anti-auxins such as triiodobenzoic acid or 2,4-dichlorophenoxyisobutyric acid. Experiments with Chenopodium have shown that the inhibition produced by continuous low-intensity light given during the dark period is also reversible by applied anti-auxins.

Experiments with the long-day plants, Hyoscyamus niger and Silene armeria, have shown that flower formation can be produced under threshold conditions by treatment with auxin indoleacetic acid. Fragmentary reports from others would indicate that auxin may affect flower formation in long-day plants in a quantitative way, but the experiments reported here appear to be the first in which a qualitative difference in flowering has been observed in response to applied auxin. The significance of auxin in the photoperiodic induction of long-day plants has been demonstrated.

Physiology of Flowering

Investigators: Lang, Liverman
Support: Lalor Foundation
Lucy Mason Clark Fellowship
Strater Fund
Frasch Foundation
General Funds

The object of these studies has been to gain further insight into the kinetics of the flowering process. One project has dealt with the relationships between flowering and temperature in long-day plants. A second group of experiments has dealt with the interrelations of the dark
period and the interruption of the dark period by flashing light in short-day plants. A third group of experiments has concerned the relationship of the flowering hormones of long-day to short-day plants. Comprehensive experiments on the interrelation between temperature and photoperiod were done in the Earhart Plant Research Laboratory with the newly discovered long-day plant, *Silene armeria*. Unlike other plants which have been studied in the past, *Silene* was found to be promoted, in general, in its flowering response by increasing temperature. Only within a restricted temperature range, 17° to 23°C, was flowering promoted with decreasing temperature as has been the case with other long-day plants.

It is known that the effectiveness of a long dark period in inducing floral initiation in a short-day plant can be inhibited by a flash of light, given near the middle of the dark period. In these experiments, a direct correlation was found between the amount of light needed to suppress the flowering response and the length of the dark period. Thus, with cocklebur, 250 candle foot minutes were required to inhibit flowering in an 8.5-hour night, whereas 1500 candle foot minutes were required to prevent flowering in a 13-hour night. These findings as well as those with the short-day plant, *Chenopodium*, are compatible with the hypothesis that the process which takes place during the dark period proceeds slowly at first and then increases rapidly. This kind of kinetics would be expected on the basis that the dark process is an enzymatically controlled one.

Further experiments have dealt with comparison of the flower hormones of long-day and short-day plants. This is done by grafting long-day and short-day plants together in various combinations.

In general it can be concluded that once a donor has been induced, it is capable of inducing flowering on uninduced receptors regardless of photoperiodic type. The floral stimuli of long-day and short-day plants thus appear to be identical.

**Physiology of Vernalization**

Investigator: Jansen
Support: General Funds

In the study of the physiology of vernalization, the promotion of flowering by low temperature, use has been made of the excised embryo technique. Previous investigators have shown that vernalization in excised rye embryos is effectively achieved only if sugar is supplied in the nutrient solution during the cold treatment. It has also been shown in earlier experiments that vernalization in whole grains is prevented under an atmosphere of nitrogen. In the present experiments the influence of
sucrose and anaerobiosis alone and in combination on the development of excised embryos was investigated. It has been found that sugar is required only during the first three weeks of the vernalization period and can be dispensed with during the second period. Aerobic conditions are required during the second period only. These facts suggested that the process of vernalization may be separated into two component or partial processes.

Fruit Growth

Investigator: Jansen
Support: General Funds

The studies on fruit growth involve the in vitro culture of excised flowers of red-currant tomato. It has been shown that the ovary of such flowers is capable of growing into mature fruits in culture on a nutrient solution containing sucrose and mineral salts. Glucose or fructose will substitute effectively for the sucrose requirement. Under certain conditions, growth of the excised ovary approximates closely the rate of development of seedless, parthenocarpically-induced fruits on the plant. No seeds develop in culture, although palmation is an essential prerequisite to the development of the excised ovary in vitro.

Biochemical Effects of Light in Plants

Investigator: Galston
Support: Rockefeller Foundation Fund

It is now known that several apparently unrelated responses of plants to light are mediated by similar, if not identical, pigments. Thus, photoperiodic control of flowering, stimulation of leaf growth, inhibition of stem growth and enhancement of lettuce seed germination are all brought about primarily by red light of approximately 6600 A and to a lesser extent by blue light of approximately 4000 A. The present investigation has utilized dark-grown pea plants in an effort to elucidate the mechanism by which these light effects are produced. Stem sections of dark-grown pea plants will almost double in length and fresh weight if supplied with about 0.1 mg./L of the plant growth hormone, indoleacetic acid (IAA). Pre-exposure of the plants to red light decreases their sensitivity to IAA about a hundredfold; i.e., it takes about 100 times as much IAA to produce a given growth effect. The total amount of growth obtainable, however, is the same in dark and in red light-exposed tissue.

Pre-exposure of the plants to red light has been found to decrease the ability of stem sections to remove IAA from solution, and also to increase significantly the activity of an enzyme, indoleacetic
acid oxidase, which destroys IAA. The significance of these findings in interpreting the effect of light on this tissue is still under investigation, but it seems possible that the observed increase in activity of the enzyme-destroying IAA could account for the decreased sensitivity of red light-exposed tissues to IAA. Thus, the primary effect of red light in etiolated pea tissue may be to increase the activity of IAA oxidase.

**Experimental Separation of Water Absorption from Cell Elongation by the Use of Benzimidazole**

Investigators: Galston, Baker  
Support: National Science Foundation

Dark-grown pea stem sections supplied with small quantities of an active auxin such as indoleacetic acid grow rapidly, approximately doubling in length and in fresh weight over a 16-hour period. The addition to the medium of $2 \times 10^{-3}$M benzimidazole, a presumed purine antagonist, inhibits growth in length about 40%, but fails to inhibit water uptake at all. In many instances, water absorption may actually be stimulated while growth in length is greatly inhibited. As a result, the sections swell greatly in the transverse direction, becoming barrel-shaped. These effects are not produced in the absence of auxin.

The addition of various purines, purine ribosides, pyrimidines and nucleotides to benzimidazole-inhibited sections fails to reverse substantially the inhibition of growth in length, and usually results in further enhancement of the already stimulated water-absorption mechanism. The effects of benzimidazole cannot therefore be attributed entirely to competitive inhibition of purine-containing systems.

Although the precise mechanism of action of benzimidazole on pea stem sections is as yet unknown, its use permits a study of auxin-catalyzed water uptake uncomplicated by cell elongation. The morphogenetic effects of this substance on tissues grown in pure culture is currently under investigation.

**The Destruction of Indoleacetic Acid in Plant Tissues**

Investigator: Goldacre  
Support: Commonwealth Scientific and Industrial Research Organization of Australia Pre-doctoral Fellowship General Funds

These studies concern the nature and properties of two systems found in etiolated pea seedlings which catalyze destruction of indoleacetic
acid (IAA). These two systems are an enzyme system, IAA oxidase, and a dialyzable system which contains several small molecular components which destroy IAA in the light but not in the dark. The relative proportions of these two systems vary according to the environmental conditions under which the plants are grown.

Indoleacetic acid oxidase, which has been described extensively in earlier work, has been found to be in all probability two distinct enzyme systems which act in parallel. One system possesses a dialyzable cofactor, the other is without such cofactor requirement. It has previously been reported that 2,4-dichlorphenoxyacetic acid (2,4-D) is able to increase the activity of indoleacetic acid oxidase. It has now been found that this property of 2,4-D is actually due to an impurity in the preparations which have been tested. The active impurity is 2,4-dichlorophenol. The phenol, which is an extremely powerful inhibitor of the enzyme catalase, is also effective in increasing the activity of IAA oxidase. Dichlorphenol is without an inhibiting influence on peroxidase. The chemical study of the dialyzable nonprotein components of the system which photoinactivate IAA has shown that several substances are involved. Activity of the dialyzable nonprotein components is decreased by titration with 8-hydroxyquinoline or other sequestrian agents. It would appear that a portion of the activity is metal-dependent and a portion metal-independent.

It is clear that in plant tissues IAA is subject to numerous pathways of destruction by probably two enzyme systems as well as by a variety of independent, small molecular weight compounds.

Chemical Basis of Anti-auxin Activity

Investigator: McRae
Support: General Funds

It has become possible to formulate with some precision conditions under which an auxin analog acquires anti-auxin rather than auxin activity. This new insight into the relations between structure and activity is based upon the concept that an active auxin is bound through two different groups to two enzymatic sites in the plant. The two reactive positions of the auxin molecule are the carboxyl group and the position on the ring system ortho to the side chain. Any molecule capable of reacting at one of the sites but incapable of reacting at both appears to act as an anti-auxin. Thus when the essential carboxyl group of an auxin is eliminated the resulting molecule is still able to combine with the enzyme through the ortho position. Similarly, when the essential ortho positions are blocked the molecule can combine with the enzyme through its carboxyl group. Further, a single-point attachment can be attained
even in molecules which possess the essential free ortho position and carboxyl group by introducing steric hindrance into the side chain. Compounds so modified will interfere with activity by preventing the auxin from making its requisite two-point attachment.

Among the compounds which have been tested and found to act as anti-auxins according to the preceding predictions are p-chloro- and 2,4-dichlorophenoxyisobutyric acid, 2,6-dichloro- and 2,4,6-trichlorophenoxyacetic acid. These have been shown to competitively inhibit both IAA and 2,4-D induced growth of Avena coleoptile sections. In addition, the compound 2,4-dichloranisole is active as an anti-auxin, but as would be expected 2,4,6-trichloranisole, which has neither a free carboxyl group nor a free ortho position, has no effect on IAA or 2,4-D induced growth of Avena sections.

Still another prediction based upon the two-point attachment concept is that different auxins may interact and compete at the same catalytically active site. By an extension of the kinetic treatment used in the investigations outlined above, it has been shown that such auxins as IAA and 2,4-D act and compete at the same catalytically active growth-promoting site when supplied together to Avena sections.

Auxin-induced Growth Inhibition, a Consequence of Two-Point Attachment

Investigators: McRae, Foster
Support: General Funds

As part of the program for elucidation of the action of auxin on plants, the phenomenon of inhibition by excess auxin has been investigated. The growth of Avena coleoptile sections, now developed into an accurate quantitative measure for auxin activity, was the system chosen for study. On the assumption that two different parts of the auxin, in this case indoleacetic acid (IAA), must be attached to two different sites of some entity such as an enzyme in order to elicit the growth response, the conclusion follows that there should be an optimal concentration of IAA and that when this is exceeded the response will decrease. This is owing to the fact that at sufficiently high concentrations of IAA there is a higher probability that two different molecules of growth substance will become attached to the pair of sites than that a single molecule can compete and consummate the necessary two-point attachment.

By an extension of the methods of enzyme kinetics of Michaelis and Menton it is possible to derive an equation which expresses rate of growth of Avena coleoptile sections in terms of substrate (IAA) concentration and a series of constants. This equation has been successfully
applied to the IAA-promoted growth of Avena coleoptile sections. As is expected by this treatment, the promotion of growth by auxin is first order with respect to auxin concentration and is therefore linear with auxin concentration at low concentrations. Auxin-induced inhibition of growth on the contrary, as would be expected from the kinetic treatment, is, as a first approximation, proportional to the square of the auxin concentration. The experimental data which have been obtained fit closely to the curve predicted from the equation over a concentration range of $10^{-3}$ to $6 \times 10^2$ mg/liter of auxin.

Influence of Auxin on Metabolic Pathways

Investigator: Boroughs
Support: Frasch Foundation

An attack on the problem of the role of auxin in the process of growth was begun in this laboratory last year by studying the fate of radioactively labeled intermediates in Avena coleoptile in the presence and absence of indoleacetic acid (IAA). This study showed that the incorporation of the label increased with time, although no net synthesis of protein occurred. The rate and total amount of incorporation of the label was the same in the tissue with or without the IAA. From this it was concluded that auxin played a minor role, if any, in the protein metabolism of excised Avena coleoptiles.

The incorporation of carboxyl-labeled acetate into the lipid compounds of Avena was studied and found to be essentially unaffected by auxin.

Further elaboration of the role of auxin was attempted by incubating Avena coleoptiles with radioacetate or radiosucrose, fractionating the tissue into various solubility classes, and chromatograming the extracts on paper and making a radioautograph. Both acetate and sucrose are incorporated by the tissue, but in all the cases examined so far no differences could be detected between treated and untreated controls.

Beginning with acetate, coleoptiles are able to incorporate the label into at least seven different detectable compounds, including components of the cell wall itself. Research on this problem is still being continued.

Growth of Rice Coleoptile under Water

Investigator: Yamada
Support: General Funds
Although it has long been known that the rice coleoptile can grow better under water than in air, the physiological basis for this phenomenon has never been elucidated. Decapitation tests carried out in air and under water and growth tests carried out with floating or submerged coleoptile sections suggest that the superior growth under water depends merely on a decreased auxin destruction. There is more residual indoleacetic acid (IAA) in culture solutions in which submerged coleoptile sections are grown than in solutions containing floating sections. Similarly, more IAA remains in coleoptile tissue infiltrated with IAA and incubated in nitrogen gas than in similar tissue incubated in air. Both these results suggest that the rice coleoptile has a high IAA-destroying activity and that more IAA is destroyed with an increased supply of oxygen. Further evidence for the participation of auxin in the growth under water is shown by the ability of anti-auxin (2,4-dichlorophenoxyisobutyric acid) to retard growth almost completely. Thus the better growth under water must be caused by the conservation of auxin in the tissue. KCN inhibition of growth was found to be the same in floating and submerged sections, suggesting that aerobic respiration must be involved in both cases. A possible working hypothesis is that growth under water is determined by the interaction of at least two factors: auxin content and oxygen supply.

**Plant Growth Responses Induced by Sterols**

**Investigator:** Helmkamp  
**Support:** General Funds

In spite of the universal occurrence of sterols and steroids in plant tissues, the role of these substances has remained almost totally obscure. The first demonstration of any physiological role or activity of sterols in plants was that of Loewe and Spohr, and others in 1926. From this early work and its later confirmation, it can be concluded that estrogenic substances including estrone when applied to certain species of plants significantly increase vegetative growth. Experiments of Bonner and Axtman, in 1936-38, show further that estrogenic substances increase the growth rate of excised pea embryos in vitro. This test has now been used to study the specificity of sterols and steroids in relation to plant growth responses.

It has been shown that the growth response of pea embryos to sterols is a quite specific one. Estrone is growth-promotive in concentrations of 1 to 10 milligrams per liter. All other sterols tested were found to be inactive or growth inhibitory. Testosterone, in particular, is inhibitory to growth at concentrations as low as 0.1 milligrams per liter. Interestingly enough, the naturally occurring plant sterols, such as sitosterol, are all inactive. A variety of crude saponin prep-
arations were tested in the pea embryo growth test. All were found to increase the growth of pea embryos.

It can be concluded from this preliminary survey that plant sterols and steroids of the kind contained in the saponin preparations do have physiological activity. It has been shown further that the physiological activity of sterols has a marked chemical specificity, and is restricted to a few individual compounds.

Structure of Plant Toxic Substances from Thamnosma montana.

Investigator: Crosby
Support: General Funds

Three crystalline compounds were isolated from T. montana by E. L. Bennett in 1948, on the basis of their high toxicity to tomato seedlings. Qualitative and quantitative analyses for the elements present and chemical classification tests carried out with the aid of ultraviolet spectrophotometry showed all three substances to be derivatives of coumarin, containing only C, H, and O and having the formulae, \( \text{A}, \) \( \text{C}_{11} \text{H}_{4} \text{O}_{3} (\text{OCH}_3)_2, \) \( \text{B}, \) \( \text{C}_{16} \text{H}_{15} \text{O}_6 (\text{OCH}_3), \) and \( \text{C}, \) \( \text{C}_{16} \text{H}_{13} \text{O}_5 (\text{OCH}_3). \) Degradation and final comparison with authentic samples showed \( \text{A} \) and \( \text{B} \) to be the purscoulmarins, isopimpinellin and byakangelicin, whereas the structure of \( \text{C} \) has not yet been finally proven. It is known, however, to be a derivative of isobergaptene.

Although these substances are ineffective against bacteria, they were found to effectively inhibit the growth of a number of dicotyledonous plants. Monocotyledonous plants were unaffected. The same effects were observed with excised shoots. Normal growth is not resumed after removal of the plants to normal growing conditions, after growth has been inhibited by the presence of the toxic substances.

Oxalic Acid Oxidation of Spinach Leaves

Investigators: Sachs, Laties
Support: Rockefeller Foundation
National Science Foundation

Most interest in the enzymatic mechanisms of the chloroplasts has centered about the photosynthetic apparatus. Some years ago, however, it was found by Laties that chloroplasts also contain an active oxidative system which is remarkable in being cyanide-insensitive. It has now been shown that this system is oxalic acid oxidase and that in the presence of cyanide peroxide is formed as a reaction product. This would be expected if the enzyme were a flavoprotein. The affinity of substrates for the enzyme was found to be great, the \( K_m \) being approxi-
mately $8 \times 10^{-4}$ molar. It is of interest that acetone powders carry out the oxidation equally as well as intact chloroplasts. These powders are free of chlorophyll.

It may be noted that the mere presence in leaves of an oxalic acid oxidase system must cause us to revise the classical concept that oxalic acid in the plant is an inert and metabolically functionless end product.

**Synthesis of Fatty Acids from Acetate by Excised Fruits**

Investigator: Kurtz  
Support: Atomic Energy Commission Predoctoral Fellowship  
General Funds

The present study concerns fat synthesis in higher plants. It has been shown that excised developing flax fruits, properly selected for age and uniformity, will synthesize fats provided that certain substrates and growth factors are supplied to them. The substrates found to be most effective in supporting fat synthesis are compounds of two- (acetate, ethyl alcohol), or three- (acetone), or four- (acetoacetate) carbon atoms. This is in agreement with the existing knowledge of fat synthesis in animals and microorganisms. The immediate fat precursors, glycerol and oleic acid, when fed along with sucrose are also effective in supporting fat formation. Sucrose alone is ineffective. This suggests that oleic acid is first broken down and then resynthesized to appropriate fatty acids which are converted to glycerides.

In all cases energy is required for the synthesis of fats, as shown by the fact that light and an energy source such as sucrose are necessary for rapid production of fats at the expense of short-chain carbon substrates. Apparently light is essential to this process through nonphotosynthetic reactions.

For maximum fat formation in excised fruits the B-vitamin, biotin, is required in addition to the light, sucrose, and substrate requirements. Biotin, when added to the culture medium in extremely small quantities (0.005 mg./liter), accelerated fat formation to such an extent that the amount of fat formed in cultured excised fruits paralleled the amount formed in fruits left on the plant. Biotin apparently is a cofactor in the enzyme system involved in the conversion of acetate to fat.

**Further Characterization of a High-molecular Weight Cytoplasmic Protein**

Investigator: Eggman

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It has been shown earlier that green leaves of various plants possess a characteristic protein component of high-molecular weight and that this protein is highly labile to mildly acid conditions, pH 6.6 to 6.0. The protein contains bound nucleic acid. During the past year the nucleic acid moiety has been shown to be of the ribose type and to be especially rich in guanine. It is therefore clear that this high-molecular weight (about 600,000) component represents a ribonucleoprotein of the leaf cell.

Analytical ultracentrifugation has been used as an analytical tool for determining the amount of ribonucleoprotein in cytoplasmic protein mixtures. With this method, it was found that the amount of ribonucleoprotein increases as leaves mature. Environmental temperature also influences concentration of ribonucleoprotein in the leaf.

A Virus-like Protein Associated with a Plant Tumor

Investigators: Camus, Durrell
Support: American Cancer Society
National Foundation for Infantile Paralysis

Methods developed in this laboratory for the extraction and characterization of plant proteins have been applied to a characteristic tumor tissue, the crown gall. It has been shown by electrophoresis and ultracentrifugation that the tumor tissue contains a characteristic high-molecular weight protein which is absent from related but non-tumorous tissue. That the tumor component may be a nucleoprotein is suggested by its electrophoretic mobility.

The crown gall tumor is elicited by a specific bacterium which acts as a vector in transmitting the disease from plant to plant. The tumor, once established in a suitable host, can be transmitted systemically and from plant to plant by grafting and in such movement is independent of the presence of the bacterium. The characteristic protein component, on the other hand, appears in the tissue as it becomes tumorous. Crown gall tumors can be evoked in a variety of host plants. The tumor protein is electrophoretically similar if not identical in the four species so far examined.

The tumor protein here described resembles the plant virus both in physical properties and in its transport within the plant. Whether it is in fact a virus, a virus for which the crown gall bacterium is the normal vector, is a question which cannot be answered without an independent method for demonstration of infectivity of the isolated protein.
Inhibition of Darkening in Leaf Extracts

Investigators: Boroughs, Camus, Eggman
Support: Frasch Foundation
American Cancer Society
General Funds

Many plant tissues and extracts become dark as a result of wounding or grinding, and during research on the nature of crown gall in which it became necessary to examine the protein extracts by means of electrophoretic and ultracentrifugal analysis, the practical problem was posed of how to reduce this darkening process without in any way denaturing the proteins. The theory was adopted that a metal enzyme was responsible for the bulk of the discoloration and that by chelating the metal or metals involved the process could be inhibited.

It was found that when spinach leaves are ground in the presence of diethyldithiocarbamate, the darkening process can be inhibited by two-thirds, as judged by the reduction in color at 440 mp. No significant difference in behavior of the treated and untreated controls was apparent when the samples were examined in the ultracentrifuge or electrophoresis apparatus, indicating that no serious denaturation of the proteins occurred, even though it is known that the bulk protein of leaf cytoplasm is extremely labile.

VIROLOGY

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The Mechanism of Genetic Recombination of Phage

Investigators: Visconti and Delbrück
Support: National Foundation for Infantile Paralysis

A theory of genetic recombination in phage has been developed according to which a mixed infection of a bacterium with different genetically
marked types represents not a simple genetic cross but an experiment in population genetics. The intracellular population of prophages undergoes several rounds of mating. Mating is random with respect to partners and, presumably, random with respect to time. Superimposed on these processes is the maturation of the prophages, which removes them from the pool of multiplying and mating prophages and which is assumed to be random with respect to genotype.

The general purpose of the theory is to explain and predict the results of crosses (mixed infections). The variables of the experiments are the markers and their linkage relations, the ratio between the infecting parental particles, and the time of inspection (at normal lysis time, or earlier, in the case of premature lysis, or later, in the case of delayed lysis).

The theory fits with some very general notions concerning the life cycle of bacteriophages, namely, the transformation into prophage and accumulation at a linear rate of mature phages from a pool of growing and recombining prophages, as derived both from genetic and from biochemical tracer studies.

The principal accomplishment of the theory is that it accounts (1) for the occurrence of particles in the progeny which combine markers from more than two parents, (2) for the deviations from genetic equilibrium and their decrease in the course of time, and (3) for the positive correlation between different recombinations in the progeny population. This positive correlation should, and does (Doermann and Hill, 1952), also show up in simple mapping experiments with two factor crosses. Map distances calculated by the theory show neither positive nor negative correlation, suggesting that there is little, if any, interference between different recombinations within the same linkage group.

One aspect of phage genetics which has not been accounted for by the theory is the work of Hershey (1951) on heterozygous phage particles in the progeny. Hershey finds that about 2% of the progeny from a bacterium infected with equal input of two marked parents are heterozygous with respect to any given factor. It may be considered unlikely that the heterozygotes should be interpreted as a sample from the zygotes in the mating pool.

From the point of view of this theory, mixed infection constitutes a very complex genetic experiment. The complications are mainly caused by the experimental difficulties which stand in the way of an analysis of single matings. Particularly, the idea that in the elementary mating process reciprocal recombinants are produced in exactly equal numbers seems at present not susceptible to any direct experimental tests.
On the whole, the theory places the genetics of phage very much in line with orthodox genetical theory, as it has been worked out for higher organisms.

Genetics of the Bacterial Virus T3

Investigators: R. Dulbecco, Fraser
Support: National Foundation for Infantile Paralysis

As a preliminary to genetic work, general information was secured on adsorption and interference in the bacterial virus T3 and its host range mutant used for genetic study. Adsorption in 1/140 M phosphate buffer, pH 7, with addition of .05% NaCl and 10^{-4} M MgSO_4, was satisfactory for T3 itself but poor for the h mutant. It was found that traces of broth increased the adsorption of the latter greatly. A reversible first step and an irreversible second step requiring the presence of an energy source are suggested. Lysis from without has been shown not to occur with T3 using multiplicities up to 56, in strong contrast to the situation with T2. In a series of mixed infections with h+ and h, increasing the h+ multiplicity, the multiplicity of h2 being constant, reduces the number of mixed bursts, suggesting by analogy with T2 that a limited number of phages can participate in reproduction. A five-minute interval in broth between h+ infection and h infection gives the first infecting phage an advantage reflected in a lower number of mixed yielders. However, the strong exclusion of the superinfecting phage occurring with T2 under similar conditions is not observed here.

The genetic work was begun by a study of the phage progeny from a single bacterium resulting from a mixed infection with h+ and an h mutant (h2} selected at random from the h+ stock. Previous work had shown the occurrence in the progeny of a new type, h3, characterized by a high rate of mutation to a new type, h1, and different from the parent h2. At least two other distinct new types have now been identified in the same progeny, also showing a high, but different, mutation rate to a phenotype resembling h1. Study of the progeny is being continued, and work on the nature and relations of the various types is in progress.

Radioactive Sulphur Tracer Studies on the Reproduction of T4 Bacteriophage

Investigators: Maaløe, Symonds
Support: Rockefeller Foundation Fellowships
National Foundation for Infantile Paralysis

When a bacterium lyses after infection by a phage it gives rise to a burst of bacteriophages which can be most easily detected...
by their ability to further infect bacteria. Along with these finished phages there may be some noninfective particles which are precursors to the finished phage but have not had time to mature. Previous attempts to identify such immature particles in lysates of the phage T4 have been made by labeling the desoxyribose nucleic acid (DNA) of the phage with phosphorus isotope ($^{32}$P), but they gave no evidence for any such particles.

In the present experiments the work has been carried further by labeling the protein of the phage with sulphur isotope ($^{35}$S). The results do indicate that sulphur-containing, but noninfective particles are present in T4 lysates. These particles adsorb on bacteria and precipitate with anti-T4 serum in a similar way to finished phage. They are, however, somewhat lighter, and have no appreciable killing effect on bacteria.

These results are interesting in the light of other recent work. Hershey and Chase (1952) have shown that on adsorption of a phage particle on a bacterium the outer protein membrane of the phage remains intact on the cell surface and only the DNA enters the bacterium. The empty protein membrane can be shaken off the bacterium and proves to be a stable structure much like the so-called "ghosts" which can be produced by subjecting phages to a sudden decrease in osmotic pressure (Anderson 1949). This fact that the phage protein constitutes a stable particle, even when separated from the DNA, suggests that the reason why we have been able to isolate noninfective particles containing sulphur, while none were found containing phosphorus, is that the immature phage particles which are precursors to the finished phage are unstable outside the host cell, and only the protein part survives lysis in a recognizable form.

**Genetics of Bacteriophage**

**Investigator:** Doermann

**Support:** General Funds

A three-weeks' visit at the Institute was spent in conferring with Delbrück and Visconti on problems of mutual interest in the field of bacteriophage genetics. In particular, discussions centered around the conformity of a number of results obtained at the Oak Ridge National Laboratory with the theory of phage genetics recently formulated by Visconti and Delbrück. Future tests of this theory were planned. A second result of the visit was the preparation of a manuscript which is concerned with genetic recombination and linkage in bacteriophage T4. The major conclusion presented in the manuscript is that the genetic loci of bacteriophage are distributed in linear sequence.
Host-Controlled Variation in Bacterial Viruses

Investigator: Weigle
Support: National Foundation for Infantile Paralysis

In a study of the lysogenic properties of the E. coli strain Kl2 a new indicator, C, for the carried phage lambda was discovered. Lambda can infect and multiply in cells of C and Kl2S with equal efficiency. When lambda is grown on cells of strain C, the resulting progeny have a new property: they no longer lyse cells of strain Kl2S except when they encounter a rare Kl2S cell in the right physiological condition. The progeny of the phage multiplying in this rare cell are transformed again: they have regained their original property of infecting Kl2S and C with equal efficiency.

The phage modified by multiplication in strain C is called lambda C. Lambda and lambda C have the same serological sensitivity, the same UV resistance, the same heat resistance. They differ only in that lambda gives plaques on C and Kl2S while lambda C gives plaques only on C (one phage in 10^4 gives a plaque on Kl2S).

A detailed analysis has shown that we are dealing with non-heritable alterations stemming directly from passage through a new host rather than with mutations. Passage through new hosts or new tissues is a widely used method for altering the properties of viruses. In some instances, selection of spontaneous mutants has been demonstrated to be the mechanism causing variation. Nonhereditary mechanisms have sometimes been postulated but since no such case had been sufficiently analyzed it was often assumed that selection of mutants is the only possible mechanism for producing virus variants. The case of lambda and lambda C definitely shows the occurrence of nongenetic host-controlled variation in bacterial viruses.

Nongenetic Nature of a Virus Variation

Investigator: Appleyard
Support: Rockefeller Foundation Fellowship

Cells of E. coli strain C can be made lysogenic by exposure to the phage lambda C. When treated with ultraviolet light, the lysogenic cells can be induced to lyse and yield lambda C. As in the case of lytic multiplication of lambda or lambda C, a few phage particles (about 2 in 10^4) are able to form plaques on Kl2S. To determine if these plaques are formed by rare phage mutants, a critical examination was made of the phage yields from small groups of induced lysogenic bacteria to determine whether these rare phage particles appear in clones. Phage particles able to form plaques on
K12S are distributed according to the Poisson distribution in bacterial bursts, and are therefore not mutants arising at random during the multiplication of the phage. The ability of a small fraction of the lambda C to form plaques on K12S appears to be due to rare physiological events distributed at random among the phage and/or the K12S cells.

The efficiency with which lambda C forms plaques on K12S is unaltered when the phage is produced by induced lysis of strains of C selected for resistance to the lytic phage T1. It can be greatly increased, however, by exposure of K12S cells to a small dose of ultraviolet light before infection.

The Production of Lysogenic Strains of Bacteria

Investigator: Lieb
Support: United States Public Health Service Fellowship

When a population of sensitive bacteria is infected with a temperate bacteriophage, only some of the bacteria will lyse. Among the surviving cells two types are found: (a) sensitive cells, indistinguishable from the original cells, and (b) lysogenic cells, which carry the phage in a latent noninfective form and transmit the lysogenic trait to all their offspring. Cells which have adsorbed phage but have not been lysed by it can be isolated immediately after infection and their progeny studied after six to eight generations.

The study of clones derived from single cells of E. coli strain K12S infected with the temperate phage "lambda" revealed that clones which contain lysogenic cells do not in addition contain cells which lyse. The formation of carried phage, resulting in lysogenicity, and the multiplication of phage leading to lysis are mutually exclusive processes in an infected clone. The decision as to which process is to prevail is made by the bacterium either before or immediately after infection. However, carried phage is not immediately established permanently in cells which have decided in favor of lysogenesis. During the first hour after infection, the establishment of lambda can be suppressed in some or all of the cells by growing the clones at temperatures above 20° C. Such temperature treatments have no effect on cells of established lysogenic cultures; there is, in fact, no method known for systematically transforming lysogenic K12 strains into sensitive strains. These findings suggest that the infecting phage which does not lead to lysis acts as a cytoplasmic particle immediately after infection, and later becomes attached to a permanent cell constituent, so that lysogenicity becomes an hereditary trait.
Inactivation of Bacteriophage by Specific Antisera

Investigator: Kaiser
Support: National Foundation for Infantile Paralysis
Predoctoral Fellowship

Bacteriophages treated with specific antisera are prevented from growing in sensitive bacteria. The first step in phage growth is their adsorption to bacteria. In order to determine whether antisera inactivated phages adsorbed normally, T2 bacteriophages labeled with $^{32}$P were used. Labeled phage was mixed with antisera; aliquots were removed at appropriate times to get phage populations which still retained one to 90% of their initial infectivity. The aliquots were mixed with sensitive bacteria and the amount of $^{32}$P adsorbed to the bacteria in 10 minutes was measured. It was found that a variable fraction of phages which could not infect bacteria owing to the antiserum was still able to adsorb. This fraction was measurable only in phage populations which were still more than 15% infective.

Cofactor Activation of Bacterial Viruses; Observation of Infected Bacteria

Investigator: McKee
Support: Atomic Energy Commission Predoctoral Fellowship
General Funds

With the use of dark-field microscope, the adsorption of a cofactor requiring a strain of the T4 bacteriophage to glass surfaces was investigated. It has been found that cofactors other than those active in promoting adsorption to bacteria are active in promoting adsorption to glass. The most active of these is DL-methionine. This indicates that the charge configuration allowing adsorption to glass is less specific than that promoting adsorption to bacteria. The ionic conditions under which the adsorption of the phage T2 takes place have been established in a semi-quantitative way by using the same technique.

The dark-field microscope was also employed to observe infected bacteria. Bacteria in this condition have characteristic spots on their surface. If the infection is carried out in buffer or if the phage particles are inactivated with UV light, these areas remain small and constant in number per cell, but if the infection is carried out in broth the spots increase in size and number.
Genetics of Two Serologically Related Coliphages

Investigator: Mandell
Support: National Foundation for Infantile Paralysis
Predoctoral Fellowship

A study of the genetics of T3 and T7 coliphages has been begun. It has been found that these phages are compatible, and when the host is infected with both phages some of the bacteria appear to liberate both kinds of input phage. In addition there are liberated other phages which seem to have an altered serological reaction, but which otherwise retain the reaction of the parents on indicator strains of the host. Further study of these aberrant progenies is in progress.

Production of Virus Plaques on Monolayer Tissue Cultures

Investigator: R. Dulbecco
Support: James Boswell Fund for Virus Research

Plaque formation by single virus particles is the basis for the analytical techniques used in the study of bacterial viruses. This formation has now been obtained with an animal virus, the virus of Western equine encephalomyelitis growing on chicken fibroblasts. This phenomenon may serve for the development of an animal virus assay much superior to any other assay now in use and for the isolation of the progeny of single virus particles.

Virus plaques are produced on a continuous fibroblastic layer grown on the bottom of a glass flask of the size of a Petri dish. A continuous cell layer is first grown in two days, following a modification of a technique devised by W. R. Earle, and then the virus is applied on it. Easily detectable round necrotic areas (plaques) are produced within two or three days of subsequent incubation.

The number of plaques produced by two different dilutions of the same virus sample is proportional to the concentration of the virus; hence a plaque is produced by a single virus particle. A comparison between the number of plaques produced and the fraction of chicken embryos infected by the same virus sample indicates that all or nearly all virus particles able to infect the embryo produce a plaque. There is therefore a 1:1 relation between infecting particles and plaques.

Plaques produced by the virus of the Western equine encephalomyelitis are different from plaques produced by one other virus tested, the Newcastle disease virus. This fact facilitates the study of the interaction between viruses.
THE STAFF

The Division of Biology Staff for the year 1951-1952 was made up as follows:

G. W. Beadle, Chairman of the Division

Professors

Ernest G. Anderson, Ph.D.  A. J. Haagen-Smit, Ph.D.
James F. Bonner, Ph.D.  Albert Tyler, Ph.D.
H. Borsook, Ph.D., M.D.  A. Van Harreveld, Ph.D., M.D.
Max Delbrück, Ph.D.  Frits W. Went, Ph.D.
Sterling Emerson*, Ph.D.  C. A. G. Wiersma, Ph.D.

*On leave of absence

Visiting Professors

David Bishop, Ph.D.  University of Massachusetts
Hans Borei, Ph.D.  Wenner-Grens Institute, Stockholm
Hans Wanner, Ph.D.  University of Zürich

Research Associates

Gordon A. Alles, Ph.D.  Lilian V. Morgan, M.A.
Charles E. Bradley, D.Sc.  Esther B. Tietz, M.D., Ph.D.
Henry O. Eversole, M.D.  Jean J. Weigle, Ph.D.
Albert E. Longley, Ph.D.

Associate Professors

A. W. Galston, Ph.D.  G. E. MacGinitie, M.A.
N. H. Horowitz, Ph.D.  H. K. Mitchell, Ph.D.
E. B. Lewis, Ph.D.  R. D. Owen, Ph.D.

Assistant Professor

Demorest Davenport*, Ph.D.

*University of California, Santa Barbara College
Senior Research Fellows

Bernard Axelrod, Ph.D.
Clara L. Deasy, Ph.D.
Jacob W. Dubnoff, Ph.D.
Renato Dulbecco, M.D.

Geoffrey L. Keighley, Ph.D.
George G. Laties, Ph.D.
Edward Novitski, Ph.D.
Howard J. Teas, Ph.D.

Gosney Fellows

Thomas C. Nelson, Ph.D.
Alfred Tissieres, M.D., Ph.D.

Marguerite M. Vogt, M.D.
Katerina I. Zarudnaya, Ph.D.

Research Fellows

Adolph Abrams, Ph.D.
Raymond K. Appleyard1, Ph.D.
William G. Ashby2, Ph.D.
Robert S. Bandurski, Ph.D.
Giuseppe Bertani3, Ph.D.
Jean M. Campbell, Ph.D.
Guy Ch. Camus, Ph.D.
E. E. Dale, Ph.D.
Wifredo de Rafols4, Ph.D.
August H. Doermann5, Ph.D.
Marguerite Fling, Ph.D.
Ernst Florey, Ph.D.
Hugh S. Forrest6, Ph.D.
Akio Fujiwara7, Nogaku Hakushi
Helen R. Garman, Ph.D.
Harold Garner8, Ph.D.
Edwin A. Goldsmith, Ph.D.
Morris Goodman9, Ph.D.
Francis A. Haskins, Ph.D.
Henry Hellmers10, Ph.D.
Joseph Holden11, Ph.D.
Herbert M. Hull, Ph.D.
Otto E. Landman12, Ph.D.

Anton Lang9, Ph.D.
Margaret Lieb6, Ph.D.
Jan Links10, Ph.D.
Peter H. Lowy, Doctorandum
Ole U. Maaløe1, Ph.D., M.D.
Jean Mauron, Ph.D.
Mary B. Mitchell, M.A.
ThadH. Pittenger11, Ph.D.
Donald Robertson, Ph.D.
Findlay Russell, M.D.
Pierre Schaeffer1, Ph.D.
Richard Schweet6, Ph.D.
Alberto Soriano1, 12, Ph.D.
John S. Stamm, Ph.D.
A. G. R. Strickland, Ph.D.
Neville D. Symonds1, Ph.D.
Albert Ulrich13, Ph.D.
Kay Verkerk, M.A.
Niccolo Visconti14, M.D.
Robert Weinstein15, Ph.D.
Emanuel Windsor, Ph.D.
Noboru Yamada16, Nogakushi

1 Rockefeller Foundation Fellow
2 United States Forest Service
3 University of Indiana
4 Del Amo Foundation Fellow
5 Oak Ridge National Laboratory
6 United States Public Health Service Fellow
7 Tohoku University
8 American Cancer Society Fellow
9 Lalor Foundation Fellow

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Fulbright Fellow
United States Atomic Energy Commission Fellow
John Simon Guggenheim Memorial Foundation Fellow
University of California
Carnegie Institution of Washington, Cold Spring Harbor
United States Chemical Corps
National Institute of Agricultural Research, Konosu, Japan

Graduate Fellows and Assistants

Bruce N. Ames¹, B.A.
Howard J. Boroughs², B.A.
George Bowen³, B.S.
Donald G. Crosby *, B.A.
George R. Dubes¹, B.S.
Luther Eggman, B.S.
George L. Ellman⁴, M.S.
Glenn Fischer, B.A.
Robert J. Foster *, B.S.
Henry Gershowitz, B.A.
Peter Goldacre⁵, B.Sc.
Norman Good, B.A.
Ronald C. Greene¹, B.S.
George K. Helmkamp *, B.A.
Claude W. Hinton¹, M.A.
David S. Hogness⁶*, B.S.
Bruce W. Holloway⁴, B.Sc.
Lionel Jaffe¹, B.S.
Leonard L. Jansen, M.S.
Robert B. Jennings *, B.S.
Burke Judd, M.S.
Armin Dale Kaiser³, B.S.
Dan L. Lindsley¹, M.A.
James L. Liverman⁷, B.S.
Joseph D. Mandell³, M.S.
John W. McKeel¹, B.S.
Dougal Harold McRae, M.A.
Robert L. Metzenberg, B.A.
Earl B. Patterson⁸, B.Sc.
Irving Rappaport, B.Sc.
Jose Luis Reissig, B.S.
Sherman H. Ripley, M.S.
Roy Sachs, B.S.
Paul D. Saltman⁹, B.S.
Philip S. Thayer ¹⁰, M.A.
David R. Viglierchio, B.S.

*Chemistry Major

¹Atomic Energy Commission Fellow
²Frasch Foundation Fellow
³National Foundation for Infantile Paralysis Fellow
⁴Nutrition-McCallum Foundation Fellow
⁵Commonwealth Scientific and Industrial Research Organization Fellow
⁶National Institutes of Health Fellow
⁷Lucy Mason Clark Fellow
⁸Atomic Energy Commission operating through Office of Naval Research
⁹DuPont Fellow
¹⁰United States Public Health Service Fellow
Graduate Students

Robert Lester, B.S.                  Fred Wolfgram, A.B.
Bruce Rogers, M.S.

Special Graduate Students

Luis E. Gregory, M.Sc.             Geronimo Terres, M.A.
Gordon Sato, B.A.

Visiting Graduate Students

Harris G. Moore, M.S.             University of Southern California
Richard H. Nieman, M.S.          University of Southern California
Carl Price, B.S.                  Harvard University

Custodians

W. A. Bowness                   Paul Pierreson

Gardeners

Clarence Edward Lippa          Edward C. Taylor
Albert Miotto

Laboratory Assistants

Stuart Bowen                     Dan Pletsch
Sherman J. Carlquist            Morris Robkin
Barbara DuBridge                 Elmer O. Slater
Gerald Dudek                     Mae C. Swanson
William H. Estabrook            James Trew
Jack T. Gordon                   Jerrold Turner
Olga Gunn                        James S. Tyler
Jan Haagen-Smit                 Annette Van Dell
Lydia Lang                       Anthonie Van Harreveld, Jr.
Jack A. Livesey                  Cora Wells
Fumio Nakahiro                 Anneke Went
Sudie B. Newman                  Meredith Wills
H. Gunther Perdigao
Laboratory Technicians

Jean Marie Belmont
Bland Ewing
Mike Gomez
William Hudson
Thomas M. Jones

Eleanor Kerr
Arnold A. Leeping
Audrey Phillips
Elfriede Scheurenberg

Librarian

Jirina Maria Evans

Microanalyst

Glenn Swinehart

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Andree Crehan, Secretary
G. Fling, Administrative Asst.
Barbara Gaddy, Secretary
Christian H. Greene, Secretary
Elizabeth Land, Secretary

Inger Larsen, Secretary
Jeanne L. McCune, Secretary
Elizabeth Morse, Secretary
Hilda Rook, Chief Clerk
Donna P. Thorpe, Secretary
Mary Walp, Secretary

Research Assistants

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Rosamond Scott Baker, B.A.
Elizabetht Ann Bartron, B.A.
Ruth Bates, A.B.
Joan Davalle Beyers, B.A.
Elizabeth Bleecker, M.D.
Ruth Ann Bobrov, Ph.D.
Barbara Bostwick, B.A.
Ruth G. Corbett, B.A.
Josephine Dulbecco
Mary E. Durrell, M.S.
Phyllis Ellman, M.S.
Ruth E. Estey
Helen R. Fahy
Margaret M. Fox, B.S.
Dorothy Fraser, M.A.
William Gencarella, B.A.
Emma Jane Griffith, B.S.
Amelia A. Gunderson, M.S.

Dorothy Haskins, B.S.
Burgess W. Heacox, M.A.
Frank Hirosawa, M.Sc.
Mary R. Hull, B.A.
Helen Jeffrey, R.N.
Marcella O. Juhren, M.A.
Jacqueline C. Kahn, R.N.
Mary G. Keighley, B.A.
Anna Kilim, B.A.
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Claire Michelson, M.S.
Adele Millerd, M.Sc.
Rachel Morgan, B.A.
John J. Norris, B.A.
Kenneth Ohki, M.A.
Marilyn B. Rusler, A.B.
Helen E. Surber, M.S.
Donald E. Sutton
Selina Weinbaum, B.S.
Shops

Anthony Nunn, Maintenance Carpenter        W. P. Thomson, Mechanic
Frank Ostrander, Machinist

Stockroom

Anna Teas, Supervisor                      G. A. Zentmyer, Assistant
R. W. Gibbons, Assistant

Superintendents

George G. Chalmers                         Kerckhoff Marine Laboratory
John F. Dale                                Biology Experimental Farm
G. P. Keyes                                 Earhart Plant Research Laboratory
Charles M. Newman                          Orlando Greenhouse
W. S. Smith                                Kerckhoff Marine Laboratory

Superintendents, Assistant

James E. Cunningham                        Earhart Plant Research Laboratory
Robert G. Hoover                           Biology Experimental Farm

Technical Assistants

Lyle C. Bacon                              Richard G. Myers
Richard H. Fuller                          Karl J. Niedermann
James Gay Helmuth                          James A. Ross
Jack Kelleher                               Robert L. Spencer
William V. Keyes                           Edith M. Wallace
Henry C. Kirk                               Jess Williams
Ken Lunan                                   James H. Wilson
Alice R. McColloch
STAFF PROMOTIONS, AWARDS AND HONORS

Renato Dulbecco  
Promoted from Senior Research Fellow to Associate Professor

George Laties  
Promoted from Research Fellow to Senior Research Fellow

Howard J. Teas  
Promoted from Research Fellow to Senior Research Fellow

G. W. Beadle  
Honorary D.Sc. degree from Northwestern University  
Elected Member of the Royal Danish Academy of Sciences

Dan L. Lindsley  
Thomas Hunt Morgan Award for 1952

Guy Ch. Camus  
Louis Rapkine Foundation Award

Ray D. Owen  
Coauthor with Adrian Srb of General Genetics

James Bonner and A. W. Galston  
Authors of Principles of Plant Physiology

Albert Tyler  
Elected Foreign Member of the Societa Italiana per il Progresso della Zootecnica

A. J. Haagen-Smit  
Appointed Corresponding Member of the Royal Academy of Science of the Netherlands  
Elected Member of the Air Pollution and Smoke Prevention Association of America

Henry Hellmers  
Elected Chairman of the Southern California Section of the Society of American Foresters

VISITING LECTURERS

Seventeen visitors gave lectures or seminars in the Division of Biology during the year. In many instances their presence at the Institute was made possible by the Eversole Lecture Fund.

The speakers and their subjects were as follows:

Professor Fritz Zernike, University of Groningen, "Recent Advances in Phase Microscopy".

Professor Regnar Granit, Director, Karolinska Institute, University of Stockholm, "Self-regulation of Reflex Muscle Contraction".

Professor Hans Borei, Wenner Grens Institute, University of Stockholm, "Respiration of the Egg in Relation to Fertilization".
Professor H. Munro Fox, Bedford College for Women, University of London, "The Biological Significance of Respiratory Pigments".
Professor John L. Webb, University of Southern California, "The Action of Acetylcholine".
Captain Ashton Graybiel, M.C., U.S.N., U.S. Naval School of Aviation Medicine, Pensacola, "The Perception of Linear Acceleration and Its Influence on Vision".
Professor Kitty Ponse, University of Southern California, "Sex Determinations in Amphibians".
Dr. R. F. Kimball, Oak Ridge National Laboratories, Oak Ridge, Tennessee, "Modifications of the Genetic and Non-Genetic Effects of Radiation upon Paramecium aurelia".
Professor Charles M. Pomerat, University of Texas, "Tissue Cultures in Experimental Biology and Medicine".
Professor Benjamin W. Zweifach, Cornell University Medical School, "Basic Mechanisms Regulating Peripheral Circulation".
Professor Melvin M. Green, University of California, Davis, "Genetical and Biochemical Studies of Mutation".
Dr. Peter M. Nossal, The University, Sheffield, England, "The Tri-carboxylic Acid Cycle in Yeast".
Dr. G. Evelyn Hutchinson, Osborn Zoological Laboratory, Yale University, "Fresh Water Plankton".
Dr. H. M. de Jong, Neuropsychiatrist, Veterans Administration, "Experimental Catatonia, a General Reaction of the Central Nervous System".
Professor Daniel Mazia, University of California, Berkeley, "Physiology of the Cell Nucleus".
Professor Paul K. Stumpf, University of California, Berkeley, "Fat Metabolism in Higher Plants".
Professor Kenneth Smith, Cambridge University, "Recent Advances in Virus Research".

HIXON LECTURES

Four lectures were sponsored at the Institute by the Hixon Fund. On January 28 and 30, Professor Roger J. Williams, Director of the Biochemical Institute, University of Texas, spoke on "Individual Metabolic Patterns and Their Significance" and on January 30 on "Alcoholism as a Genetotrophic disease".

Two lectures were given by Dr. John C. Lilly, School of Medicine, University of Pennsylvania, on February 5 and 7. They were entitled: "Moving Figures in the Electrical Activity of the Brain" and "Correlation of Behavioral Subjective and Electrical Activity of the Brain".
The papers presented at the Hixon Fund Symposium on "Cerebral Mechanisms in Behavior" have been edited by Dr. P. E. Mindlin and will be published in the fall of this year, by the Stanford Press, under the title, Psychoanalysis as Science.

TEACHING

Following the revision of the Biology Option that went into effect in the fall of 1951, a few further changes have been made, some of which were made to take care of the time required by the Air Force ROTC program.

Professor Horowitz gave the lectures in Biology 1 and Professor Beadle again took charge of the laboratory instruction. Efforts are being constantly made to improve instruction in this course, which is the first course in biology and one required of all science option sophomores.

During the year Professor Galston taught his regularly assigned plant science course and in addition took over the course that would normally have been given by Professor Went. In both he did outstandingly well, judging by the enthusiastic comments of the students who took these courses.

Microbiology, taught by Professor Dulbecco, has become a regular part of the biology curriculum now that Professor Dulbecco is a member of the regular teaching staff. This course, too, has been received with enthusiasm on the part of the students.

GRADUATES

Thirteen men were awarded B.S. or Ph.D. degrees in Biology in June, 1952. Their names, degrees and plans for future work are as follows:

Sherman Butler, B.S., Air Weather Service, Air Corps
George L. Ellman, Ph.D., Research, Dow Chemical, Midland, Michigan
Norman Good, Ph.D., Postdoctoral work, University of Minnesota
J. B. Hatcher, Ph.D., Research, Jet Propulsion Laboratory
Gilbert Kitching, B.S., Graduate School, University of Southern California Medical School
Edwin B. Kurtz, Ph.D., Assistant Professor, University of Arizona
James L. Liverman, Ph.D., Postdoctoral work at C.I.T.
Dan L. Lindsley, Ph.D., National Research Council Postdoctoral Fellowship, Princeton University
Earl Patterson, Ph.D., Postdoctoral work at C.I.T.
Jose L. Reissig, Ph.D., Research, Instituto de Investigaciones, Fundacion Campomar, Buenos Aires
Donald Sutton, B.S., Graduate School, Department of Wild Life Management, University of Wisconsin
Philip S. Thayer, Ph.D., Merck Postdoctoral Fellow, University of California, Berkeley
Frederick Wolfgram, Ph.D., Postdoctoral work, Johns Hopkins University

Gosney Fellowships

The following held Gosney Fellowship appointments during the year 1951-1952:

Thomas C. Nelson, Ph.D., research on bacterial genetics.
Alfred Tissieres, M.D., Ph.D., research on chemical genetics.
Marguerite M. Vogt, M.D., research on bacterial virology.
Katerina I. Zarudnaya, Ph.D., research on chemical genetics.

McCallum Foundation - Nutrition Foundation Fellowships

George L. Ellman held the second McCallum Predoctoral Fellowship until January, 1952, when he finished his work for a Ph.D. degree in chemistry with Professor H. K. Mitchell.

The third McCallum Fellowship is held by Bruce Holloway, of the Waite Agricultural Research Institute, Adelaide, Australia, who is working for a Ph.D. degree in genetics.

McCallum Foundation Summer Scholarships

The fund established for a second year by Mr. and Mrs. Arthur McCallum has provided support for 15 graduate students during the summer of 1952. The usual award is $100 per month for the three-month period. These scholarships meet a long-felt need by making it possible for graduate students to spend summers working on their thesis and research programs.

Lucy Mason Clark Fellowship in Plant Physiology

This fellowship, established in 1949, was held during 1951 for a second year by James L. Liverman, who finished his Ph.D.
work under Professor Frits W. Went in June.

**DuPONT FELLOWSHIP**

Established in 1951, this predoctoral fellowship was held for the second year by Mr. Paul Saltman, who is working for his Ph.D. in plant biochemistry with the Bonner group.

**SEELEY W. MUDD SCHOLARSHIP**

For several years, Doctor Seeley G. Mudd has provided funds for a tuition scholarship for an undergraduate interested in some phase of medicine. During the past year this scholarship was held by a sophomore in the Biology option, Mr. James H. Wilson.

**OTHER FELLOWSHIP APPOINTMENTS**

In addition to the fellowship appointments listed above, there are a number of research workers who hold the title Senior Research Fellow or Research Fellow, whose stipends come from grants made to the Institute in support of an area of research rather than specifically for a fellowship.

During the year 1951-1952, 12 men held predoctoral fellowships awarded by an agency operating outside the Institute, usually on a national basis. These were distributed as follows:

- Atomic Energy Commission Fellowships: 7
- Commonwealth Scientific and Industrial Research Organization Fellowship: 1
- National Foundation for Infantile Paralysis Fellowships: 3
- United States Public Health Service Fellowship: 1

Seventeen persons held nationally awarded postdoctoral fellowships during this period. These were distributed as follows:

- American Cancer Society Fellow: 1
- Atomic Energy Commission Fellow: 1
- Del Amo Fellow: 1
- Fulbright Fellow: 1
- John Simon Guggenheim Memorial Foundation Fellow: 1
- Lalor Foundation Fellow: 1

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Additions to the Library this year totalled 375 books and 750 bound periodical volumes. An estimated 250 of the latter came from the binding of current issues, the balance from purchases of back volumes and sets. Twenty-eight volumes of the Archives de Biologie were acquired, completing our set except for volumes 1, 34 and 36. A 33-volume purchase completed our run of the Arkiv for Botanik. Biological Reviews was completed by the purchase of volume 1, number 1, a gap of long standing. Our run of the Comptes rendus of the Société de Biologie was extended by a 20-volume acquisition, although we still lack volumes 1 through 77 of this series. Seventy-five volumes of Wilhelm Roux' Archiv für Entwicklungsmechanik der Organismus were purchased through the Walter J. Johnson Co. of New York, and 32 volumes of the Roux' Vorträge und Aufsätze through Anna Marie Schnase, a Berlin dealer. Other sets acquired were the first 28 volumes of the Zeitschrift für menschliche Vererbungs- und Konstitutionlehre and 78 volumes of the Zentralblatt für Bakteriologie, Parasitenkunde und Infektionskrankheiten.

FINANCIAL SUPPORT

The total amount spent by the Division of Biology on instruction and research in the fiscal year 1951-1952 was in excess of $500,000. Approximately half of this was derived from Institute endowment income and half from special short-term grants or from government contracts. The funds used during the year came from the following sources:

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General Electric Company

Ibec Research Institute Fund

International Minerals and Chemical Corporation

Lasker Foundation Fund

Eli Lilly Fund

Loomis Institute for Scientific Research

Los Angeles County Air Pollution Control District

F. S. Markham Fund

Merck & Company Fund

McCallum Foundation

Malcolm McNaghten Fund

Seeley G. Mudd Fund

National Foundation for Infantile Paralysis

National Science Foundation

National Science Foundation

National Science Foundation

Nutrition Foundation

Light utilization by plants

Plant physiology

Creatine research

Fellowships, scholarships

Amino acids, peptides

Special biology expenditures

Smog constituents and smog plant damage

Electroencephalography

Chemically induced mutation, new growth factors

Scholarships

Scholarships

Premedical scholarship

Virus research

Animal physiology

Plant physiology

Virus research

Biochemical genetics of Neurospora
Nutrition Foundation-McCallum Foundation

Research Corporation - Williams-Waterman Fund for the Combat of Dietary Diseases

Riker Laboratories

Rockefeller Foundation Fund

Edward G. Robinson Fund

Leonard G. Strater Fund

Elbridge and Mary Stuart Foundation

University of California - Beet Sugar Fund

United States Atomic Energy Commission (Administered by Office of Naval Research, United States Navy)

United States Atomic Energy Commission (Administered by Office of Naval Research, United States Navy)

United States Chemical Corps

United States Department of Agriculture, Forest Service

United States Navy, Office of Naval Research

United States Public Health Service

United States Public Health Service

Predoctoral fellowships

Tryptophane-nicotinic acid metabolism

Veratrum growth and alkaloid formation

Joint chemistry-biology program

Electronarcosis

Plant physiology

Milk protein research

Sugar-beet research

Radiation genetics

Protein synthesis

Plant physiology

Plant physiology

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