BIOLOGY
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AT THE CALIFORNIA INSTITUTE
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A Report for the Year 1956-1957
on the Research and other Activities of the
Division of Biology
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INTRODUCTION

In living things, including man, genetic individuality is determined at the moment of fertilization of the egg. How is this process accomplished? What are the subtle changes in the surface of the egg that allow one sperm to enter but exclude the next? Answers to these questions are important to our understanding of the process of development and may also make it possible for man to limit his numbers in a manner less ruthless than the wars and famine that in the past have kept him from too far outstripping his food resources.

What is the nature of the genetic information that is brought together from the two parents at fertilization? Biologists are beginning to understand the structure of the molecules that are the primary genetic material in all living systems. These molecules are similar in viruses and man. They are enormous by one standard—millions of times larger than molecules of hydrogen—yet are below the limits of direct observation by the most powerful light microscope. They are marvelously built to carry information in the form of a special kind of submicroscopic code. They alone among all known molecules are able to serve as templates or models for the construction of exact replicas of themselves. This is the basis, at the molecular level, of all biological reproduction.

The hereditary material in a human cell consists of perhaps 10,000 to 100,000 kinds of genes. In the replication of these units of heredity errors are sometimes made. These are analogous to typographical errors in a message typed or printed in the 26 letters of our alphabet. The molecular material that is the gene has the property of replicating such errors. Most of them are deleterious but one in a million or so may be advantageous. Indeed it is such favorable errors, such mutations, that are believed to make evolution possible.

High energy radiation—ultraviolet radiation, X-rays, gamma rays, neutrons, electrons, cosmic rays, etc.—increases the probability of errors in the copying of genetic information. This is why geneticists worry about the hazards of such radiation. It is immaterial whether it comes from the fallout of nuclear weapons, from therapeutic or diagnostic X-rays, from power reactors, or from the natural background barrage of radioactivity from which none of us can completely escape. There is no level of radiation so low that it does not produce some increase in mutation rate. How great may be the long-time hazard to a human population from low levels of radiation such as we are presently exposed to as a result of medical radiation or fallout, no one knows for sure. With the inevitable increase in peacetime uses of nuclear energy—and regardless of whether nuclear weapons are used in war—it is urgent that this question be answered.

Are gene mutations in body cells responsible for some or all malignancies? The answer is not known. The question is important, for if gene mutation is indeed responsible, one might well expect a direct linear relation between exposure of the cells of an individual to ionizing radiation and the chance of developing a malignancy such as leukemia. A linear relation at all levels of exposure would mean that there is a real hazard even at levels as low as those of background. Present radioactive fallout from testing of nuclear weapons is perhaps only one-tenth of background, but if the relation is linear at all levels, this would increase the incidence of malignancy by a small but real amount. On the other hand, if there is a threshold below which no effect is produced and if that threshold level is higher than background plus medical radiation plus fallout, there may be little to worry about in this regard. Pres-
ently available data for man are insufficient to answer the question of linearity at all levels. The data are consistent with a linear relationship but they are also consistent with the hypothesis that there is a threshold at low levels.

How is the information in genes used in the many processes of development differentiation and functioning that are essential to the complete organism? Somehow the information must be transferred to such large molecules as proteins, which then serve as structural elements and as the catalysts known as enzymes. How are proteins—for example, the hemoglobin of red blood cells—synthesized? What is the relation between gene structure and the configuration of the protein molecule? Through studies on hemoglobin synthesis and structure, investigations of enzymes and in other ways the information necessary to answer such questions is being slowly but surely accumulated.

Viruses are among the smallest and simplest living systems. Some viruses appear to be little more than submicroscopic units of genetic material wrapped up in a highly specific protein coat. The multiplication of a virus is in all cases dependent on a living host cell, which must be of a specific kind for each virus. The host may be a bacterium, a higher plant, or an animal like man. It is now known for a number of viruses that only the core of genetic material needs enter the host cell. Once inside the material takes over control of the metabolic machinery of the host cell and directs it to produce more virus genetic material and to fabricate protein of the virus type. This process yields a new generation of virus particles.

Viruses once produced may remain latent within the host cell. The virus genetic material then divides synchronously with that of the host cell. In fact the genetic material of the virus may combine with that of the host. If conditions are modified—and this is often subject to experimental control—the replication of virus material can be speeded up so that it exceeds that of the host cell. The host cell is then killed and complete virus particles are released.

In the presence of virus genetic material in a latent form, host cells may be modified in appearance and behavior. In some instances modification is such that the cell becomes malignant. A classical example is the Rous sarcoma of the chicken. It is currently believed by some investigators that viruses may be causally involved in the production of the cancerous state in many more instances than are now recognized. It is obviously of great importance to medicine to know if these views are right.

It has long been known to surgeons and to experimental biologists that tissue transplants, although routinely successful when transferred from one part to another of the same individual, do not often persist when made between two individuals of the same species, even though the donor and recipient are closely related. Skin transplants in man behave in this way. The basis of the difference in the two cases is genetic. Transplants between identical twins that are alike genetically are as successful as are those involving only a single individual. The failure of transplants in cases in which donor and recipient are genetically different is due to the development of immunity by the recipient. This immunity is directed against specific cellular antigens present in the donor and absent in the recipient, and results in rejection of the transplant.

Within the past several years, methods of desensitizing experimental animals to foreign transplants have been discovered. As a specific example, the injection of spleen cells from an adult mouse into a newborn mouse of different genetic strain induces tolerance to later skin transplants from the donor strain. It is important to understand the mechanism underlying this phenomenon, not only because of its general biological importance but also because of its possible potential medical application. Such applications include that in skin transplantation and even more importantly that in therapeutic bone marrow transplantation following exposure to levels of radiation that would otherwise be lethal or highly injurious.
How does a cell perceive and react to specific stimuli? Bacteria, algae and protozoa react to light. Plant roots grow down and their stems grow up. The retina of the human eye responds to a few light quanta and the stimulus is transmitted to the central nervous system. The answers are being sought through many approaches. One of these is through studies of the growth responses to light and gravity of the spore-bearing stalk of a mold, Phycomyces. This lowly plant is an especially favorable material for investigating the physical and chemical principles that are involved.

One of the many ways in which man is distinguished from other organisms is in the extraordinary development of his nervous system. This has enabled him to supplement biological inheritance with a highly developed cultural inheritance that includes language, religion, art, music, technology and science. How is the nervous system constructed? How does it function? These are questions of tremendous importance both to biology in general and to the future of the culture that is unique to man. Answers are being sought at levels ranging all the way from individual molecules to the whole organism.

The insistent curiosity of man is a powerful motivating force in the development of science. So are his physical needs. Plants are of interest to science merely because they exist. But they are of interest to man also because they feed him and other animals on earth. How do plants grow? Why do they flower? What are their relations to the environments in which they grow? Can they be made more efficient in producing food for the ever increasing numbers of people that inhabit the earth? It has long been clear that the acquisition of the knowledge needed to answer these and related questions could be enormously accelerated. One of the ways in which this can be done is to investigate the behavior of higher plants under fully controlled environmental conditions in laboratories such as the Earhart Plant Research Laboratory of the Institute. There are now many examples that demonstrate how science and agriculture can profit by more rigorously controlling the many factors that influence the outcome of experiments with higher plants.

The research carried out by members of the Division of Biology staff in attempts to answer questions of the kind posed in the foregoing paragraphs is summarized in the following section of this report. The 178 separate projects are somewhat arbitrarily classified under the headings animal physiology, biochemistry, biophysics, embryology, genetics (including immunogenetics), marine zoology, plant biochemistry and physiology, plant ecology and physiology, psychobiology, and virology.

A significant part of the work of the Division is concerned with the training of the next generation of biologists. This involves undergraduate teaching, direction of graduate study and collaboration with postdoctoral Research Fellows.

The research, teaching and other activities of the Division were taken care of by a total staff of 254. Of these, 97 held faculty appointments. There were 12 undergraduate students in the Biology Option and 25 graduate students registered for Ph.D. degrees in the Division.

A major addition to the faculty of the Division was arranged for in the appointment of Robert L. Sinsheimer of Iowa State College as Professor of Biophysics. Laboratories on the first floor of Kerckhoff have been remodeled specifically for his work on nucleic acids. His activities will complement in an important way the studies of several other groups in the Divisions of Biology and Chemistry.

From the beginning of the Biology Division at the Institute, plans have provided for an additional laboratory wing extending north from Kerckhoff. With the completion of the Norman W. Church Laboratory for Chemical Biology, the proposed connecting wing is the only section needed to complete the Biology unit as originally conceived. A U. S. Public Health Service construction grant made to the Institute
for this addition greatly improves the prospects of its becoming a reality. The Public Health Service grant is contingent on matching funds being obtained. Approximately $500,000 is needed for this purpose.

With the relinquishing of temporary quarters for virus research, borrowed from the Huntington Memorial Hospital Institute for Medical Research, and the consolidation of all virus workers in the Church Laboratory, the basement and sub-basement of the Biology section of this building are now fully occupied. Space in Kerckhoff made available through the moving to Church Laboratory of virologists and Division Offices is being used for the new biophysics laboratories and for the general relief of groups formerly overcrowded. Proposed future activities of the Division in biochemistry, virology, biophysics, physiology and psychobiology will require that the connecting wing be completed as soon as matching funds can be obtained, plans made and construction carried out.

Another long anticipated need of the Division is a consolidated center for research on plants to be located adjacent to the Dolk-Clark-Earhart laboratories. The last installment of the Arcadia experimental farm, sold to the Temple City School District in 1954, will be relinquished by June 30, 1959. To partially replace the farm and the obsolete and inconvenient plant-growing facilities on Orlando Drive, a new greenhouse and an outdoor garden space are needed. The urgency of this development is intensified by the need for a smog-free greenhouse space in which to grow plants for Professor Sinsheimer's work on plant viruses.
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for the year 1956-1957

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Much of the research work summarized in this section has not yet been reported in print, in many instances because it is not yet complete. For that reason this report is not intended as a publication and should not be cited as such. Individual projects should be referred to only if specific permission to do so is obtained from the investigator responsible for the material. References are made here to published papers bearing on the projects reported, but a complete list of publications by members of the Division is not given here. Such lists are published in the Institute's bulletin Publications of the Staff, copies of which may be obtained upon request.
1. CHANGES IN THE VOLUME OF CORTICAL NEURONAL ELEMENTS DURING ASPHYXIATION

Investigator: A. Van Harreveld
Support: National Institutes of Health, Public Health Service
General Funds

About three minutes after circulatory arrest a surface negativity of the cerebral cortex develops, accompanied by an increase in the electrical resistance of the cortex. About one-third of the cortical conductivity may be lost in a few minutes. It was concluded previously that this loss is due to a decrease of intercellular ions, which are responsible for most of the conductivity of a tissue. These intercellular ions are believed to be transported into the cortical cells and fibers, accompanied by water to maintain osmotic equilibrium. It has been attempted to demonstrate the predicted swelling of cortical elements. The distribution of water in the tissue was preserved as well as possible by a process of quick freezing and alcohol fixation at about -20°C. Preparations were made of cortices frozen before and after the resistance change had taken place. The diameters of nerve cells and of apical dendrites were considerably larger in preparations of cortices after the resistance change. The mean increase in the volume of the nerve cells was 40%, that of the dendrites about 70%. The transport of ions from extra- into intracellular spaces as indicated by these volume increases is ample to account for the resistance change observed after cortical asphyxiation.

2. CHANGES IN THE VOLUME OF CORTICAL NEURONAL ELEMENTS DURING SPREADING DEPRESSION

Investigator: A. Van Harreveld
Support: National Institutes of Health, Public Health Service
General Funds

Spreading depression, which is propagated slowly over the cortex, is characterized by a deep depression of all functions of the cerebral cortex. This depression is accompanied by the development of a surface negativity and by a marked increase in cortical resistance. These phenomena resemble the potential and resistance changes observed after cortical asphyxiation, suggesting that also during spreading depression a movement of ions and water from the extra- into the intracellular compartment takes place.

The cerebral cortex was quickly frozen when the resistance change accompanying spreading depression had reached a maximum at a predetermined point. The cortex was then fixed as described in the preceding section. The diameters of apical dendrites were measured at the region of maximal cortical resistance. They were compared with the diameters of apical dendrites at a corresponding place on the heterolateral cortex which acted as a control. The diameters of the apical dendrites on the experimental side were consistently greater than those on the control side, indicating that a similar mechanism causes the cortical resistance increase during spreading depression and after cortical asphyxiation.
3. SPONTANEOUS REINNERVATION OF THE SMALL LARYNX MUSCLES

Investigator: A. Van Harreveld
Support: Harlan Shoemaker Fund
          General Funds

Around 1880 it was observed that removal of one of the two nerves innervating one of the small larynx muscles (m. cricothyroideus) in the rabbit does not result in a noticeable muscle degeneration. Exner proposed two explanations: 1) that the majority of muscle fibers of this muscle would be innervated by both motor nerves supplying the muscle, and 2) that nerve fibers of the intact motor nerve would form sprouts supplying the muscle fibers which had lost their innervation by the removal of the other motor nerve. Although Exner considered the last explanation the less likely one, it was shown in the last decades that such a reinnervation does occur in the rabbit and can result in a fargoing functional repair of muscles which had lost a considerable part of their innervation.

In the present investigation one of the motor nerves of the cricothyroideus muscle was removed and two months later the force of contraction on stimulation of the remaining motor nerve was compared with the force of contraction of the symmetrical control muscle on stimulation of the symmetrical motor nerve. Stimulation on the operated side caused a considerably stronger contraction, showing that this nerve had adopted additional muscle material. In histological preparations the sprouting of nerve fibers was observed. These new fibers formed endings on muscle fibers which were much simpler than the endings observed on the muscle fibers in normal control muscles.

4. SPONTANEOUS REINNERVATION OF PARETIC MONKEY MUSCLE

Investigators: A. Van Harreveld, Ruth E. Estey
Support: Harlan Shoemaker Fund
          General Funds

Edds claimed that the spontaneous reinnervation of muscle, discussed above, is not a prominent feature in monkey muscle which has lost part of its nerve supply. This was based on a comparison of the nerve fibers in end branches of the motor nerve and the number of endplates formed by such a branch. It is tacitly assumed that nerve sprouting only takes place in the end ramification of the motor nerve. In the present investigation a search was made for newly formed nerve fibers in the large intramuscular branch of the motor nerve. Although greatly contributing to the reinnervation of the muscle such a form of reinnervation would not be indicated by an increase in the ratio of endings and nerve fibers in a terminal branch.

In monkeys the sartorius muscle was partly denervated and 14 days later the muscle was removed and stained with a silver method which makes it possible to distinguish newly formed nerve fibers from old ones (Cajal). In these preparations numerous new nerve fibers ending in simple endings on the muscle fibers were found in the terminal branches. Also newly formed nerve fibers were observed in the intramuscular part of the motor nerve. These findings suggest that the reinnervation in monkey muscle may be more pronounced than is indicated by the work of Edds.

Publications:


5. **MEMBRANE POTENTIAL CHANGES DURING EXCITATION OF THE SLOW NERVE FIBER OF CRUSTACEANS**

Investigators: Graham Hoyle, C. A. G. Wiersma
Support: Rockefeller Foundation
National Science Foundation
General Funds

The single nerve fibers causing slow muscular contractions in crustacean muscles have been isolated by micro-dissection and stimulated with the aid of micromanipulated electrodes. The responses of individual muscle fibers have been examined with the aid of internal capillary micro-electrodes. The membrane potential changes were recorded after direct-coupled amplification with a cathode ray oscillograph. Muscles examined were the closers, benders, and extensors. Species used included Panulirus interruptus, Cambarus clarkii, Cancer antennarius, C. anthonyi, Pachygrapsus crassipes.

The slow nerve fiber gives rise to very small junctional potentials, when stimulated at low frequencies in most of the muscle fibers examined. At higher frequencies of stimulation the potentials are larger, having increased in size by a process of facilitation. In some of the muscles, e.g. the extensor of Panulirus, every muscle fiber shows a response and the responses are fairly uniform in appearances. In others, e.g. the closer of Pachygrapsus, many muscle fibers show no noticeable response on low frequency stimulation and a number none even on high frequency. Also in this muscle, a few of the fibers respond to single slow fiber impulses with quite large responses. In most of the closer muscles there is a very considerable variation in the sizes of the responses of different fibers to the same frequency of stimulation.

In some of the muscles the decay of the junctional potentials can be attributed solely to the passive electrotonic spread effect, i.e. transmitter action is brief. In others, e.g. in the bender of Cancer, the decay is much slower than can be accounted for by electronic decay. In these cases there must either be a lingering of the transmitter substance or else the transmitter must set up a relatively long lasting reaction in the membrane.

A few of the larger responses in Palinuris closer have been observed to lead to the development of spike responses from the muscle fibers during stimulation at frequencies above 60 per second.

General characteristics of the slow responses include: a) great degree of facilitation, b) slow development of fatigue; c) relatively large over-all membrane depolarization at low frequencies.

6. **MEMBRANE POTENTIAL CHANGES DURING EXCITATION OF THE FAST NERVE FIBERS OF CRUSTACEANS**

Investigators: Graham Hoyle, C. A. G. Wiersma
Support: Rockefeller Foundation
National Science Foundation
General Funds
Single fast nerve fibers supplying closer muscles of Panulirus interruptus Cambarus clarkii, Cancer antennarius, C. anthonyi, Pachygrapsus crassipes and others have been stimulated and the electrical responses of single muscle fibers studied with the aid of internal electrodes. The benders of Panulirus and Cancer antennarius and the extensor of Panulirus have also been examined.

The fast nerve fiber gives rise to small junctional potentials in most of the muscle fibers examined, when stimulated at low frequencies. Only in the closer muscles of Pachygrapsus and Cambarus have large junctional potentials been observed. In these muscles, the large potentials were found in only a small proportion of the muscle fibers. Of these, many gave rise to subsequent large spikes even when excited with single shocks. The spikes usually just fail to reach the zero baseline.

The smaller fast-fiber responses show a considerable degree of facilitation, up to four or five times the original size. This facilitation occurs quickly. But the responses soon start to decline during continued stimulation.

In most of the muscles examined the magnitude of the responses at their maximum varied greatly between the muscle fibers.

7. THE BIOPHYSICS OF INHIBITORY ACTION IN CRUSTACEAN MUSCLE

Investigators: Graham Hoyle, C. A. G. Wiersma
Support: Rockefeller Foundation
National Science Foundation
General Funds

Inhibitory nerve fibers in the crustaceans have been known since the last century. Although Biedermann found that inhibitory nerve-fiber activity could be associated with positive potential changes, all recent investigations failed to confirm this, even ones utilizing the internal electrode technique. In the present investigations, the inhibitory nerve fibers of the openers, stretchers, benders, and closers of several species have been isolated and stimulated whilst recording electrical changes in single muscle fibers with the aid of internal capillary micro-electrodes.

In some muscle fibers of all the muscles examined potential changes associated with inhibitory action have been observed. In Panulirus these are always positive (polarizing) whatever the normal membrane resting potential. They are, however, reversed in sign when the membrane potential is artificially increased by passing a polarizing current across the membrane with the aid of a second internal electrode.

In Cancer and Cambarus both polarizing and depolarizing potentials were observed. These are evidently related to the resting potential magnitude and can be reversed in sign by polarizing or depolarizing past the critical exchange potential level.

This means that in these muscle fibers the resting potential can be higher than the equilibrium potential for the ion whose permeability is increased by the inhibitory transmitter substance.

8. THE COUPLING BETWEEN MEMBRANE POTENTIAL CHANGE AND MECHANICAL CONTRACTION IN CRUSTACEAN MUSCLE

Investigators: Graham Hoyle, C. A. G. Wiersma
Support: Rockefeller Foundation
National Science Foundation
General Funds
The generally accepted hypothesis of the coupling between surface membrane potential and shortening in muscle is that a lowering of the membrane potential is the agent which initiates the process.

This hypothesis has been tested in several crustacean preparations by isolating and subsequently stimulating single excitatory and inhibitory nerve fibers whilst recording both the membrane potential changes of individual muscle fibers and the tension developed by the whole muscle.

The tension in many instances can be correlated with depolarization. Contraction starts only after a certain amount of change in the mean level of the membrane potential. Inhibitory action occurs by counteracting the depolarizing effect of the excitatory action.

Inhibitory nerve impulses cause an attenuation of the excitatory potentials in some instances when their timing is close. But, mechanically, inhibition is equally effective whether there is attenuation or not. When there is attenuation there is a greater degree of repolarization.

In the closers of Randallia and Cambarus excitation of the fast nerve fiber at low frequencies fails to evoke a contraction although it elicits large junctional potentials. By contrast stimulation of the slow nerve fiber at the same frequency evokes a good contraction but the junctional potentials are minute. The mean level of depolarization is also, in Randallia, less in the second case.

It is concluded that in the Crustacea the excitatory and inhibitory actions occur by way of an intermediate process in the membrane, which is not associated with appreciable membrane potential changes. When these occur, due to permeability changes, they are probably indirect, and are not the precursors of shortening or inhibition.

9. NUMBER OF NERVE CELLS IN THE CENTRAL NERVOUS SYSTEM OF THE CRAYFISH

Investigator: C. A. G. Wiersma
Support: National Science Foundation
General Funds

It has long been known that the number of nerve cells in the arthropod central nervous system is small compared to vertebrates, but actual figures are few and limited to individual ganglia. In this investigation a serial section was made through all the ganglia of the central nervous system of one crayfish from the brain to the telson. The nerve cells were stained with toluidine blue. Each abdominal ganglion contained about 500 cellbodies, each thoracic 2,000, the subesophageal 6,000 and the brain about 10,000. In addition small cells were observed in thick layers in brain and subesophageal ganglion. These cells were too small and numerous to be counted individually. Estimated number of these cells for the brain is 65,000, for the subesophageal 1,000.

The total number of nerve cells counted in all "normal" cell layers was 28,722. Since it is possible that the small cells have a neurosecretory function this may be the full complement of central nerve cells available, with the exception of cell layers present in the ganglia of the eye.
10. THE BIOLOGICAL SYNTHESIS OF PROTEIN

A. Erythropoietic Factor. This year the major effort has been devoted to the isolation of the erythropoietic factor in the plasma of rabbits made anemic by phenylhydrazine. Progress in this field had been retarded by the lack of a suitable assay. The test that has been used, the production of polycythemia in normal rats, consumes too much time—18 days—and too much material—at least 200 ml of anemic plasma—to be useful as a guide to isolation procedure.

A more useful assay has now been devised. It is based on the reticulocyte count in the blood of normal, adult mice. The material to be tested is injected subcutaneously daily for three days; on the fourth day the reticulocyte percentage of the circulating erythrocytes is counted. Typical figures are: saline controls, 2.0 ± 0.9; 1 ml. daily of boiled filtrate of the plasma of anemic rabbits, 7.0 ± 1.5. With this test as a guide the filtrate from boiled anemic plasma was fractionated as follows: the proteins were precipitated by saturated ammonium sulfate at pH 4.0; the precipitate was dissolved and dialyzed free of ammonium sulfate, and precipitated again by 20% ethanol at pH 4.0; this precipitate was dissolved in water and then fractionated on a modified cellulose cation exchange column. All the activity emerged in one fraction, and is the purest material obtained so far. In rats 0.5 mg daily for two weeks causes a polycythemia; 20 µgm daily for three days causes an increased reticulocytosis in mice. The purest fractions so far contained more than one protein (paper electrophoresis).

The active fractions, as the original active plasma filtrate, stimulate the uptake of radioactive iron (Fe^59) into the circulating hemoglobin in rats. The active material gives, therefore, a positive response by the three types of test commonly used to measure a stimulant of erythropoietic activity: production of polycythemia, reticulocytosis, and Fe^59 uptake. So far the group has been unable to separate erythropoietic activity from protein.

The mouse assay has made possible the testing of whole plasma. This is not feasible in the long, polycythemic test in rats, because whole plasma induces a
depression of hemoglobin, presumably by an immunologic reaction. Measured by reticulocytosis in mice whole plasma of anemic rabbits is much more active than the filtrate after boiling the latter material. With the mouse assay the group has detected, indeed measured, the erythropoietic stimulating material in the plasma of normal animals—horse, human and rabbit.

The plasma of rabbits made anemic with phenylhydrazine is not a practical source of the erythropoietic factor for clinical trials. It is too expensive and too laborious to prepare. A source such as normal horse blood, even if it contains only 1% of that in anemic rabbit plasma, may be more useful. In any event it is necessary to compare the erythropoietic material in whole plasma, whether from anemic or normal animals, with that in the filtrate of boiled in anemic plasma. The mouse assay makes this possible. Accordingly the group is proceeding with fractionation of whole plasma and of the filtrate from boiled anemic rabbit plasma.

This work was done in collaboration with Ashton Graybiel, Captain, M.C., U.S. Navy.

B. Hemoglobin Synthesis. As heretofore studies have been made of hemoglobin synthesis in rabbit reticulocytes in vitro, as an example of protein synthesis in general and because of interest in hemoglobin synthesis per se. In Biology 1956 there appeared an analysis of the factors in plasma and in liver that stimulate hemoglobin synthesis in rabbit reticulocytes in vitro; the total stimulation exerted by both liver and plasma had been accounted for by known substances. It was now possible to examine the process of hemoglobin synthesis more closely.

Over the last 15 years accumulating evidence has implicated ribonucleic acid (RNA) in protein synthesis. Rabbit reticulocytes that synthesize hemoglobin (and other proteins) actively contain 600 to 700 mg percent of RNA; mature rabbit erythrocytes in which no protein synthesis is detectable (in such short-period experiments as four hours) contain about 40 mg percent RNA. Accordingly the relation, if any exists, between RNA synthesis and hemoglobin synthesis has been examined. For this purpose C14-labeled nucleotides and nucleosides were used to measure RNA synthesis, and C14-leucine to measure hemoglobin synthesis. In these experiments the following substances were isolated, purified and their C14 content measured: hemoglobin, RNA, free nucleotides (the mono-, di- and tri-phosphates). For analysis the RNA and nucleotides were hydrolyzed and their constituent purine and pyrimidine bases isolated.

Some authors have suggested that concomitant RNA synthesis is obligatory for protein synthesis. The group undertook to measure the number of internucleotide bonds formed per peptide bond synthesized in hemoglobin. If concomitant RNA synthesis is necessary for protein synthesis one may expect that only one or only a few internucleotide bonds would be formed per peptide bond synthesized. The measurements indicate that one internucleotide RNA bond is formed while 100 to 200 peptide bonds are synthesized in hemoglobin.

Exclusion of histidine from the reaction medium reduced hemoglobin synthesis almost to zero, but there was no reduction in RNA synthesis.

Whether the label used for RNA synthesis was guanosine or guaninemonophosphate, no radioactivity could be found in the cells four hours later as free nucleoside; it was all either in RNA or in the mono-, di- or triphosphate nucleotides, mostly in the two latter forms. The same relation was found with the adenine, uracil and cytosine nucleotides. Evidently there are in reticulocytes mechanisms for rapidly converting nucleosides into nucleotides, and these, aided possibly by nucleosidases, leave little or no free nucleosides in the cells.

The ratio (specific activity of purine or pyrimidine base in RNA/specific activity of the corresponding base in the free nucleotides) was 1 to 3%. Although many more peptide bonds are synthesized than internucleotide bonds, approximately
the same value was found for the analogous ratio of leucine in hemoglobin-free leucine. These ratios may be considered as "Turnover Numbers."

Publications:


11. INCORPORATION OF RADIOACTIVE LYSINE INTO PROTEIN

Investigator: R. S. Schweet
Support: National Science Foundation
American Heart Association

Studies of the soluble enzyme system from guinea pig liver which incorporates lysine into protein were continued. The linkage of the incorporated lysine by its ε-amino group to the γ-carboxyl of a glutamic acid in the protein, reported last year, has been confirmed. The dipeptide isolated from the protein containing C14-lysine was shown to be ε-N-γ-glutamyllysine by comparison with the synthetic compound. Both the α- and γ-glutamyllysine peptides were synthesized for comparison.

The recent report that ε-linked lysine has been found in collagen provides evidence for the physiological significance of these studies. The hypothesis now under study is that this lysine incorporation is an intermediate stage in collagen synthesis.

Publication:


12. ANTIBODY SYNTHESIS

Investigators: R. S. Schweet, R. D. Owen
Support: National Science Foundation
U. S. Navy, Office of Naval Research
American Heart Association
General Funds
Antibody formation is one of the systems which appear to be specially suitable for the study of the steps involved in general protein synthesis, because a new protein which can be easily isolated is formed. An analysis of this problem in relation to the known stages of protein synthesis has led to the formulation of a theory of antibody synthesis.

Publication:


13. AMINO ACID ACTIVATION

Investigators: R. S. Schweet, Esther H. Allen
Support: National Science Foundation
American Heart Association

This problem involves the study of enzymes from pancreas which catalyze a reaction between adenosinetriphosphate (ATP) and amino acids. The product of this reaction is thought to be the "activated" amino acid, which in the whole cell is the first intermediate in protein synthesis. This first stage is measured either by the formation of amino acid hydroxamides or by the incorporation of radioactive pyrophosphate into ATP.

An enzyme which is specific for tyrosine "activation" has been isolated and appears to be homogeneous in the ultracentrifuge and by paper electrophoresis. Using this enzyme, the mechanism of "activation" has been investigated, and soluble enzymes involved in the next stage of protein synthesis have been demonstrated. This second stage is the transfer of the "activated" amino acid to ribonucleic acid or nucleoprotein.

This system provides the first opportunity to analyze the intermediate stages in protein synthesis.

Publications:


14. PHOSPHOLIPIDS CONTAINING AMINO ACIDS OTHER THAN SERINE

Investigators: J. J. Wren, H. K. Mitchell
Support: National Science Foundation
General Funds

The literature contains abundant evidence for the presence of chemically bound non-hydroxy, amino acids in phospholipids from widely diverse sources. Since it is possible that they may be concerned in protein synthesis it is desirable to elucidate the nature of the lipid-amino acid linkage.

Chromatography of crude lipid extracts on silicic acid has eliminated the need for precipitation methods in phospholipid preparation and has fractionated components as well. It has led to the discovery of a wider variety of amino acids than previously found (Biology 1956, p. 23). Various observations, including those of infrared spectra, suggest that the amino acids are not joined in polypeptide chains to ethanolamine or serine residues in "cephalins," but may be joined
separately to fatty acid chains. Linseed oil, freed of phospholipids by chromatography, also contains traces of amino acids which may be bound thus.

Publication:


15. GENETIC CONTROL OF XANTHINE DEHYDROGENASE IN DROSOPHILA MELANOGASTER

Investigators: Edward Glassman, H. K. Mitchell
Support: American Cancer Society
National Science Foundation
General Funds

Crude extracts of two eye-color mutations in D. melanogaster, rosy (ry) and maroon-like (ma-1), have previously been reported as being deficient in xanthine dehydrogenase (see Forrest, Glassman, and Mitchell, Biology, 1956, p. 25). This enzyme has now been purified approximately 50-fold from wild-type flies, using charcoal, ammonium sulphate, and calcium phosphate gel. No activity was detected in purified extracts of the mutants.

Antibodies to the enzyme were prepared by injection of purified extracts into rabbits. Preliminary data indicate that extracts prepared from ma-1 adults contain cross-reacting substances which remove the antibody to the enzyme, whereas ry does not.

This is a somewhat unique situation in which mutation at two different genetic loci results in a deficiency of the same enzyme and it provides a basis for further studies on the genetic control of enzyme formation.

Publications:


16. BIOSYNTHESIS OF PYRIMIDINE NUCLEOTIDES IN NEUROSPORA

Investigators: Alice L. Tuttle, H. K. Mitchell
Support: American Cancer Society
General Funds

Studies have been continued on the enzymic character of the pyrimidine-requiring mutants of Neurospora with particular reference to the intermediate steps in the synthesis and utilization of orotic acid. Previously it had been demonstrated with one of the mutants, 36601, that the decarboxylation of orotidylic acid did not occur as in the wild-type strain and two other mutants of the pyrimidineless series. Later, when the culture of 36601 spontaneously began to grow like wild type, isolation by genetic procedures of the strain capable of this growth permitted demonstration of some decarboxylase activity, though significantly less than for wild type. The genetic and biochemical evidence suggested a partial reversion at the locus concerned with the initially observed block.

Recent work has dealt with steps in orotic acid synthesis involving ureido-
succinic acid and dihydroorotic acid. A method of colorimetric analysis for the
ureido grouping has been applied to extracts of mutants to detect accumulation of
these compounds during growth on pyrimidine-supplemented medium. Extracts
have also been tested both colorimetrically and spectrophotometrically for activity
in the conversion of ureidosuccinic and dihydroorotic acids to orotic acid. The ef­
fect of the temperature maintained during growth of some of these mutants has
also been investigated.

Finally, the entire series of mutants originally classified as pyrimidine­
requiring has been tested for growth on media supplemented with the compounds
under study. The relative effectiveness of supplementation in nitrate versus Fries
minimal medium has been noted. In addition, comprehensive tests of supplemen­
tation by several amino acids in both types of media have been initiated following
reports that various 4- and 5-carbon amino acids can support growth of certain
mutants maintained in the pyrimidineless series. With a number of these mutants,
considerable growth takes place upon addition to minimal medium of one, or of
any of several, amino acids tested. The question of a possible sparing action
exerted by these amino acids on the effective level of pyrimidine supplementation
is now being studied.

17. PURINES AND PTERIDINES IN DROSOPHILA

Investigators: E. Hadorn, H. K. Mitchell, Edward Glassman
Support: Rockefeller Foundation
           American Cancer Society
           National Science Foundation
           General Funds

Cooperative investigations on the biochemical genetics of Drosophila were con­
tinued with a brief visit here last summer by Professor Ernst Hadorn. Hadorn noted
that transplantation of small bits of living normal Drosophila tissue into larvae
of the rosy mutant resulted in the production of normal eye color in the mutant
whereas a deficiency of the enzyme xanthine dehydrogenase had been noted here
for the mutants maroon and maroonlike. These observations have now been cor­
related by demonstration that all three mutants have similar biochemical pheno­
types as well as similar eye colors. All have been observed to have deficiencies
with respect to the enzyme, uric acid, and isoxanthopterine while all accumulate
2-amino-4-hydroxy-pteridine, xanthine and hypoxanthine. Hypoxanthine is ac­cumu­
lated in particularly large quantities and is also excreted in large quantities. These
results are in accord with earlier conclusions that pteridines are derived from
purines and they also demonstrate that purine degradation is not essential to sur­
vival of the fly.

Experiments were also carried out to detect substances in normal tissue that
might induce normal eye color in the rosy mutant. Results so far have been nega­
tive with only living tissue transplants serving to produce this effect.

18. A FUNGISTATIC SUBSTANCE PRODUCED IN PLANT MERISTEMS

Investigators: Elizabeth M. C. Turner, H. K. Mitchell
Support: American Association of University Women
           General Funds

In an investigation into the cause of resistance of oats to the fungus Ophiobolus
graminis (the cause of the "take-all" disease of wheat and barley) it was found that
sap expressed from oat seedlings contains a substance which is strongly fungistic
in particular large quantities and is also excreted in large quantities. These
results are in accord with earlier conclusions that pteridines are derived from
purines and they also demonstrate that purine degradation is not essential to sur­
vival of the fly.

This inhibitor has now been isolated. When assayed against Neurospora
crassa it is active at a concentration of about 1γ/ml, against O. graminis at 0.2γ/ml. It is produced in the meristems of roots and leaves of oats and has also been found in smaller amounts in the few other plants which have been examined.

Further work on purification of this substance is in progress.

19. ON THE BIOCHEMICAL ROLE OF B₁₂

Investigators: J. W. Dubnoff, Louise Phillips
Support: National Institutes of Health, Public Health Service

Two B₁₂-containing fractions believed to be the biochemically active forms of vitamin B₁₂ have been partially purified. These fractions replaced the more complex system of vitamin B₁₂, glutathione, and glucose-6-phosphate required to reactivate sulfhydryl enzymes in aged cells of E. coli mutant 11B-3. Triose phosphate dehydrogenase, a crystalline enzyme normally reactivated by cysteine, was partially reactivated by these B₁₂ preparations. The reactivity of the sulfhydryl groups of glutathione and cysteine is greatly enhanced by these preparations. Two methods for the quantitative assay of this activity in tissues and blood have been developed.

The adaptive nature of tryptophane peroxidase in rat liver was studied. It was found that this enzyme was very labile. Tryptophane stabilizes the enzyme to a marked degree. This fact and the finding of high levels of enzyme on the injection of cortisone in the absence of tryptophane argue against a direct participation of tryptophane in the synthesis of the enzyme.

20. FREE ENERGY CHANGE IN HYDROLYTIC REACTIONS: THE UN-IONIZED COMPOUND CONVENTION

Investigator: Frederick H. Carpenter
Support: General Funds

Perhaps unique to the field of biochemical energetics is the concept of the "group transfer potential" which is designed to give a measure of the relative tendency or potential of a group or radical to participate in a transfer reaction. In practice this transfer potential is measured by determining the free energy change involved in the transfer of the various groups in the various compounds to the same recipient compound, water. In other words, the free energy change involved in the hydrolytic removal of the group in question is used as a measure of the transfer potential of that particular group in the compound [Klotz, "Energetics in Biochemical Reactions," Academic Press, Inc., N. Y., N. Y., 1957]. When a relatively large, negative number is obtained for the free energy change involved in the hydrolytic splitting of a bond, the bond has been designated as a "high energy bond" (Lipmann, Adv. in Enz., 1, 99, 1941).

The actual numerical value of the free energy change involved in a hydrolytic reaction depends upon the convention used to describe the reaction. A frequently used convention has been to consider the hydrolysis reaction at a specified pH value, generally pH 7 [Oesper, "Phosphorus Metabolism," Johns Hopkins Press, Baltimore, Md., Vol. 1, 1951]. In a large number of hydrolysis reactions, new acidic and/or basic groups are liberated. Part of the over-all free energy change involved in the hydrolysis at a fixed pH value can be attributed to the ionization and neutralization of these groups. From a study that was made of possible conventions for the hydrolysis reactions which would lead to a correct estimate of the contribution of ionization-neutralization reactions to the over-all free energy change at a fixed pH value, the un-ionized compound convention was derived: The free energy change involved in the hydrolysis of un-ionized reactants at unit
activity in water by water in the liquid state to un-ionized products at unit activities in water. The use of this convention leads not only to an accurate estimate of the contribution of ionization-neutralization reactions (which may account for a considerable proportion of the "transfer potential") to the over-all free energy change at a fixed pH value but it is also of considerable use in the comparison of the free energy change involved in the hydrolysis of similar bonds in related compounds. Comparison of such reactions by the un-ionized compound convention results in a much narrower range of values than that obtained by the pH dependent convention which includes the variable contribution of ionization-neutralization reactions.
BIO-ORGANIC CHEMISTRY

STAFF:
Professor: A. J. Haagen-Smit
Research Associates: Charles E. Bradley, Henry Klostergaard
Research Fellow: Joseph A. Sacher

21. CONTROL OF PLANT EMISSIONS

Investigator: A. J. Haagen-Smit
Support: General Funds

Last year has seen the confirmation of this laboratory's findings that Los Angeles smog is largely due to a photochemical oxidation of hydrocarbons under influence of oxides of nitrogen. These research results have pointed at burning of gasoline, oil and gas as major smog producers.

For the application of these findings to the practical control of smog a laboratory was built at the Southern California Edison Company's El Segundo steam station. Dr. Haagen-Smit was granted a leave of absence from the Institute to direct this pioneering air pollution endeavor. At present pilot-scale equipment designed to remove the main pollutants from flue gas is being built and operated.

22. TOXICITY OF LATEX

Investigator: C. E. Bradley
Support: General Funds

Sapium biloculare is a genus of the Euphorbiaceae and is native of southern Arizona and northern Mexico. Samples of this latex for analysis were obtained in the Organ Pipe Cactus National Monument near Ajo, Arizona. Traditions dating from the early Spanish explorers of this region claim that the latex or "juice" of this plant is highly poisonous and that the Apache and Opata Indians used it as an arrow poison, calling it "Yerba de la Fleche," and also used it to stupefy fish--taking the work out of fishing.

Two toxic materials have been found in this latex--an alcohol soluble resin poisonous to warm-blooded animals, and a water-soluble saponin toxic to fish. The toxic resin has a strong physiological action resulting in a general loss of muscular control. Fish are stupefied by very dilute solutions of the saponin.

Publication:


23. MILK PROTEINS

Investigator: H. Klostergaard
Support: Carnation Company

Immunological Studies. β1- and β2-lactoglobulin have so far proven indistinguishable in immunological tests with rabbits, whereas α-lactalbumin is distinct from either one. This test is the most sensitive for detecting contamination of crystalline lactoglobulin preparations with α-lactalbumin.

Allergy Studies. This work is on a new schedule, which should give better
control of the tests. It has been in operation for only about three months.

The two studies above are being made with Dr. Dan Campbell's group at the Institute.

X-ray Studies. a-Lactalbumin crystals have been prepared and the X-ray pattern indicates an arrangement of possibly 10 to 12 molecules in the unit cells and a complete lack of symmetry.

β-Lactoglobulin crystals have been made with Nb₆Cl₁₄ and Ta₆Cl₁₄. Attempts to make crystalline preparations with phospho-tungstic and phosphomolybdic acids have not yet yielded any success, but are still not complete.

This study is a part of Dr. Corey's program at the Institute.

Fractionation procedure. A patent was granted for a fractionation procedure developed outside the Institute. It is therefore now possible to publish details of this new method. It involves using sodium chloride at pH 3.5 to 3.8. Applications are numerous for fractionation and crystallization as well.

Physical-Chemical Studies. Work on electrophoretic and sedimentation properties of milk proteins has been continued.

Publication:


24. RELATIONSHIP BETWEEN AUXIN AND MEMBRANE PROPERTIES

Investigator: Joseph A. Sacher
Support: Lalor Foundation
General Funds

The senescence and abscission of leaves and fruits have long been known to accompany a drop in auxin content of the tissue. Recent investigations provide an explanation of the role of auxin in these inhibition phenomena. The observation that added auxin greatly increased the longevity of isolated segments of bean pods led to the discovery that auxin effects the maintenance of the selective permeability of cell membranes. Without added auxin, membrane-integrity in the bean segments is lost in three days, as manifested by displacement of intercellular air due to exosmosis of cellular substances. Cellular dissociation follows rapidly. The latter may be attributable to the action of pectinases on the middle lamella. The normal in vivo source of auxin in bean pods appears to be the seeds.

The hypothesis that abscission is the result of a particular susceptibility of cells in the abscission zone to loss of membrane-integrity was confirmed by debbling experiments with Coleus, Fuchsia and Cestrum. This removal of the distal supply of auxin resulted in liquid-logging of the intercellular spaces only in the abscission zone. This is followed by cellular dissociation and abscission. The sequence is inhibited by application of auxin to the petiolar stumps.

Publication:

25. GROWTH RESPONSES OF PLANTS TO LINOLENIC ACID

Investigator: Joseph A. Sacher
Support: Lalor Foundation
General Funds

Cytohistological Studies. The intumescence induced by application of linolenic acid to the endocarp tissue of bean pods (Haagen-Smit, et al.) is a result of both cell enlargement and cell division. Only the outer 7 to 10 cell layers are involved in the response. Mitoses occur largely within 12 to 24 hours after treatment, although cell enlargement continues up to 72 to 96 hours. During the latter phase, membrane-integrity is maintained only in the tissue of the intumescence. In the rest of the endocarp, cellular dissociation and degeneration speedily progresses.

Growth Responses. Linolenic acid has pronounced stimulating effects on the growth of excised pea embryos. Maximum stimulation of root growth (34%) occurred at a concentration of 1 part per million (ppm) linolenic, of shoot growth (76%) at a concentration of 5 ppm. Inhibitory levels for roots were found at concentrations over 15 ppm, and at over 125 ppm for shoots. Lateral root formation was stimulated by 1 to 15 ppm linolenic.

26. INVESTIGATIONS ON THE CLIMACTERIC OF FRUITS

Investigator: J. A. Sacher
Support: National Institutes of Health, Public Health Service

Histological studies reveal that the maturation of certain fleshy fruits is attended by a liquid-logging of the intercellular spaces and cellular dissociation, similar to that described above as experimentally induced in isolated bean segments. Investigations are in progress to ascertain if the loss of selective permeability of cell membranes may be causal of respiratory changes occurring in maturing fruits.

27. "PROTECTIVE" MILK STUDIES

Investigator: H. Klostergaard
Support: Carnation Company

It has been stated that the cow is capable of producing antibodies against a number of antigens when the latter are placed in the udder of the cow. Further it has been stated that such milk can transmit an immunity-protection to humans when ingested in the normal way.

A project is being started which covers these two phases: (a) A cow is being injected with an antigen and the possible titer of the milk followed with time.
(b) Milk thus obtained will be ingested by animals and the titer of their blood will be checked.
BIOPHYSICS

STAFF:
Professor: Max Delbrück
Research Fellows: René Cohen, Helga Harm
Graduate Student: David S. Dennison

28. THE DISTRIBUTION OF STRETCH, TWIST, AND GROWTH RESPONSE IN THE GROWING ZONE OF PHYCOMYCES

Investigators: R. Cohen, M. Delbrück
Support: National Science Foundation
General Funds

A study is being made of the stimulus-response system in the growing zone of the sporangiophores of Phycomyces. The stimulus is light, the response is growth.

In previous studies the growth responses of Phycomyces to various illumination programs were studied by measurements on the sporangium. These reflect the integrated responses of all parts of the growing zone and do not reveal where the responses occur. The present studies aim at a more detailed description. Points on the growing zone are marked by starch grains applied with a micromanipulator, and observed micrometrically. The steady state (constant illumination) was studied first. Growth is described in terms of two local characteristics of growth: the stretch rate and the twist rate. The stretch rate is zero at the top of the growing zone, rises sharply to a maximum, declines to a plateau, and then decreases gradually to zero at the bottom of the growing zone. The twist rate undergoes curious rapid changes in the uppermost portion of the growing zone (including typically even a change in sign), rises to a maximum located somewhat lower than the stretch maximum, and then declines to zero at the bottom of the growing zone.

The growth response, contrary to prevailing beliefs, does not parallel the distributions of stretch rate. There is no growth response in the upper third of the growing zone, where the stretch rate is maximal. In the lower two-thirds the growth response is fairly uniformly distributed.

Other details of the growth responses are being studied with the aim of analyzing the organizational principles by which the growing zone of Phycomyces as a whole manages to produce orderly responses to light stimuli.

29. PHOTOTROPIC EQUILIBRIUM AND PHOTOTROPIC-GEOTROPIC EQUILIBRIUM IN PHYCOMYCES

Investigator: David S. Dennison
Support: Arthur McCallum Fund
General Funds

Phycomyces sporangiophores are positively phototropic and negatively geotropic. When confronted by two distinct light sources, or by one light source and gravity, their behavior should provide knowledge useful in further work, as well as additional insight concerning the mechanism of phototropism and geotropism. The purpose of this work is to gather data characterizing tropic equilibrium in Phycomyces.

Two Equal Lights. If a sporangiophore is illuminated by two equal lights, its average direction of growth will bisect the angle between the two lights.
(provided this bisecting line is roughly vertical). However, most specimens will oscillate about this equilibrium direction. The period of oscillation in a young sporangiophore (growth speed at least 2 mm per hour) is usually 60 to 70 minutes, and it falls gradually to 30 to 40 minutes after 10 to 15 hours, during which time the growth speed doubles. The net growth of a sporangiophore during a single cycle remains roughly constant at 2.5 mm throughout its growing period. The amplitude of oscillation may reach 30 degrees from the mean direction. Much greater amplitudes are achieved when the lights are from 30 to 90 degrees apart than when the lights are more than 90 degrees apart. However if this angle is 0 degrees (a single vertical source), the regular oscillation is replaced by an irregular wandering of small amplitude.

Two Unequal Lights. If one light is brighter than the other, the equilibrium direction is no longer half-way between the two lights but is between this midline and the brighter light. A mathematical expression has been developed that fits fairly well the observed relation between the ratio of intensities of the lights, the angle between them, and the deviation of the sporangiophore from the midline. This expression is based on the following hypothesis: the plant grows at such an angle that on the average the total light energy received by the light sensitive area from one source is equal to the total energy received from the other. The total energy received from a light source is determined mostly by the angle at which the light rays strike the photosensitive region, but it is also affected by the shadow cast by the spherical sporangium at the top of the photosensitive region. This work should be useful in any further experiments in which Phycomyces makes a comparison between the phototropic effectiveness of two light sources (e.g., action spectrum studies) since it gives an estimate of the sensitivity of such a technique.

One Light and Gravity. If a sporangiophore is illuminated by a single horizontal light, its equilibrium position is about 25 degrees above horizontal. This light-gravity equilibrium is characterized by the same regular oscillations as in the case of two lights. In fact the gravitational stimulus appears to have an effect identical to that of a light directly above the sporangiophore and having an intensity equal to one-half that of the real light; further, this quantitative relation remains unchanged while the intensity of the real light is varied over a range of 10^9.

In the absence of light, the geotropic response is weak and variable, unless the gravitational stimulus is increased by means of a centrifuge.

30. GENETICS IN PHYCOMYCES

Investigator: Helga Harm
Support: National Science Foundation

Phycomyces blakesleeanus is a favorable subject for biophysical studies of phototropism and light-growth responses; however, little is known about the genetics of this organism. Investigations of the genetics may be of interest from two points of view: (1) biochemical mutations may be helpful in elucidating the biochemical processes involved in phototropism; (2) recent advances in microbial genetics have emphasized the need for intensive studies of materials in which tetrad analyses can be made. According to the work of Burgeff about 30 years ago, Phycomyces might be suitable for such investigations. Therefore, experiments in several directions, involving the life history and cultural conditions, cytology, and hybridization of mutants, were performed.

1) Genetical investigations are rendered difficult by the long dormancy of the zygote (usually 6 months or more). Specific temperature and light treatment reduced the dormancy to about 2-1/2 months. Efforts will be made to reduce the dormancy still further.
2) The results of crosses involving two genetic markers are compatible with the hypothesis of Burgeff that the 500 to 2,000 haploid spores of the zygote sporangium originate from a single diploid nucleus, although many diploid nuclei are formed in the zygote. For the reason mentioned above a more critical test of this hypothesis is of great importance. Therefore, three-factor crosses, involving a newly isolated mutant ("slow growing") were performed. In this case not more than four genotypes within a single zygote sporangium should occur if Burgeff's hypothesis is correct. The results of the experiments will be available in a short time.

3) Using the sexual type (+) or (-) as genetic markers, there was difficulty in obtaining clear results from the zygote sporangiospores, because some of them behave as neutral. When hypoxanthine is added to the medium (Robbins and Kavanagh, 1942), the sexual type is expressed in almost all cases.

4) Cytological observations showed that the number of nuclei in individual spores varies from 2 to 6 in a (+) and in a (-) wild-type strain of different origin. This was found in the vegetative spores as well as in the zygote sporangiospores. However, in one mutant (-) strain 1 to 12 nuclei per spore were found. This strain exhibits also the abnormality that some of the spores (of the order of 1%) have fusion-like connections with another spore.

A manuscript entitled "Phycomyces blakesleeanus, its life history, cultural characteristics, and some genetical considerations" is in preparation and will be published when the three-factor crosses have given a definitive answer concerning the origin of the zygote sporangiospores.
EMBRYOLOGY

STAFF:
Professor: Albert Tyler
Research Fellow: Gerald Bernstein
Research Aides: Lorraine Bernstein, Mary A. Jones, Betsy H. Laties

31. ELECTRICAL AND IONIC CHANGES UPON FERTILIZATION IN ECHINODERM EGGS

Investigator: Albert Tyler
Support: National Institutes of Health, Public Health Service

In last year's report an account was given of the demonstration of the existence of a resting potential between the inside and outside of the unfertilized egg, of its changes upon fertilization, of its reversible depolarization by addition of potassium ion (K+) externally, of the high K+ and low sodium ion content of the egg, and of membrane resistivity and capacitance. The results have now been published in detail and indicate that the process of fertilization has features in common with the stimulation of other excitable tissue such as nerve and muscle.

The technical difficulties of inserting microelectrodes into the cells were largely overcome by the utilization of a manometric micropipette (described in the first publication listed below) as a suction device for holding the egg in desired position for lateral approach in the field of the microscope. This device is of general utility in various types of experiments involving microdissection and microinjection, some of which are being explored here and elsewhere.

The above-mentioned similarity of the electrical properties of the egg to those of nerve and muscle leads to the expectation that, upon fertilization, movements of certain ions would also be similar to those found upon stimulation of the excitable tissues. This appears to be the case for potassium which has now been investigated. Eggs of the sea urchin Arbacia were loaded with radioactive potassium (K42) and, after washing, aliquots were fertilized, samples centrifuged rapidly at various times and the radioactivity of the supernatant determined. The results of seven sets of experiments were consistent in showing that the rate of release of the K42 increases very markedly starting within 1-1/2 to 2 minutes after fertilization. There is also consistently a reduction during the 5th to 8th minute followed again by a rise.

Aside from this special interest for fertilization the various experiments suggest that certain general features of the process of excitation in cells may be profitably and conveniently studied with this kind of material.

This work was done in collaboration with Professor Alberto Monroy, Instituto di Anatomia Comparata, Universita di Palermo; Dr. C. Y. Kao, Rockefeller Institute for Medical Research, New York; and Professor Harry Grundfest, Department of Neurology, College of Physicians and Surgeons, Columbia University, New York.

Publications:

32. FERTILIZINS AND FERTILIZATION

Investigators: Albert Tyler, Gerald S. Bernstein, Mary A. Jones
Support: National Institutes of Health, Public Health Service
General Funds

Fertilizins of sea urchins are now being prepared in purified form in sufficient quantity for the exploration of the possibility of obtaining active fragments of small molecular size. Previous work has shown that, in several species, the substance is composed of elongate (about 20:1 axial ratio) molecules of about 300,000 molecular weight, and possessing a valence of approximately 15 effective negatively charged groups at pH 8. It belongs to the category of glycoproteins, being composed, in most species so far examined, of 14 different amino acids and 2 sugars. Fertilizins can be converted into a non-agglutinating, but still specifically combining form by exposure to heat, proteolytic enzymes and ultraviolet irradiation. However, the active material is still of large (non-dialyzable through cellophane) molecular size, nor has there been any evidence of marked differential elimination of amino acid or sugar components. Tests of the simpler sugars and amino acids, found as constituents of the fertilizin molecule, show no specific combining activity. This program has reached the state where a large series of partial hydrolysates must be investigated.

In collaboration with Dr. D. W. Bishop, now of the Department of Embryology, Carnegie Institution of Washington, Baltimore, evidence was obtained for the presence of fertilizins in eggs of mammals. This work has now been published.

Early experiments had shown that when sea-urchin eggs are deprived of the gelatinous coat by brief exposure to low acidity they require higher densities of sperm for fertilization. This has been interpreted on the basis of loss of much of the specific receptor material (fertilizin) from the surface. While many other kinds of experiments (with enzymes, with antifertilizin, with fertilizin-treated sperm) previously reported, are consistent with their interpretation, the possibility has been suggested that the low pH treatment causes some other, unspecified, damage to the egg. This has been examined again in experiments in which cleavage rate and later development have been followed in eggs exposed for several minutes (up to 10) to solutions acidified to various degrees ranging from pH 5 to 3.4. Upon fertilization the eggs were observed to develop as normally as the controls. These and other previously reported experiments find their simplest interpretation in terms of fertilizin serving as the specific sperm receptor on the surface of the egg.

Publications:


33. MONOGRAPH ON FERTILIZATION

Investigator: Albert Tyler
Support: National Institutes of Health, Public Health Service
General Funds

A monograph is in preparation, for the Protoplasmatologia series, dealing with the biological and biochemical properties of sperm and eggs and with their interactions. The relation to studies in immunology and virology is to be included, as is work on artificial parthenogenesis, for which a fairly comprehensive table of methods and results has been prepared.
34. ANTI-DEVELOPMENTAL ACTION OF FERTILIZIN ANTISERA IN SEA URCHINS

Investigators: Albert Tyler, Mary A. Jones, Betsy H. Laties
Support: National Institutes of Health, Public Health Service
General Funds

It is well known that antisera prepared against whole cells, or various crude extracts thereof, of various organisms, can exert a cytotoxic action. For many kinds of studies relating to normal and abnormal growth and differentiation of cells it is of importance to prepare such antisera against purified constituents of known location in the cell. Last year it was reported that antisera prepared against the fertilizin of several species of sea urchins were capable of inhibiting cell division and development. The experiments have been extended with antisera prepared in 11 additional rabbits (total 42) against purified fertilizin preparations. All consistently produced inhibitory antisera of high titer. A three-week series of nine injections with a total of 0.8 to 1 mg of fertilizin sufficed to produce potent antisera. Such antisera, when used undiluted, completely inhibit the division of the de-membranated fertilized eggs after about 15 minutes exposure (17°C). When the freshly fertilized eggs are cultured in increasing dilutions of antiserum, development is blocked at progressively later stages. For example, dilutions of 2- to 16-fold generally block before the first division; 256- to 512-fold in late cleavage (32- to 128-cell stage); 1024- to 2048-fold in blastula to prism stages.

Antisera against sperm or constituents thereof (antifertilizin, nucleoprotein), or against blood and epidermal tissue of the adult have not exhibited the anti-developmental effect. Those against various egg constituents other than fertilizin have proven generally less effective and more variable. Present studies show that embryos of later stages of development retain on their surface at least some of the antigenic structures characteristic of the unfertilized egg and of the fertilizin molecule, and can be killed by brief exposure to the antisera against fertilizin. Various types of experiments are in progress, for the purpose of attempting to isolate, from the antisera, antibodies having stage-specific and tissue-specific effects, but clear-cut selective action has not yet been demonstrated. For example, in one type of experiment the three different types of cells of the 16-cell stage were isolated but all were found to be similarly affected by the antisera.

General considerations concerning immunological studies of early development are presented in an article cited below which contains also some of the results of these investigations. Another article, on Gila monster venom, discusses also the concept of natural auto-antibodies in relation to growth and differentiation.

Publications:


35. ACTION ON UNFERTILIZED SEA-URCHIN EGGS OF ANTISERA AGAINST FERTILIZIN

Investigators: Albert Tyler, Mary A. Jones
Support: National Institutes of Health, Public Health Service
General Funds

When unfertilized sea-urchin eggs are exposed for short periods (5 to 30 minutes) to antisera against homologous fertilizin their fertilizability is progres-
sively destroyed. This loss of fertilizability can be accomplished without visible cytolytic effect on the eggs. The fertilizability can be partially restored by exposing the antiserum-treated eggs to trypsin. Presumably this treatment has removed from the surface some of the antibodies that were bound to the sperm-receptor sites.

Prolonged treatment of unfertilized eggs with antisera against fertilizin results in their cytolysis. After shorter treatments, the duration of which depends upon the strength of antiserum employed the unfertilized eggs (prepared by trypsin treatment, or in some cases de-jellified by exposure to low acidity) exhibit to various degrees changes corresponding to activation. These changes involve initially formation of the hyaline (ectoplasmic) layer, mitotic activity, and in a small percentage of cases cytoplasmic division. One interesting feature is a later fragmentation of the activated eggs into numerous very small, generally spherical, bodies some of which are nucleated or contain chromatin bodies. These are being examined further cytologically.

While there are a great variety of agents that can activate unfertilized eggs there is special interest in the action of an agent that is specifically directed against a known constituent of the cell, such as fertilizin.

36. **THE EFFECT OF VERSENE AND 2,4-DINITROPHENOL ON SEA-URCHIN SPERM**

**Investigator:** Gerald S. Bernstein  
**Support:** Population Council  
National Institutes of Health, Public Health Service

Diluted sea-urchin sperm have a short life span and an initially high respiratory rate that declines as the sperm age. Versene prolongs the motile life of diluted sperm and reduces and maintains sperm respiration. Two years ago it was noted that 2,4-dinitrophenol (DNP) increased the low respiratory rate of Versene-treated sperm but had little effect on the high respiratory rate of nonversenated sperm.

Since DNP is known to uncouple oxidation from phosphorylation and, in some instances, to stimulate the activity of enzymes that dephosphorylate adenosine-triphosphate, the finding raised the possibility that either the respiration of diluted sperm was not completely coupled to the production of energy-rich compounds or that these compounds were broken down with wasteful rapidity. Since Versene and other chelating agents protect diluted sperm, the effects of dilution are attributable to certain metal ions present in sea water.

In previous work sperm were diluted with sea water, which contains bicarbonate as a buffer. The pH of sperm suspension gradually increases in a manometric experiment where respiratory CO₂ is absorbed with alkali, because CO₂ is eventually removed from the buffer system. The rate of change depends on the respiratory rate of the sperm, and since Versene-treated and untreated sperm respire at different rates they exist at different pH levels. When DNP is used it is necessary to maintain pH at a constant level, because the undissociated DNP molecule penetrates cells more readily than ionized DNP, and the degree of ionization is determined by the pH of the medium. Under the conditions of the experiments the amount of undissociated DNP was sometimes twice as great in untreated suspensions as in suspensions of versenated sperm, and it seemed necessary to repeat the tests under more rigorously controlled conditions.

The experiments were repeated using CO₂-free sea water buffered with glycylglycine. Although glycylglycine is a metal-chelating agent it had little effect on the respiration of the sperm used in this work. In buffered sea water Versene decreased sperm respiration even more than in ordinary sea water. The results
obtained with DNP were the same as those obtained earlier, that is DNP greatly stimulated the respiration of Versene-treated sperm but had little effect on the relatively high respiratory rate of untreated cells.

These experiments showed that the amount of stimulation induced by DNP was inversely related to the respiratory rate of the sperm. However the low respiratory rate of aged sperm was not increased by DNP.

Experiments were also done to determine whether Versene lowers the adenosinetriphosphatase (ATPase) activity of sea-urchin sperm. Using frozen thawed sperm as an enzyme source, it was found that Versene reduced the amount of phosphorus liberated from ATP. This reduced activity was not stimulated by DNP. Work is now being done with the ATPase of isolated sperm tails.

37. EFFECT OF VERSENE ON HUMAN SPERM

Investigator: Gerald S. Bernstein
Support: Population Council

Last year it was reported that at 10^{-2} and 10^{-3} M the metal-chelating agent Versene (ethylenediamine tetracetic acid) inhibits the motility of human sperm. Further studies have been made on the mechanism of this inhibition by treating immobilized sperm with various ions (Mg^{++}, Ca^{++}, Zn^{++}, Cu^{++}).

When inhibited sperm were left in 10^{-3} M Versene they were reactivated by all of the above ions, but when the inhibited sperm were washed and treated in a Versene-free diluent only calcium reversed the inhibition. This indicates that at 10^{-3} Versene inhibits motility chiefly by depriving sperm of calcium, that at least some of the calcium-Versene complex is removed by washing, and that the other ions reactivate sperm indirectly by displacing calcium from its Versene complex.

When sperm are immobilized by 10^{-2} M Versene and then placed in a Versene-free diluent, calcium causes an immediate but temporary reactivation. Magnesium induces a more prolonged reactivation but only after a rather long latent period. Certain mixtures of calcium and magnesium produce the effects of both ions, i.e. a brief reactivation due to calcium followed later by a prolonged reactivation due to magnesium. In other mixtures the ions may act synergistically. This indicates that at 10^{-2} M Versene deprives sperm of both magnesium and calcium, and that some of the chelated calcium remains within or attached to the sperm where it can be displaced by excess magnesium.

It has been found that Versene and copper each protect sperm against the toxic effect of the other. Thus, while both copper and Versene at 10^{-3} inhibit sperm motility a mixture of the two at this same final concentration is nontoxic.

Publication:


38. PHYSIOLOGICAL STUDIES OF HUMAN SPERM WITH IMPAIRED MOTILITY

Investigator: Gerald S. Bernstein
Support: National Institutes of Health, Public Health Service

In a continuation of work begun last year, sperm from two more infertile patients have been studied. The semen of both patients contained virtually no
motile sperm; the washed sperm of one patient did not metabolize fructose, the metabolic substrate normally present in seminal plasma, while sperm from the other metabolized this sugar at about half the normal rate. This is in contrast to a patient studied last year whose sperm, although immotile, metabolized fructose at the same rate as normally motile sperm. Additional data will be obtained from these and other patients.

This work was done in collaboration with Dr. Edward T. Tyler, U.C.L.A. Medical School.

39. THE CARBOHYDRATE COMPONENTS OF FROG-EGG JELLY

Investigator: Gerald S. Bernstein
Support: Population Council

Quantities of jelly were obtained from the oviducts, eggs, and egg water (water in which eggs have been standing) of two species of frogs, and the jelly prepared in powdered form. As reported previously, three reducing sugars were found when hydrolysates of jelly from Rana clamitans oviducts were analyzed by paper chromatography. One sugar was identified as fucose while another was identified as a hexosamine. The third reducing sugar has now been identified as galactose while the hexosamine spot has been resolved into two components, possibly glucosamine and galactosamine. Jelly from R. clamitans eggs contains the same sugars as oviducal jelly.

Although R. pipiens eggs and oviducal jelly appear to contain the same sugars as R. clamitans jelly, the R. pipiens material contains much less fucose. The material obtained from egg water has not yet been analyzed.
40. ON THE USE OF TRANSLOCATIONS

Investigator: E. G. Anderson
Support: U. S. Atomic Energy Commission
           General Funds

Over a period of years, a large number of translocation stocks have been
accumulated. The greater part have been processed and are now being prepared
for storage for future use, either as objects of study or as useful tools for scientific
studies and practical maize improvement. Some of their uses as tools may be
described as follows:

1. Use in mapping the chromosomes. Translocations are formed by the
   reciprocal interchange of a portion of one chromosome and a portion of a non-
   homologous chromosome. In the pairing of such altered chromosomes with their
   corresponding normals at meiosis, homologous parts must pair, so a "cross-
   shaped" figure is formed. On separation at the completion of meiosis, spores or
gametes are formed of four chromosomal constitutions. These are formed in equal
frequency and may be designated as:

<table>
<thead>
<tr>
<th>Chromosomes 1, 2</th>
<th>Normal gamete</th>
</tr>
</thead>
<tbody>
<tr>
<td>&quot; 1^2, 2^1</td>
<td>Translocation gamete</td>
</tr>
<tr>
<td>&quot; 1, 2^1</td>
<td>Duplicate-deficient gametes</td>
</tr>
<tr>
<td>&quot; 1^2, 2</td>
<td></td>
</tr>
</tbody>
</table>

The duplicate-deficient gametes abort, giving rise to characteristic semisterile
pollen and ears. Only the normal and translocation gametes function. Any gene
near the position of the break in either chromosome will be linked with the semi-
sterility. Thus the semisterility which marks the translocation can be placed on
the linkage map of either chromosome by ordinary linkage test-crosses. The
position of the translocation on the chromosome itself can be observed cytologically
at the mid-prophase of meiosis when the chromosomes are closely paired. This
gives a point on the linkage map which corresponds to a visible point on the chromo-
some. By mapping a series of cytologically observed translocations on the linkage
map of a chromosome, a cytological map of gene locations may be made. Other
techniques, most of which likewise involve translocations are also used to give
additional information. All ten chromosomes have been mapped to some extent,
and additional information is being added. Summaries of available information
have been published for chromosomes 9 (1938), 1 (1939), 2 (1940), 3 (1940), 4
(1954), 6 (1955), 7 and the short arm of 1 (1941), 7 and also a summary of in-
formation bearing on the location of centromeres (1945). Data on chromosomes
2, 5, 6 and the long arm of 1 have not yet been published; much additional informa-
tion is largely available to workers in the field through the medium of the Maize
Genetics News Letter.

2. Use in marking genes for scientific study. In the study of the functioning of a gene in the development or expression of any inherited trait, the most important stages for study are before the trait has reached its full expression. Thus with a morphological trait such as tassel-seed it is important to study the early differentiation of the terminal inflorescence before the trait itself can be recognized. With a biochemical trait it is equally important to assay the tissues during the stages of development of the trait. In such cases it is always useful and often necessary to detect the embryos or plants carrying the trait much earlier than the trait itself can be detected. Once the gene for the trait has been located, it can be followed through any operation by tagging the portion of the chromosome in which it has been located. This can be done most readily by the use of a translocation in which one break is near the gene under study and the other break is near the gene chosen as the most desirable marker.

An excellent example of the above technique is found in the procedure used in the biochemical study of the effect of the sugary gene on the developing endosperm. It was found that the assays of starchy and sugary seeds began to differ significantly about two weeks after pollination, and a careful comparison was planned for the period which followed. Sugary and starchy seeds could not be distinguished until much later. But at two weeks the difference between yellow and white endosperm became apparent. So a translocation was used to make the gene yl serve as a marker for su. A yellow inbred line was crossed with a white sugary stock of T4-6a, and the F1 pollinated by a white sugary line. Except for infrequent crossovers, the yellow seeds were starchy and the white seeds sugary. Since the seeds were grown on the same ear, very precise comparisons could be made. A similar technique is being used at Brookhaven by Dr. O. L. Stein in the study of the embryo and early germination stages of dwarf 1. Here again the dwarf 1 gene is linked to white endosperm by means of a 3-6 translocation. The same type of procedure would be applicable if it were desired to set up a seeding treatment experiment to try to modify the effect of a tassel-seed gene. The seeds could then be separated into normal and tassel-seed before planting by color or texture of endosperm.

The use of such a technique is contingent upon having available a translocation which will have the breaks in each chromosome so placed as to link together the two specific genes, the one under study and the one chosen as a marker. About 720 random translocations would be required to give an even chance of having the two breaks fall within any two quarter chromosomes selected. The number of translocations which have been isolated and are now being processed is about 900.

3. Use in locating genes to chromosome. The use of translocations instead of genes has several advantages. Two chromosomes are tested instead of one. Semi-sterility is a better marker than most genes; it is more widely applicable and has fewer viability complications. The available translocations give a much better choice of distribution than do the available genes. There is often a reduction of crossing over in the proximity of the translocation, thus increasing the length of chromosome over which the test is effective. This technique has been widely used by maize geneticists. Frequently the set of translocations used will cover the 10 chromosomes with a break near the mid-point of each arm. The coverage is excellent--much better than could be obtained by the use of available genes. The efficiency, based on the expected average amount of work required to locate a gene, is good, especially for mature plant characters of good viability.

With the gene-marked translocation technique, it is possible to combine nearly all the advantages of the use of translocations with the ease of handling of the best marker genes. The greatest difficulty has been the assembling or building up of a proper series of translocation stocks, each with the same gene marker. A full series for all chromosomes with wx used as a marker gene is now on hand. These translocations are homozygous waxy with one break in chromosome 9 not far from the waxy gene. The other breaks are distributed to give one break in each arm of
chromosome 1, and one in the central region of each of the others. The new mutant gene is crossed to each of these testers, the F₁'s selfed or testcrossed; and the resulting seed separated for waxy is planted, and the resulting distribution observed.

If for instance an unplaced albino is crossed to waxy and the F₂ generation gives an equal frequency of albinos from waxy and non-waxy seeds, it can be said that albino is not linked to waxy and that the gene is not in the middle region of chromosome 9. Then if the albino is crossed to the series of tester stocks wx l-9S, wx l-9L, wx 2-9, wx 3-9, etc., all but one of the resulting F₂ populations should also show random frequencies of albino from the waxy and non-waxy seeds. If these are planted alongside, a mere inspection of the bench is usually sufficient to place the gene to chromosome. In general, counts need not be made, except for the one cross which shows linkage, so that work is reduced to a minimum. The efficiency of this technique is very high. The coverage of the chromosomes seems to be adequate for present purposes, and could be increased by the addition of more testers.

4. Use in the detection and localization of genes for agronomic traits. Searching for new genes of economic or other interest is not essentially different from the process of locating known genes, and usually the same techniques can be applied. But since most of the traits dealt with are not simple and clear-cut, the techniques must be applied more carefully, and the coverage for a given translocation may be less extensive. The most generally useful are the translocation and the gene-marked translocation techniques. Both techniques have been successfully employed at Minnesota, Wisconsin, Cornell, Iowa State, Illinois, Purdue, Pennsylvania, Missouri, U. S. Department of Agriculture (Beltsville), Pioneer HiBred Corn Company, etc., utilizing the translocation stocks developed at Caltech. For examples of precision work with gene-marked translocations there may be cited 1) the early work of Ullstrup and Brunson (1947) on resistance to Helminthosporium carbonum, which was followed by the use of the A-B translocations by Roman and Ullstrup (1951), and 2) the localization of a gene for high-amylose starch by Kramer et al. (1956). For examples of the use of chromosomal techniques in the analysis of complex and difficult agronomic traits there may be cited 1) the work on resistance to Helminthosporium turcicum by Jenkins et al. (1952-1957), and 2) the work on resistance to the European corn borer by Ibrahim and by Penny and Dicke. More recent work utilizing gene-marked translocations has localized the most important major gene for restoration of fertility to cytoplasmic sterile lines.

The usefulness of the translocation techniques to practical corn improvement hinges primarily on their effectiveness for gene detection and location as applied to the kind of traits with which an agronomist or corn breeder must deal. To test their practicability a number of trial tests have been made involving such traits as the differences between inbred lines and those between cornbelt inbreds and exotic lines, and such individual traits as time of maturity, tillering, wilting in dry climate, etc. Due to lack of space these tests were carried out on too small a scale but the results were promising and indicate that the techniques are applicable. Some of the more promising leads will be followed up by Dr. Pettet and others at the California State Polytechnic College.

5. Use in the transfer of favorable genes to adapted inbreds. Traits of agronomic importance in exotic or unadapted varieties of maize are of little value unless they can be transferred to adapted inbred lines. Fortunately, if the location of a gene is known, it is a simple matter to mark the chromosome so that its presence can be detected through a back-crossing program. A translocation close to the locus of the gene can be used effectively. The translocation may be crossed to the inbred line, and the F₁ back-crossed to the inbred as the recurrent parent, using semisterile plants. When the conversion is sufficiently complete, the translocation can be made homozygous. Then it can be used in place of the inbred line in a cross with the desirable gene and also in the back-crossing program. This time semisterility will mark the normal chromosome which carries the gene.
When sufficiently converted, the translocation chromosomes can be eliminated, which would leave the converted stock homozygous for the desired gene. This is essentially the technique regularly employed in handling genes for lethal, poorly viable, or otherwise difficult traits where it is much easier to follow the translocation than the gene itself.

These techniques make possible the introduction into our adapted lines of valuable traits from exotic varieties of maize.

Publications:

41. CONVERSION OF MUTANT INFLORESCENCE TYPES

Support: U. S. Atomic Energy Commission
General Funds

A series of mutant types which differ from the normal in the morphological structure of the tassel and ear have been described on a midwestern dent background (Nickerson and Dale). It was considered desirable to have a comparative description of one or more somewhat simpler and more primitive backgrounds. Parker Flint was selected as a typical 8-rowed Northern Flint, and Argentine Pop as a primitive small-seeded type from South America. Each mutant inflorescence type has been recurrently back-crossed to Parker Flint and to Argentine Pop. Both series are approaching the stage where the non-mutant sibs are substantially like the recurrent parent.

42. THE GAMETOPHYTE FACTOR OF CHROMOSOME 5

Investigator: A. E. Longley
Support: U. S. Department of Agriculture
U. S. Atomic Energy Commission

In Zea mays the translocation 5L. 09-9L. 06 brought into one linkage group the characters shrunken, waxy, gametophyte, brittle, necrotic and red aleurone. The necrotic character is a seedling abnormality observed by E. G. Anderson that causes parts of the young leaf tissue to become watery and die, and it produces zebra-like bands of lighter green tissue in the leaves of mature plants. The gametophyte character is detected only when pollen carrying this character fall on pistils that possess an inhibitor, preventing it from effecting fertilization. This inhibitor factor seems to be dominant and at present has not been tied to any linkage group. Since it seems to eliminate all pollen carrying the ga factor it eliminates all other genes on the same chromosome.

Since egg transmission is normal for semisterile plants carrying the inhibitor or the ga factor or both, therefore selfed plants heterozygous for ga and for a second linked character and the inhibitor show directly one-half the recombination by the departure from 50% of the second character in seed or progeny classes, if the second character is not on the ga bearing chromosome, or by the departure from 0% if the second character is on the ga chromosome. When the recombination value is too low to show in some small samples these were not included in determining the value and consequently the determinations in this group given below are slightly too high. Back-cross seeds or progenies, when pollen from semisterile plants was used on testers carrying the inhibitor, gave a direct measure of the recombination value between ga and a second linked character by the departure of the second character from 100% or 0%.

A table follows showing recombination values of linked characters:

<table>
<thead>
<tr>
<th>Character</th>
<th>Recombination value</th>
</tr>
</thead>
<tbody>
<tr>
<td>sh - ga</td>
<td>Back Cross %</td>
</tr>
<tr>
<td></td>
<td>27.1</td>
</tr>
<tr>
<td>wx - ga</td>
<td>7.6</td>
</tr>
<tr>
<td>- ga</td>
<td>9.0</td>
</tr>
<tr>
<td>ga - bt</td>
<td>3.0</td>
</tr>
<tr>
<td>ga - nc</td>
<td>4.3</td>
</tr>
<tr>
<td></td>
<td>7.2</td>
</tr>
</tbody>
</table>
The Rockefeller Foundation as part of its agricultural program for Latin America has assembled a large collection of Mexican and Central American varieties and strains of maize in their "corn bank" at their Chapingo laboratory near Mexico City. Many of these are local and unusual strains which are rapidly disappearing from cultivation. Some tests are being made for traits of economic value, such as disease resistance, drought resistance, cold tolerance, etc. As valuable traits accumulate, it becomes increasingly important to have an effective technique for transferring these traits to commercial strains of maize. The use of gene-marked translocations for detection and localization of genes, and their transfer to commercial lines would appear to be the best available technique. As the tester stocks are not adapted to the climatic conditions in Mexico, they are being converted to a Mexican background to be available for future use. The variety Zapaluta chica was chosen because of its unusually wide range of climatic adaptation. It is native to the northern Mexican plateau but grows well in Central Mexico, and can be grown without difficulty in much of the United States, including a large portion of the corn belt.
PART B.--DROSOPHILA

STAFF:
Research Fellows: Lawrence Burton, Frank Friedman
Graduate Student: Ellsworth H. Grell
Curator of Stocks: Rhoda F. Grell
Research Aides: Dorothy W. Brainerd, Ann T. Glassman, Theodore C. Henderson, Jr., Margaret M. Phillips, Tamiko Sueoka, Jess Williams
Student Assistant: Seymour A. Rapaport
Laboratory Assistant: Caroline L. Eggeman

44. PSEUDOALLELISM AT THE "hairy" LOCUS IN DROSOPHILA

Investigator: A. H. Sturtevant
Support: General Funds

One of the most striking recent developments in genetics is the increasing number of "pseudoallelism." The characteristic feature here is that two separately arisen recessive mutants, that do not give a wild-type phenotype in the first generation when they are crossed, nevertheless can be shown to occupy separate but neighboring loci and to produce the wild-type by crossing over.

This relation is under intensive study in this laboratory. During the year another example has been found, involving the two mutants long known as "hairy" and "hairy-2." It appears that "hairy" lies just to the right of "hairy-2." The case is in some respects not too favorable for further analysis, but it does add another to the list, and is of some interest because the two types differ in the pattern in which the extra hairs occur.

45. REVERSE MUTATION AND GENE DUPLICATION

Investigators: E. B. Lewis, Ann Glassman, Ted Henderson
Support: U. S. Atomic Energy Commission
General Funds

Studies of certain genes affecting eye-color pigmentation in Drosophila are providing information on processes which resemble reverse mutation. Normally, the eye color of this organism is red. A well known mutant gene, white, removes the eye pigments entirely. Another gene, spotted-white, produces a variegated eye color ranging from light orange to brown and appears to occupy virtually the same position in the chromosome as does white. Both white and spotted-white are reasonably stable through the germ line; that is, they rarely, if ever, return to normal. On rare occasion among the progeny of females carrying white and spotted-white, recombinant types occur that indicate that white and spotted-white are different genes, yet genes which lie exceedingly close together in the chromosome. In addition, two changes resembling partial reverse mutations have been detected among the progeny of such females and these also arose in association with a recombination of the parental X chromosomes. It has been established by direct examination of the salivary gland chromosome that these partial reversions are reasonably large tandem duplications of a region of the X chromosome which includes the loci of white and spotted-white.

The evidence indicates that these "white duplications" arose by a kind of illegitimate crossing over between the two parental chromosomes. Thus, an accident in the regular and orderly process of recombination of paternal and maternal chromosomes can sometimes lead to chromosomes containing an increased gene content. Moreover, such accidents may, in cases like the present one, lead
to products which resemble reverse mutations or "conversions" as they are sometimes called. The "white duplications" should prove valuable tools for the analysis of gene action of both the mutant and normal forms; for example, by the process known as unequal crossing over they can be used to construct chromosomes having still higher numbers of repetitions of the white region. Already it has been possible to identify by chromosome studies a derived product which appears to be a triplication of the white region, that is, contains three doses of the white regions in the same chromosome.

46. DETECTION OF TRANSLOCATIONS OF CHROMOSOME IV OF DROSOPHILA

Investigator: E. H. Grell
Support: E. I. du Pont de Nemours and Company

Translocations, the exchanges of sections between two or more chromosomes, are important products of ionizing radiation. In large experiments the standard method used to detect them is laborious. Therefore a test was made of a promising method of detecting translocations involving chromosome IV of Drosophila.

There were indications that most flies, which carry one broken fourth chromosome and the mutant gene, ci\(^W\), on the other fourth chromosome, lack most of a certain wing vein. Wild-type males were X-rayed and mated to ci\(^W\) females. A total of 30,035 males from this mating were examined. Only .3% of the males whose fathers had been given 3,000 roentgen units of X-rays lacked part of the vein. Tests indicate that most of the .3% did carry a translocation of chromosome IV. Cytological examination of salivary gland chromosomes shows that the breaks must be within limited regions of both the fourth and the other chromosome for the vein to be affected. Since breaks outside these small regions are not detected, the method is not as efficient as first contemplated.

47. AN ATTEMPT TO INVESTIGATE A GENE INTERACTION

Investigator: E. H. Grell
Support: E. I. du Pont de Nemours and Company

Drosophila which have prune eye color and carry the dominant gene Prune-killer do not live beyond the middle of larval life. The mode of action of Prune-killer when combined with prune is entirely unknown. Without prune, Prune-killer is indistinguishable from its normal allele. Since prune is an eye-color factor which affects the pteridine pigments, it was suspected that the combination with Prune-killer might further affect pteridines. As an attempt to test this hypothesis, eye buds from prune larvae were injected into Prune-killer hosts and wild-type hosts and Prune-killer eye buds were injected into prune hosts and wild-type hosts. When the hosts had become adults, the transplanted eyes were dissected from the host abdomens and the pigments of the transplanted eyes were then chromatographed.

The type of host did not appear to influence the pigmentation of the transplanted eyes. This may mean that either Prune-killer has no effect on pteridines or the action of Prune-killer is not autonomous.

48. A STRAIN OF DROSOPHILA WITH A HIGH SPONTANEOUS MUTATION RATE

Investigator: E. H. Grell
Support: E. I. du Pont de Nemours and Company

Genes are normally very stable. Radiation and certain reactive chemicals are known to induce mutations at a high rate, but in most strains of Drosophila about
1 spontaneous visible mutation is found among 20,000 flies.

Now a strain has been found which has a much higher spontaneous mutation rate under certain conditions. The mutations are most often expressed in mosaics. One side of the fly is mutant and the other side is normal. As high as 1% mosaics have been observed from a cross of this strain with another strain. About one-fourth of the mosaics transmit the mutation to their offspring. Several new mutant loci have been obtained from this strain as well as new alleles of previously known genes.

Flies of this mutator strain contain a much greater amount of the amino acid lysine than normal flies. Experiments show that flies of the mutator strain cannot dispose of the excess lysine in their bodies. Future work will be directed toward finding out if the mutations are caused by the abnormal lysine metabolism or if they are unrelated.

49. NON-RANDOM ASSORTMENT OF NON-HOMOLOGOUS CHROMOSOMES

Investigator: Rhoda F. Grell
Support: U. S. Atomic Energy Commission

Normally, the meiotic mechanism operates in such a manner as to insure that the distribution of one member of a pair of chromosomes to a gamete is random with respect to the distribution of any other member of any other pair. There have been slight indications that this law is not completely valid for the sex chromosomes and the fourth chromosomes of Drosophila.

A favorable set of conditions for studying this non-random assortment has been discovered. When females are constructed which carry a Y chromosome in addition to their two normal X-chromosomes and are heterozygous for a translocation involving the third and fourth chromosomes, a considerable degree of non-random assortment of the Y chromosome and the fourth chromosomes takes place. Analysis of the progeny of such females shows that in accordance with expectation the free fourth chromosome segregates at random with respect to the fourth chromosome involved in the translocation and the Y is recovered in 50% of the progeny. When, however, the Y and non-Y classes are compared, it is found that haplo-IV and triplo-IV progeny are not present in each class in equal numbers as expected. Instead the ratio of haplo-IV's in the Y class to haplo-IV's in the non-Y classes is 16.9:1 and the ratio of triplo-IV's in the Y to non-Y classes is 1:19.9. Furthermore, an analysis of the diplo-IV progeny which are present in equal numbers in the Y and non-Y classes discloses that the Y-bearing gametes carried the translocated fourth chromosome 2.6 times as often as they carried the normal fourth chromosome; while, conversely, the non-Y-bearing gametes carried the free fourth chromosome 1.7 times as often as they carried the translocated fourth chromosome. Under these conditions, therefore, the normal Y chromosome tends to go to the opposite pole from the normal fourth chromosome much more often than is expected on a random basis.

Publication:

50. RED CELLS IN DROSOPHILA

Investigator: E. B. Lewis
Support: General Funds

The many different kinds of cells which make up a complex organism are
usually if not always identical with one another in their gene content. For example, if an organism normally develops an eye pigment, then the genes necessary for the production of that pigment are present, as far as known, in all the cells of the body, not just in those cells which actually make the pigment. One of the many possibilities for explaining this somewhat paradoxical situation assumes that there exist genes whose function is to control whether or not a given cell is permitted to make eye pigment. A possible example of this type of gene has been found in Drosophila in the form of a mutant which causes the fly to develop a red pigment in certain fat cells which are scattered throughout the body and which are normally colorless. The pigment appears to be identical with one of the eye pigments and is produced in the form of numerous red granules inside fat cells. The mutant, known as "red cells," may help to provide clues about the mode by which genes supervise the growth and development of an organism. In collaboration with Dr. Jack C. Jones of the National Institutes of Health, a detailed account of these cells has been prepared.

Publication:


51. LEUKEMIA AND IONIZING RADIATION

Investigator: E. B. Lewis
Support: General Funds

It has long been suspected that cancer may in some cases arise as the result of a somatic mutation. Thus, in a malignant disease, a part of the body contains cancerous cells which act as if they were permanently altered in their growth rate compared to normal body cells. Such a cancerous line of cells presumably stems from a mutation in one (or more) of the normal body cells. X-rays and other ionizing radiations are known to be very effective agents in producing mutations in the body cells as well as in the germ cells. Hence, on the somatic mutation hypothesis for the origin of cancer, it is not surprising that ionizing radiations prove to be powerful cancer-producing agents.

Abundant evidence is now at hand that the malignant blood disease, leukemia, can be induced by ionizing radiations. A review of this evidence has been undertaken in order to study how the increase in incidence of this disease after irradiation is related to the exposure dose. The conclusion is that the relation appears to be one of direct proportionality, at least over the range of doses which have been studied. In particular, levels of radiation exposure which have often been claimed to be well below a threshold dose for the induction of leukemia are probably effective in inducing it after all. A detailed account of these studies has been published.

Publication:


52. TUMORS IN DROSOPHILA

Investigators: H. K. Mitchell, Tamiko Sueoka
Support: National Institutes of Health, Public Health Service
American Cancer Society, California Division
General Funds

A. Tumor-Inducing Factor. Previous reports and publications (Biology 1956, p. 47) by Burton and Friedman described an assay for and the purification of a tumor-inducing factor in Drosophila. Independent experiments failed to reproduce
the results reported and experiments done jointly with Burton and Friedman were, at best, inconclusive. One possible factor that influenced these results is that the original tumor host strain was lost. A new stock of this strain is now available and a repetition of critical experiments is now in progress.

B. Attempts at Chemotherapy of Drosophila Tumors. A strain of Drosophila obtained by Burton and Friedman and subsequently inbred consistently yields higher than 99% abnormal melanotic bodies in adult flies. On the assumption that these are tumors 12 substances that are known to have some effect in tumor chemotherapy (Cancer Research, 15 Supp. 3, 1-106 [1955]) were obtained through the courtesy of Eli Lilly and Company and were tested in the Drosophila system by feeding and by injection into larvae. None of the compounds had a significant effect on the production of the melanotic bodies in the Drosophila strain investigated. The substances tested were ethionine, azaserine, colchicine, 8-azaguanine, benzimidazole, cortisone, desoxypyridoxine, 6-mercaptopurine, \( \beta \)-2-thienylalanine, triethylene-melamine, \( N \) methylformamide and triethylenethiophosphoramide. The chelating agent diethyldithiocarbamate also had no effect. Most of the compounds were tested up to semi-lethal concentrations.
PART C.--IMMUNOGENETICS

STAFF:
Professor: Ray D. Owen
Research Fellows: Leslie Brent, John B. Loefer, Paul Margolin
Graduate Student: James H. Berrian
Research Aides: Mildred Barnes, Lucette Ghekiere, Ruth Ann Kerns

STUDIES IN HOMOLOGOUS TRANSPLANTATION IMMUNITY AND TOLERANCE

Investigator: Leslie Brent
Support: Rockefeller Foundation
General Funds

Studies Included: A. The reaction of splenic grafts against their tolerant hosts. The intravenous injection of adult tissue cells into newborn mice renders the recipients tolerant of the donor strain's tissues; for example, mice so injected with homologous spleen cells are unable to react against skin grafts transplanted to them from the donor strain later in life. The situation is, however, complicated by the fact that a number of the injected mice sicken and die a few weeks after birth. The evidence suggests that death is brought about as the result of an immunological reaction by the implanted adult spleen cells against the tissues and organs of their tolerant hosts (which, by definition, are unable to "protect" themselves). The experimental data obtained in this recent investigation entirely confirm this hypothesis, and make it possible to rule out the suggestions that death of the injected mice is caused by non-specific factors or by the transfer of pathogens into highly susceptible hosts.

B. The relationship between transplantation antigens and tissue cell hemagglutinogens: There is a considerable body of evidence in favor of the concept that the hemagglutinins which appear in the serum after the destruction of homografts are not causally connected with the breakdown of the homotransplants. In line with this trend of thought is the demonstration that the antigens responsible for eliciting homologous transplantation immunity (antigens which are known to be located in the nuclei of tissue cells) have very different properties from those extra-nuclear antigens which cause the formation of circulating hemagglutinins. Since both types of tissue antigens appear to be controlled by the same genetic loci, it seemed possible that some kind of immunological relationship might exist between them.

This does not appear to be the case. Thus, while it proved possible to absorb hemagglutinins from immune sera with living spleen cells or splenic cytoplasmic preparations, this could not be achieved with cell fractions consisting almost entirely of nuclear fibers. Similarly, repeated injection of nuclear preparations into homologous mice did not bring about a significant antibody titer. The distinction between nuclear transplantation antigens on the one hand, and extra-nuclear hemagglutinogens on the other, is therefore upheld by these experiments. The lack of an immunological relationship between two sets of antigens determined by the same "histocompatibility genes" is a matter of some interest.

C. Attempts to transfer transplantation immunity passively with disintegrated immune lymph node and spleen cells: The demonstration that lymph nodes and spleens from immune mice can "adoptively" transfer transplantation immunity to normal mice points strongly to these organs as being directly involved in homograft
destruction. The apparent absence of cytotoxic antibodies in the sera of immune animals makes it all the more likely that the immune lymph node and spleen cells are carriers or manufacturers (or both) of cytotoxic antibodies. Several attempts have therefore been made to transfer transplantation immunity with immune lymph node and spleen cells first totally disintegrated by ultrasound. Relatively large doses of immune tissue, and several suspending media, were used. The negative results so far obtained confirm that the transfer of immunity is associated with the viability of the cells, which must continue to function within their hosts. Unless it is assumed that the ultrasound inactivated the cell-bound antibodies, these experiments suggest that the amount of pre-formed antibody present within or on the cells is at any one time likely to be very small. Further work is in progress.

54. **CELL-BOUND ANTIBODIES IN HOMOLOGOUS TRANSPLANTATION IMMUNITY**

Investigators: James H. Berrian, Leslie Brent
Support: United States Navy
         Rockefeller Foundation
         General Funds

Skin grafts transplanted from individuals of one inbred strain of mice to those of another are destroyed by an immune reaction directed against the cellular antigens of the grafts. Cytotoxic antibodies are not demonstrable in the serum of immune individuals, but their spleens and lymph nodes will, upon transfer to other non-immune mice of the same strain, render the recipients immune to later grafts. Hypothetically, the immune cells should contain or produce antibodies, but the existence of such cellular antibodies has so far not been demonstrated. In the present experiments the transplantation antigens were extracted from tissue cells and incubated in vitro with immune cells derived from mice which had previously been specifically immunized against the antigens. The cell-free extracts were then tested for antigenicity by injection into homologous mice; i.e. their capacity to immunize mice against skin grafts was used as a measure of the inactivation which had occurred. A limited number of experiments have so far indicated that the transplantation antigens are removed or inactivated by incubation with immune cells, but not with cells derived from normal, non-immune animals.

55. **SERUM ANTIBODIES AND HOMOLOGOUS TRANSPLANTATION IMMUNITY**

Investigator: James H. Berrian
Support: United States Navy
         General Funds

The presence of serum antibodies was sought in the sera of animals immunized with tissues from donors of the same species. Pairs of rabbits were cross-immunized with each other's tissues and tissue extracts. At various times after immunization the aggregation reactions of molecules in tissue extracts and their corresponding antisera were studied by light scattering. Autologous extracts were used as controls. The serum samples were titrated in the light-scattering apparatus with the tissue extracts so that a wide range of proportions was examined. In some proportion zones reactions were observed only between the immune sera and the tissue extracts against which the animals had been immunized. In other proportions, reactions were observed between serum and autologous extracts as well as the homologous extracts.

The data thus far collected do not support the hypothesis that serum antibodies are regularly present in homologous immunity. Further study of the system is planned to inquire whether the autologous reactions involve complementary molecular
configurations as required by the auto-antibody hypothesis of cellular growth and development.

56. EFFECTS OF ADJUVANTS UPON IMMUNE RESPONSES TO RED CELLS

Investigators: Leslie Brent, Ray D. Owen
Support: Rockefeller Foundation
U. S. Navy, Office of Naval Research
General Funds

As indicated above, a current problem in the field of tissue transplantation immunity is the relationship between serum antibodies, as hemagglutinins or precipitins, commonly formed in response to homologous antigens, and the apparently cell-borne and cell-mediated channel of immune response involved in transplant rejection. This distinction becomes especially puzzling with regard to antigens controlled by a histocompatibility locus in the mouse, which on the one hand induce serum antibodies capable of distinguishing individual differences in the red cells, and on the other, play a dominant role in graft incompatibility. The red cell antigens do not immunize an animal for tissue transplants from the same donor.

The use of certain adjuvants, containing aquaphor, parafin oil, and heat-killed tuberculosis organisms has been found in some systems to divert the immune response of the recipient into the channel of delayed hypersensitivity. The well known analogy of transplantation immunity to delayed hypersensitivity reactions suggested the possibility of testing for possible effects of adjuvants in this system. In several experiments, mice were injected with whole blood or with pure red cells, with or without adjuvant. The recipients were then tested to skin transplants from the same donors. The results of these experiments served to confirm the prior reports by Billingham, Brent, and Medawar that the immunizing capacity of whole blood for tissue transplantation resides in the white cell fraction. The use of adjuvants did not divert the immune response to the red cell antigens into the channel of tissue transplantation immunity.

57. SCALE TRANSPLANTATION IN GOLDFISH

Investigator: W. H. Hildemann
Support: National Institutes of Health, Public Health Service
General Funds

Early in the year under review, Dr. Hildemann, who received his Ph. D. degree in June 1956, completed his studies of immunogenetic systems in goldfish, along the lines described in detail in Biology 1956. Four publications have since appeared in this connection.

Publications:


58. MECHANISMS OF TOLERANCE TO TISSUE TRANSPLANTS

Investigators: Ray D. Owen, Lucette Ghekiere, Mildred Barnes
Support: U. S. Navy, Office of Naval Research

General Funds

Studies have been continued on procedures that reduce or eliminate antibody responses in experimental animals, particularly procedures involving embryonic or neonatal exposure of animals to a variety of antigens. Further evidence has been obtained that when newly-hatched chicks are injected with human O blood, this neonatal injection may have profound effects on the nature of serum antibody responses to this antigen in later life. As compared with uninjected controls, the neonatally injected chicks display a delay in the development of saline agglutinating antibody and a disproportionate development of "incomplete" antibody, which may be detected by means of an antiglobulin technique.

A paper dealing with implantation of bone marrow into irradiated recipients, in collaboration with Lindsley, Odell, and Tausche, of the Oak Ridge National Laboratory, appeared during the course of the year, and several other current publications dealing with tolerance and related phenomena are listed below.

Publications:


59. ANTIBODY INACTIVATION OF TOBACCO MOSAIC VIRUS

Investigator: Ray D. Owen
Support: Rockefeller Foundation
General Funds

Antisera to certain strains of tobacco mosaic virus were provided the U. C. L. A. plant virus group, who used them in a comparative study of antibody inactivation of strains of tobacco mosaic virus (TMV). Five strains of TMV were inactivated by three different antisera. All the strains were related antigenically and fell into at least three distinct groups. The masked and common strains of TMV could not be distinguished by the inactivation method. The data indicated that the amino acids histidine and methionine were probably not involved in any major way in the antigenic structure associated with the infectivity of Holmes' ribgrass virus.

This work was done in collaboration with Irving Rappaport, Albert Siegel, and S. G. Wildman, Department of Botany, University of California, Los Angeles.

Publication:

60. SEROLOGY OF TETRAHYMENA

Investigators: John B. Loefer, Paul Margolin, Ray D. Owen, Lucette Ghekiere
Support: National Science Foundation
General Funds

Studies of three conjugating strains of Tetrahymena pyriformis grown in a bacterial medium demonstrated the presence of a system of alternative immobilizing surface antigens. Each strain was found to have the potential for the expression of three serologically unrelated antigens. These consisted of a high temperature antigen, a low temperature antigen, and an antigen induced by growth in the presence of antiserum. Normally, only one of these antigens could be detected by the immobilization reaction as present on any one animal at any given time. The high temperature antigens of two of the strains were serologically related, while that of the third strain was unique. The low temperature antigens of the three strains were serologically related, as were the induced antigens.

Studies of inbred hybrids, provided by Dr. D. L. Nanney of the University of Michigan, indicated a potential for still more antigenic possibilities, as well as an ability for the induced antigen to appear spontaneously in the absence of the inducing antiserum. The three strains grown in an axenic medium indicated the existence of a more complex system in this medium than in the bacterial medium previously used. The possibility is suggested that more than one immobilizing antigen may be present simultaneously on organisms grown in the axenic medium. This possible breakdown of the "mutual exclusion" mechanism invites further study.

In addition to the strains of Tetrahymena pyriformis previously studied, the investigation has been extended to 18 cultures representing other genera or species, including two unidentified strains. Certain cross-reactions in these cultures and their corresponding antisera indicate taxonomic relationships; for example, so-called T. vorax strains displayed a relationship with a series of interrelated T. pyriformis strains. The serological indications are in line with observations on silverline specimens made with Dr. W. H. Furgason, of U.C.L.A., which show that these strains resemble the pyriformis type morphologically rather than the vorax type. The degree of diversity and of specificity recognizable in the immobilization system is indeed remarkable.

61. STUDIES WITH FLUORESCENT ANTIBODY

Investigators: I. J. Mayfield, James H. Berrian
Support: National Science Foundation
U. S. Navy
General Funds

Attempts were made to use fluorescent-dye-labeled antibody as a tracer to detect a small percentage of a given cell type in a mixture of cells. The system investigated was the genetically determined C and D antigenic group on red blood cells of an albino rat strain. Although the sensitivity of the method was not sufficiently high, in this case, to give reliable results, it was hoped that a system with higher antigen density might provide a clue to survival times of cell implants and, perhaps, to the mechanism of long-term immunity.
PART D. -- NEUROSPORA

STAFF:
Professors: N. H. Horowitz, H. K. Mitchell
Research Fellows: Elisabeth Bautz, M. Fling, Howard Gest, Donald C. Hawthorne,
Mary B. Mitchell
Graduate Student: Noburu Sueoka
Research Aides: Anna K. Nazarian, Victoria J. Reinking

62. ACTIVATION AND THERMOSTABILITY OF NEUROSPORA CRASSA
TYROSINASE

Investigator: Howard Gest
Support: National Institutes of Health, Public Health Service

The crude cell-free tyrosinases from two strains (T\textsuperscript{S} and T\textsuperscript{L}) of Neurospora crassa were found to be activated by gentle heating (59°C for 1.5 minutes) or by exposure to sodium ion, urea, or certain organic nitrogenous bases. Non-activated preparations show an increasing rate of dihydroxyphenylalanine oxidation activity during assay, whereas activated enzyme displays the "reaction inactivation" kinetics characteristic of many copper oxidases. Activation by sodium ion (Na\textsuperscript{+}) is antagonized by potassium ion and is readily reversible. Urea activation is also reversible, but the activation due to heating appears to be irreversible. The thermostability of crude tyrosinase from strain T\textsuperscript{S} is markedly increased by Na\textsuperscript{+} and the half-life of thermal inactivation at 59°C varies between wide limits (1 minute to approximately 70 minutes), depending on the ionic environment. The relatively heat-labile tyrosinase of strain T\textsuperscript{L} shows qualitatively similar behavior, but is quantitatively different with regard to stabilization by Na\textsuperscript{+}. Previous studies of the T\textsuperscript{S} and T\textsuperscript{L} strains have indicated that the thermostability property is determined by a pair of Mendelian alleles and it seems probable that the difference observed in the present experiments (with regard to stabilization toward thermal inactivation) is a reflection of differences in the structural organization of the enzyme in the two strains.

63. GENETIC CONTROL OF TYROSINASE SYNTHESIS

Investigators: N. H. Horowitz, M. Fling, Victoria Reinking
Support: Nutrition Foundation
U. S. Atomic Energy Commission
Rockefeller Foundation
General Funds

Previous reports summarized studies on two naturally occurring forms of tyrosinase in Neurospora crassa, differing in their thermostability. The thermostability difference is based on a structural difference between the two forms (designated as T\textsuperscript{S} and T\textsuperscript{L}, respectively) and is inherited in a simple Mendelian way. In order to obtain further evidence on the nature of the genetic mechanism involved, a search for other variant forms of the enzyme in natural strains of Neurospora has been undertaken. Preliminary evidence for the existence of several new types of the enzyme has been obtained. These differ from one another in thermostability and/or in their migration in paper strip electrophoresis. Two of these, designated Puerto Rico-15 and Baton Rouge-1, are being studied at the present time. PR-15 differs from the original T\textsuperscript{S} and T\textsuperscript{L} forms in both thermostability and electrophoretic migration: it is intermediate between T\textsuperscript{S} and T\textsuperscript{L} in thermostability, and it moves more slowly in the electric field than either of them. Mixtures of PR-15 with T\textsuperscript{S} or with T\textsuperscript{L} can be resolved electrophoretically. BR-1 is similar to T\textsuperscript{S}, but appears to be slightly more thermostable, and there is also an indication of a small electrophoretic difference.
No differences were found among the four tyrosinase preparations with respect to inactivation by a rabbit anti-TL serum (provided by R. D. Owen).

Technical difficulties in connection with the paper electrophoresis studies, due to strong absorption of the enzyme by filter paper, have been overcome by pre-treating the paper with bovine serum albumin.

Genetically, the following questions are of interest: Are all modifications of a given property of the enzyme—e.g., thermostability—referable to changes at the same locus? Does recombination occur between different properties of the same enzyme? Results from six tetrads, two half-tetrads, and four random spores from a cross of TL by BR-1 have shown monogenic segregation of the thermostability and electrophoretic properties, without recombination between them. Genetic study of PR-15, which should be of great interest, has begun. Due to low fertility of PR-15 in crosses to standard laboratory strains, it was necessary to carry out a series of backcrosses as a preliminary step.

A mutant of N. crassa which produces no detectable tyrosinase (in some genetic backgrounds a small amount of activity is found) has been sent by Professor Westergaard. In addition to the lack of tyrosinase activity, the strain carries a morphological character ("velvet") and is female sterile. The three characteristics may be manifestations of the same mutation, but more data are needed. The tyrosinaseless character is not due to the production of an extractable inhibitor of tyrosinase. Preliminary data indicate that the tyrosinaseless character is inherited independently of the T-locus (see above), which controls the thermostability of tyrosinase. Since it is known that the T-locus determines at least part of the enzyme structure, the following possibilities are open with respect to the new gene: 1) It cooperates with the T-locus in determining the structure of tyrosinase; 2) it influences tyrosinase synthesis indirectly—e.g., by controlling the metabolism of copper, a constituent of the tyrosinase molecule. In connection with the latter hypothesis, an attempt to demonstrate the presence of an enzymatically inactive, but serologically cross-reacting, form of tyrosinase in crude and purified extracts of the mutant gave negative results.

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64. LACCASE IN NEUROSPORA

Investigators: Noboru Sueoka, N. H. Horowitz
Support: Arthur McCallum Fund
General Funds

Further studies of the "second" phenol oxidase of Neurospora (Horowitz and Fling, 1953) have been continued. This year, the following information has been obtained:

1) The enzyme is identified as laccase by its substrate specificity and inhibition pattern. 2) Beside the commonly known substrates of laccase, it can also oxidize 3-hydroxykynurenine and 3-hydroxyanthranilic acid. 3) Copper deficient medium has been prepared by treating medium with calcium carbonate (Steinberg, 1919). Reduction of copper content of the medium to 10% of standard causes a greater than 90% decrease of laccase production, while the growth is not appreciably affected. Tyrosinase activity is affected less drastically, but its production by the culture is retarded several days. These results indicate that growth can occur in a medium which does not support the production of the copper-containing enzymes, laccase and tyrosinase. This suggests the possibility that copper is non-essential for growth of Neurospora. 4) A potent anti-laccase serum has been obtained from rabbits. This serum does not affect Neurospora tyrosinase. Antiserum against Neurospora tyrosinase does not inactivate laccase. No cross-reacting material to this antiserum has been found in a strain which does not produce laccase. 5) Among 21 wild-type strains of Neurospora crassa which have been collected from various parts of the world, 9 strains produce laccase. Characterization of the enzyme of these strains
is being undertaken. 6) A preliminary experiment suggests that p-cresol may induce laccase production in some strains and not in others of Neurospora crassa.

65. ABERRANT RECOMBINATION IN NEUROSPORA

Investigator: M. B. Mitchell
Support: U. S. Atomic Energy Commission
General Funds

Experiments reported in Biology 1955 and 1956 (and in Proc. Natl. Acad. Sci., 41:215-220, 41: 935-937, 1955, and Compt. rend. Lab. Carlsberg, Ser. Physiol., 26: 285-298, 1956) indicated that two phenomena were involved in the recombination of the pyridoxin mutants and some other closely linked mutants used as markers. Recombination of the markers with one another and with the pyridoxin mutants appeared to take place as a result of crossing over (exchange of segments occurring between homologous chromosomes at meiosis) and therefore to represent classical genetic behavior. Both crossover products could be recovered together. (In Neurospora the eight ascospores resulting from one meiotic, and a subsequent mitotic, division, remain together for a time enclosed in a sac, the ascus. Thus, by analyzing complete ascii, it is possible to examine all the products of a single meiosis in order to see whether two reciprocal recombinants occur, as is required by the theory of crossing over.) Atypical recombination appeared to take place between the pyridoxin mutants themselves since ascii were found in which the wild type was present; but the reciprocal recombinant, the double mutant, was apparently absent. There seemed, however, to be a relation between the two types of recombination since they tended to occur together. In more than one third of the cases in which the pyridoxin mutants recombined there was also recombination of the adjacent markers. The more likely explanation appeared to be a facilitation of mutation (to give wild segregants with respect to the pyridoxin mutants) in certain heterozygotes, by a pre-crossover event, possibly an especially intimate chromosome pairing in the region, which might or might not lead to a detectable crossover effect. It was towards an understanding of this relationship that recent experiments were directed.

It now appears, from observations of other mutants, that the neat distinction between the two recombination phenomena in the pyridoxin case may have been fortuitous. Recombination of two phenotypically identical histidine mutants appears to be classical, but recombination of either of these with a closely linked adenine mutant appears classical by some criteria and aberrant by others. It will be seen that the distinction between the two types of recombination in the pyridoxin case rests upon the ability to distinguish the double mutant from the non-recombinant or parental mutant segregants in the same ascus. The pyridoxin double mutant was assumed to be absent since each pyridoxin mutant segregant, when backcrossed to the two parents, gave wilds in one of the two crosses. Wild segregants would not be expected from crosses of the double mutant to either parent since neither parent produced wilds when crossed to itself. By this test a recombinant ascus from the cross, hist 2 x hist 3, appeared to contain, in addition to a pair of wild segregants, one double mutant pair and one pair of each of the two parent types. This is the result expected, on the basis of classical theory, from a single crossover event. With respect to a closely linked visible marker, sn, the gene order appeared to be sn hist 2 hist 3, since hist 3 but not hist 2 had recombined with sn. Three hist strains were then crossed to the closely linked mutant, ad 3, the sn hist 2 parent of the above cross, a sn hist 3 isolate from a cross of the hist 3 parent to sn, and the double hist mutant, sn hist 2 hist 3. In recombinant ascii from the first two crosses the wild segregants were accompanied, as expected, by one adenine, and one histidine pair and by a third mutant pair exhibiting the double requirement for adenine and histidine, presumably the double mutant. The gene orders found in the three crosses were, respectively, sn hist 2 ad, sn hist 3 ad and sn hist 2 hist 3 ad, as expected. Even the map distances which could be calculated were fairly consistent, that from hist 2 to ad being 6.6 units, and from hist 3 to ad, 4.2 and 3.6 units respectively. (The distance hist 2 to hist 3 previously found was 4 units.) Also as expected, the back cross of sn hist 2 ad to hist 2 gave no wild progeny.
Crosses of sn hist 2 ad and two isolates of sn hist 3 ad to the original hist 3 strain (the hist 3 parent in the sn hist 2 x hist 3 and sn x hist 3 crosses) gave, on the other hand, irreconcilable results. In the sn hist 2 ad x hist 3 cross a reversal of the gene order of hist 3 and ad appeared to take place. Instead of being located within the 6.6 unit region between hist 2 and ad, hist 3 now appeared to be located 25 units beyond ad. Further, the two hist 3 x sn hist 3 ad crosses, instead of giving no wild progeny as expected, gave 17.8 and 21.7% wilds. The strains were retested and the three crosses repeated with essentially the same result. As further checks crosses of hist 3 to sn hist 3 and to two standard wild strains were examined. The hist 3 x sn hist 3 cross gave 7.9% wilds. The cross to one standard wild type gave 50% mutant progeny as would be expected if hist 3 were a straight-forward, unreverted single-gene mutant, but in the second outcross hist 3 behaved as a double mutant by giving 67% mutant progeny. To summarize, hist 3 is thus found to give wild progeny when crossed to three descendants of itself, sn hist 3 and two isolates of sn hist 3 ad, but not when crossed to a fourth, sn hist 2 hist 3; it gives a different gene order in the two crosses, sn hist 2 hist 3 x ad and sn hist 2 ad x hist 3; it behaves as a single mutant in one cross to standard wild but as a double mutant in another.

It appears, then, that classification of a particular segregant as a double or a single mutant may depend as much upon the criterion used as upon the actual constitution of the segregant itself. A similar mixture of classical and aberrant results obtained from a series of crosses, involving another adenine mutant (ad 5), a lysine (lys 4), and an arginine (arg 3) mutant and hist 2, suggests that this situation is not uncommon. An analysis of the pyridoxin case in more detail seemed strongly indicated. Fortunately the recombinant asci (they are not very frequent) had been saved and a reexamination is being made. The most striking result so far is a cytological observation made by Dr. A. E. Longley, who found that asci from the back cross of a wild segregant to one of the parent mutants appeared to be triploid rather than diploid as N. crassa asci are thought regularly to be. Another wild segregant carrying one of the markers, co, was found to give atypical ratios of recombinant and nonrecombinant progeny when crossed to a mutant (arg 3) in a different linkage group. Instead of the expected result of 25% co, 25% wild and 50% arg and arg co, the frequencies found were 14.5%, 28.8% and 56.6% respectively. This cross has not yet been examined cytologically but the above result would be less puzzling if it is triploid rather than diploid. A good deal of the aberrant recombination found in yeast has been attributed to polyploidy and to irregular behavior at meiosis. These explanations were thought to be ruled out in the pyridoxin case because the closely linked markers behaved in a regular fashion. It now appears that the behavior of the markers may not be a sufficiently reliable criterion to warrant this conclusion. It seems important to know, if possible, to what extent behavior resulting from irregularities in the chromosome complement may have been mistaken for both mutation and crossing over. The study is being continued.

66. HEAT SHOCK AND GENE CONVERSION IN NEUROSPORA

Investigator: H. K. Mitchell
Support: U. S. Atomic Energy Commission
General Funds

During some investigations of mutagenic effects of heat shock on Neurospora use was made of the genetically marked pyridoxine mutant system described by M. B. Mitchell (see Biology 1956 and this report). It was observed that under certain conditions heat shock treatment of either the maternal or paternal parent prior to a cross heterozygous at the pyridoxine locus reduced conversion to essentially zero without altering crossover values between markers. The findings were presented and discussed at the Symposium on the Chemical Basis of Heredity at Johns Hopkins University. A reconsideration of the data may now be in order in view of recent observations on abnormal segregations in Neurospora. (See M. B. Mitchell, this report.)
OBSERVATION OF MITOCHONDRIA IN RESPIRATORY MUTANTS OF NEUROSPORA

Investigator: Elisabeth Bautz
Support: Deutsche Forschungsgemeinschaft

Earlier cytological observations of cytoplasmic particles in yeast have shown that normally respiring yeast cells contain short rod-shaped and round mitochondria. On the other hand strains with respiratory defects, such that they can only ferment, have long threadlike mitochondria. Because the sphere-like yeast cells are not well suited to cytological work, it was decided to observe mitochondrial variants in the thin hyphae of other fungi, such as the respiratory mutants of Neurospora found by Mitchell and Mitchell. Studies showed that in the cytoplasmic mutant poky as well as in the nuclear-gene mutant C117 of Neurospora, the shape and the cytochemical reactions of the mitochondria are changed. Normally respiring wild type contains round or short rod-shaped mitochondria, poky rod-shaped and short threads, and C117 very long threads besides a few shorter ones. The long threadlike mitochondria observed with phase contrast microscopy could not be stained with Janus green B; only the round, rod-shaped and some of the short threads could be stained, and therefore only these can be actually respiring mitochondria. With the Nadi reaction (indophenol blue reaction), another living reaction which indicates the amount of oxygen consumption per cell, it was proved that cells with mostly long threads must have a lower oxygen consumption than those with short mitochondria.

With acriflavine, long threadlike mitochondria could be produced in wild type and poky, but only when cells were growing in medium containing acriflavine. Since acriflavine is known to inhibit the separation of cytoplasmic particles, it is assumed that the long threads are growing or reproducing mitochondria which cannot separate.

MAPPING OF SOME METHIONINE MUTANTS OF NEUROSPORA

Investigators: M. Fling, N. H. Horowitz, Victoria Reinking
Support: Nutrition Foundation
U. S. Atomic Energy Commission
Rockefeller Foundation
General Funds

Four methionineless mutants are known which show approximately the same linkage to a morphological mutant (C102) on the right arm of Linkage Group IV. These mutants have been studied further to determine if there is any correlation between their position on the chromosome and their biochemical properties, since such a relationship has been reported in other organisms (Demerec).

The four methionineless mutants fall into two biochemical groups, cystathionineless and homocysteineless. The two cystathione-requiring mutants are distal to the morphological marker. When crossed to one another they have yielded no wild-type spores in about 10,000 spores. They behave as separate occurrences
of the same mutation. The homocysteine-requiring mutants are proximal to the morphological marker. Crosses between these two mutants yield about one wild-type in 2,500 spores. Crosses between the two types of methionine-requiring mutants indicate a distance of 15 to 25 units between the two, with the morphological mutant approximately in the middle. Thus these mutants are not part of a series of closely linked genes with related biochemical requirements.

69. ABERRANT SEGREGATION RATIOS IN YEAST

Investigator: Donald C. Hawthorne
Support: National Science Foundation
General Funds

In yeast as in Neurospora, it is possible to recover the products of an individual meiosis. Normally, a diploid ascogenous cell heterozygous for a given gene will yield four haploid spores, two carrying the dominant gene and two with the recessive gene; occasionally, however, segregation ratios other than the expected 2:2 are observed. It has been demonstrated in yeast that the aberrant ratios can be due to a variety of events: polyploidy or polysomy, mitotic crossing over, post meiotic nuclear division, and simply polymeric or complementary genes. Nevertheless, irregular segregations which are unexplainable in terms of these classical genetic concepts are encountered at a frequency of about 1 or 2% for a given gene. For example, for the five loci most frequently studied the following ratios were observed.

<table>
<thead>
<tr>
<th>Mating type (A/a)</th>
<th>356(2:2)</th>
<th>1(3:1)</th>
<th>1(1:3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Histidine (HI1/hI1)</td>
<td>733(2:2)</td>
<td>4(3:1)</td>
<td>2(1:3)</td>
</tr>
<tr>
<td>Tryptophane (TR1/tr1)</td>
<td>674(2:2)</td>
<td>2(3:1)</td>
<td>2(1:3)</td>
</tr>
<tr>
<td>Uracil (UR1/ur1)</td>
<td>900(2:2)</td>
<td>10(3:1)</td>
<td>8(1:3)</td>
</tr>
<tr>
<td>Methionine (ME1/me1)</td>
<td>813(2:2)</td>
<td>7(3:1)</td>
<td>3(1:3)</td>
</tr>
</tbody>
</table>

To account for these aberrant ratios, it has been proposed that when the dominant and recessive genes are closely associated during meiosis, the duplication of one of the genes may be patterned after its allele so as to yield a tetrad of three of one kind and one of the other. In Neurospora, there has been demonstrated a significant correlation between the occurrence of aberrant ratios and crossing over in the immediate region of the chromosome. This has suggested that the common condition inducive to aberrant recombination and crossing over is an intimate pairing of the homologous chromosomes. No comparable study in yeast could be made with the above genes because suitable linked markers were not available. However, two groups of three closely linked genes were known, and preliminary studies were made with these loci.

From the cross of a sucrose and alpha-methyl-glucoside fermenter, SU₅ ma₃ MG₂, by a maltose fermenter, su₅ MA₃ mg₂, 50 asci with four surviving spores were obtained. There were two asci with aberrant segregations; both contained one fermenter to three non-fermenters of alpha-methyl-glucoside. Unfortunately, there was no crossing over between these loci so their linear order on the chromosome could not be determined.

With the other linkage group, th₁, S-tr₁, and py₂, genes controlling requirements for thiamine, tryptophane, and pyridoxine respectively, the order was determined to be as given above. Although only 28 asci were obtained, there were two aberrant segregations, 1(3:1) and 1(1:3), for the middle marker, S-tr₁, and in both of these asci there were crossovers between the outside markers. However, nine other asci had exchanges between these two genes, th₁ and py₂, and there were three asci with 3:1 segregations for pyridoxine independence. Both the frequency of aberrant asci and the amount of crossing over were much greater than what was anticipated from previous observations.
70. SENSITIVITY OF MARINE ANIMALS TO BRIGHT LIGHTS

Investigator: G. E. MacGinitie
Support: General Funds

Work on the reaction to bright light of animals with no known light receptors has continued. The common rocky shore "legant" anemone, which lives in shallow water and during low tide periods is often exposed to sunlight for several hours (although it does cover its body with bits of shell and debris), does not react strongly to bright light; on the other hand, a species of Tealia that lives in deeper water, expands in dim light and closes when exposed to bright light. When extended in search of a place to attach a byssus thread, the foot of the common mussel will immediately withdraw when a bright light is shone upon it and will not resume the search until the light is removed; only when the byssus thread is actually being attached will the foot not withdraw, but withdrawal occurs as soon as the thread is completely attached and the foot is not extended again.

Certain fishes respond curiously to bright lights. The sheephead slaps its tail violently and opens its mouth to its fullest extent in the frightening attitudes the fish uses to drive off intruders.

71. COMPARATIVE SUSCEPTIBILITY OF FRESH WATER AND MARINE FISHES TO CARCINOGENIC AGENTS

Investigator: G. E MacGinitie
Support: General Funds

The problem of whether fresh water fishes are more susceptible to injections of carcinogenic agents than salt water fishes has never been investigated. At the Kerckhoff Marine Laboratory preliminary preparations are being made for this study on several marine species, while the fresh water study is being made at the College of Medical Evangelists at Los Angeles. Intensive investigation will begin in October. The work is being done in collaboration with Findlay E. Russell, M. D.

72. MOLLUSKS OF POINT BARROW, ALASKA

Investigator: Nettie MacGinitie
Support: General Funds

The problem was that of identifying approximately 115 species of mollusks collected by G. E. MacGinitie at Point Barrow. Three new species were described and another given a new name. It was found that many species named by American workers had previously been described from Europe. The circumpolar range of many species was thus shown. Arctic mollusks, both gastropods and pelecypods, exhibit extreme variation within a species and this characteristic has resulted in the
description of many varieties as full species. The present study reduced a number of names to synonomy. Whenever possible, the egg-laying season was given. It was found that the majority of the Arctic snails lay their eggs in capsules and that a few snails and a few pelecypods are ovoviparous.

Publication:
Nettie MacGinitie, Smithsonian Miscellaneous Publications (in press).

73. BEHAVIOR IN THE LIMPET

Investigator: Harry K. Fritchman II
Support: General Funds

Studies on the limpet, Acmaea digitalis, and a supposed variant of this species have revealed that there are considerable differences in the behavior patterns of the two animals. Both types are negatively geotactic, a sloping surface acting as a definite stimulus to movement. Both animals are also positively rheotactic. However, under conditions of both submergence in still water and exposure to air on a moist surface, A. digitalis is approximately twice as active as the variant. Only when the variant is subjected to current on a sloping surface does its movement equal that of A. digitalis.

These differences, for the most part, correlate well with the differences in the ecology of the two animals. A. digitalis is a splash zone animal, is seldom submerged and feeds on a relatively sparse film of diatoms. This ecological niche requires considerable movement in foraging while the animal is exposed to air. The variant is an upper mid-tidal dweller, is regularly submerged and often found in tide pools; hence its indifference to submergence and its inhibition to movement when exposed. Its reduced movement is probably related to the increased abundance of algal film used for food. Their negative geotactic responses, operating with their reactions to submergence and exposure, possibly act to zone the two animals intertidally.

It is suggested by these differences, plus several morphological ones, that the variant animal deserves a specific designation.
74. CARBON ISOTOPE FRACTIONATION IN NATURE

Investigators: Roderic B. Park, Samuel Epstein*
Support: Woods Hole Oceanographic Associates
General Funds

A number of workers have noted that in land plants the ratio of the heavy carbon isotope (C\textsubscript{13}) to the normal isotope (C\textsubscript{12}), as determined by a mass spectrometer, is less than that of air carbon dioxide (CO\textsubscript{2}). To determine how this fractionation is affected by various ecological changes, so that the C\textsubscript{13}/C\textsubscript{12} ratios of both living and fossil carbons might be better interpreted, a device has been constructed in the Earhart Laboratory in which tomato plants may be grown from seed in an atmosphere containing CO\textsubscript{2} of known isotopic composition. Variations in CO\textsubscript{2} concentration from 0.15\% to 1.5\% produced small but regular changes in isotopic composition; the plants were more enriched in C\textsubscript{12} when grown in the higher CO\textsubscript{2} concentrations.

Respired CO\textsubscript{2} was found to be somewhat heavier than the plant as a whole and to vary in isotopic composition with time. The dissolved CO\textsubscript{2} of the plant was also heavier than the plant as a whole.

Plants were divided into chloroform soluble, ethanol soluble and various cell wall fractions, and the isotopic composition of each separately determined. The only fraction which differs significantly in carbon composition from that of the plant as a whole is the lipid fraction, which is considerably enriched in C\textsubscript{12}. This is also true of red, brown, and green algae, phytoplankton, ferns, grasses and conifers, and is apparently universal. It is of interest in relation to the origin of petroleum. Petroleum of both marine and terrestrial origin has long been known to be more enriched in C\textsubscript{12} than present-day marine or terrestrial plants. Petroleum cannot therefore have arisen from whole plant material. The lipid fraction of present-day plants possesses the carbon composition of petroleum and the indication is that petroleum may have arisen from this plant fraction.

75. NITROGEN FIXATION IN AZOTOBACTER STUDIED BY ELECTRON PARAMAGNETIC RESONANCE SPECTROSCOPY

Investigators: Roderic B. Park, Terry Cole**
Support: Woods Hole Oceanographic Associates
General Funds

*Division of Geological Sciences, C.I.T.
**Graduate student, Division of Chemistry, C.I.T.
Azotobacter is one of a number of organisms that is able to use molecular nitrogen as its sole nitrogen source. Molecular nitrogen is an extraordinarily stable material. It is of interest for this and other reasons to know the initial step by which the enzymatic system reduces nitrogen to the level of ammonia. Electron paramagnetic resonance spectroscopy is presently being used to determine whether nitrogen is dissociated to free radicals during the fixation process. Azotobacter grown on ammonia, which is used by the organism in preference to molecular nitrogen, provides a control experiment. Preliminary experiments have indicated that free radical formation is associated with nitrogen fixation in Azotobacter.

76. THE INTERNAL STRUCTURE OF MICROSOMAL NUCLEOPROTEIN PARTICLES

Investigators: Paul O. P. Ts'o, Jerome Vinograd
Support: National Institutes of Health, Public Health Service

The purification and characterization of microsomal nucleoprotein particles has been reported earlier. The internal structure of these particles has now been studied. Answers to the following questions were sought: (1) Is it possible to degrade the microsomal particle into smaller stable subunits? Can such subunits be reconstituted to form the original particle? What are the forces which bind the subunits together? What are the shapes, sizes and the chemical compositions of such subunits? (2) What are the forces which bind ribonucleic acid (RNA) to protein in the particle?

In answer to the first group of questions, it has been found that the original 75S particles may be degraded by slight increase in pH (6.5 to 7.5), and slight increase in ionic strength (0.02 to 0.1 M) to form 60S and 40S components together with a small amount of a 5S component. Such degradation can be reversed by merely returning the mixture to the pH and ionic strength under which the original particles are stable or by addition of magnesium ion to the mixture. Dialysis also breaks down the 72S particles to 60S and 5S components and this also is reversed by adding magnesium ion. In versene solution, the 75S particles are degraded to the 40S component and further to a 25S component. In strong salt solution, the particles largely degrade to components smaller than 10S, with some residual 40S material. Degradation by strong salt is, however, also reversible. In alkaline buffer of pH 10, especially in the presence of magnesium binding agents, the particles rapidly break down to a range of small components, 25S to 5S.

Ultraviolet absorption optics have been used to identify the nucleic acid-containing moieties of the degradation mixtures. The 60S and 40S materials contain nucleic acid; the 5S material does not. It may be concluded that the microsome can be reversibly degraded to smaller subunits which are themselves nucleoprotein complexes. Electrostatic attraction and the binding through magnesium ions are the forces which hold the subunits together in the original 75S particles.

It is possible to remove 80 to 90% of the protein from the RNA by use of the hot detergent or phenol extraction methods. These methods are being perfected in order to determine how free from the protein the RNA can be without involving breakage of chemical bonds.

Trypsin and papain attack the microsomal particles and the early products include the 40S and the 60S components. It is also possible to remove the RNA from the protein by ribonuclease. This cannot be used preparatively since, in dilute buffer, the protein aggregates and precipitates from solution.

*Division of Chemistry, C.I.T.
Publications:


77. ELECTROPHORETIC ANALYSIS OF PEA MICROSOMES

Investigators: John M. Clark, Jr., Jerome Vinograd*  
Support: Kirk Macomber Fund  
General Funds

Differential centrifugation of pea epicotyl homogenates in water yields ultracentrifugally homogeneous preparations of microsomes. The present investigation has been made to find out if the preparation is also electrophoretically homogeneous. The microsomes tend to break down during electrophoresis in phosphate buffer, but this breakdown is minimized by the addition of magnesium ions to the system. In the presence of magnesium ions, the material moves with a single front.

Versene treatment of microsomal preparations has been shown to result in degradation of the particles to a variety of different products. Electrophoretic analysis of microsomes in the presence of versene has revealed that about 5% of the material moves as free nucleic acid but that the majority of the material moves with a single front. The mobility of this front indicates that the material is a nucleoprotein complex. Its mobility is greater than that of the intact particle.

*Division of Chemistry, C.I.T.

78. PHOSPHOROUS METABOLISM IN PEA SEEDLINGS. I. RNA, DNA AND PROTEIN CONTENT OF DIFFERENT CELLULAR COMPONENTS

Investigators: Paul O. P. Ts'o, Jane R. Pilcher, Clifford S. Sato  
Support: National Institutes of Health, Public Health Service  
Tobacco Industry Research Committee  
Frasch Foundation

A fractionation of pea epicotyl tissue into nuclei, mitochondria, microsomes and supernatant has been developed on the basis of sedimentation analysis (by ultracentrifuge) and location of desoxyribonucleic acid (DNA). Nucleic acids measurements were done by analyses of phosphorus, ultraviolet absorption and sugar assay. The three methods agree within 5%. The time course of the extraction of ribonucleic acid (RNA) and of DNA by perchloric acid has been followed and the procedures for quantitative extraction rigorously established.

In the apical portion of the seedling epicotyl (top two inches), each gram fresh weight of tissue contains about 30 micrograms of DNA, more than 90% of
which is located in the nuclear fraction by this fractionation procedure. Each gram of tissue also contains 500 micrograms of RNA, 65% of which is located in microsomes, 20% in the apical mitochondria, 13% in the nuclei and 3% in the supernatant. Within the apical two-inch section, the top half inch contains six times more RNA (1.2 milligram per gram of fresh weight) than the lower portion. The microsome fraction of the tip section also contains a higher percentage of the total cellular RNA than does the microsome fraction of the older tissue.

The protein content of the tip section is 3.8 milligram per gram fresh weight—twice as high as that of the lower stem section. Protein is distributed rather uniformly through the different cellular components. The nuclear and supernatant fractions each contain 30 to 35% of the total cellular protein.

Of the total trichloroacetic acid-precipitable phosphorus of the cell, 82% is present as RNA, 10% as phosphoprotein and 8% as DNA.

These studies provide a basis for investigation of the metabolism of macromolecular phosphorus compounds in pea stem tissue.

79. PHOSPHORUS METABOLISM IN PEA SEEDLINGS. II. UPTAKE OF PHOSPHATE AND RNA SYNTHESIS

Investigators: Paul O. P. Ts'o, Jane Pilcher, Clifford S. Sato
Support: National Institutes of Health, Public Health Service
Tobacco Industry Research Committee
Frasch Foundation

The kinetics of the uptake of phosphate, the partition of this phosphate into diffusible and non-diffusible forms, and the kinetics of incorporation of phosphate into trichloroacetic acid (TCA)-precipitable phosphorus has been studied in pea stem sections by the use of radioactive phosphorus (P32). It was found that the diffusible phosphate of the tissue equilibrates with the phosphate of the external medium within 10 to 15 minutes but that the amount of non-diffusible tissue phosphate (probably contained in the vacuole) increases linearly with time for at least three hours. The amount of labeled phosphorus in the TCA-precipitable phosphorus fraction (about 10% of the non-diffusible phosphate fraction) also increases at a constant rate with time. If we assume that the incorporation of P32 into the TCA-precipitable phosphorus fraction is a reaction with first order kinetics, then the present data indicate that the P32 phosphate available for incorporation into TCA-precipitable form comes from the pool of diffusible phosphate rather than from the pool of non-diffusible phosphate. This interpretation is further supported by the finding that when tissue is moved from labeled phosphate to unlabeled phosphate solutions, so that the diffusible phosphate fraction of the tissue becomes unlabeled, then incorporation of P32 into TCA-precipitable form does not increase even though the section retains 95% of its P32 as non-diffusible material.

The P32-labeled ribonucleic acid (RNA) of the several cellular fractions was hydrolyzed with base and the nucleotides separated by paper electrophoresis. The individual nucleotides were then eluted and their specific activity determined. The specific activity of RNA nucleotides from the nuclear fraction was significantly higher than that of the various cytoplasmic fractions. Thus the nuclear RNA was found to possess a specific activity four times greater than that of the microsomal RNA and twice as great as that of supernatant RNA. These findings, which are in agreement with those on RNA synthesis in animal tissues, indicate that the nucleus is the most active site of RNA synthesis.
80. THE INCORPORATION OF AMINO ACIDS INTO THE VARIOUS CELLULAR COMPONENTS OF PEA SEEDLINGS

Investigators: Paul O. P. Ts'o, Jane Pilcher, Clifford S. Sato
Support: National Institutes of Health, Public Health Service
Tobacco Industry Research Committee
Frasch Foundation

Incorporation of labeled amino acids into the several cellular constituents of pea seedlings was studied after preliminary investigations had established appropriate washing procedures for rigorous purification of each fraction. Randomly labeled C14-leucine and C14-phenylalanine were used as the amino acids. In experiments in which tissue was incubated for varying periods from 15 minutes to 3 hours it was found that mitochondrial and nuclear protein possessed a higher specific activity (counts per minute per milligram of protein) than did the microsomal or supernatant protein fractions. Carbon-14 of the two amino acids was incorporated to only a very slight extent into the ribonucleic acid.

81. STUDIES ON AMINO ACID ACTIVATION

Investigator: John M. Clark, Jr.
Support: Kirk Macomber Fund
General Funds

In order for amino acids (AA) to be incorporated into protein, they must receive energy from an external source. This step in protein synthesis--enzymatic (Enz) amino acid activation--appears to involve the following mechanism:

\[ \text{Enz} + \text{ATP} + \text{AA} \rightleftharpoons \text{Enz - AMP} - \text{AA} + \text{PP} \]

which results in the formation of an acylated amino acid. Proof of the above mechanism has been obtained by quantitative study of the exchange of pyrophosphate into adenosine triphosphate (ATP). Plant tissues have been found to contain enzymes for activation of 12 different individual L-amino acids. The corresponding D-isomers are unaffected. In pea epicotyl homogenates and in tobacco leaf homogenates these enzymes are largely associated with the non-particulate fraction of the cells. The partial purification of amino acid activating enzymes from spinach acetone powders has been achieved.

82. RIBOSE NUCLEOSIDES AS PRECURSORS OF THE CARBON SKELETON OF DNA

Investigator: Walter S. McNutt, Jr.
Support: National Science Foundation

The question as to whether the deoxyribosides of deoxyribonucleic acid (DNA) arise from nucleosides of ribose has been examined through the use of two mutants of Neurospora. One of the mutants is unable to grow in the absence of a pyrimidine source. This mutant was fed uniformly labeled C14-cytidine. The ribosides of the ribonucleic acid (RNA) and the deoxyribosides of the DNA were isolated and their specific activities were determined. Cytidine, deoxycytidine, uridine and thymidine were highly labeled and had molar specific activities which were very similar to one another. Furthermore, the distribution of labeling between the pyrimidine and sugar portions of the nucleosides was similar to that of the administered cytidine, showing that the carbon skeleton of the pyrimidine deoxyribose of the DNA arose from intact cytidine molecules.

An unanticipated finding has been that the pyrimidine nucleosides of the nucleic acids from this completely blocked mutant were only about 0.7 as radio-
active as the administered cytidine. Thus it is evident that though the mutant cannot
grow in the absence of cytidine, it nevertheless has some capacity to synthesize the
pyrimidine nucleoside.

The second mutant used is one unable to grow in the absence of a purine
source. This mutant, when fed uniformly labeled C14-adenosine, was found to have
the purine ribosides of the RNA and purine deoxyribosides of the DNA similarly
labeled. The distribution of labeling between the purine and sugar portions of the
nucleosides of the RNA and DNA was again very similar, though with this mutant
the sugar portion of the nucleosides was diluted to a greater extent than were the
purine portions.

Attempts are being made to isolate enzymes from these organisms which
bring about these conversions.

83. THE BIOGENESIS OF A PTERIDINE

Investigator: Walter S. McNutt, Jr.
Support: National Science Foundation

The yeast Eremothecium ashbyii incorporates the pyrimidine ring of adenine
into the pyrimidine portion of the ring system of riboflavin. In the search for
intermediates between adenine and riboflavin a substance showing characteristics
of a 2,4-dioxypteridine has been isolated from cultures of this organism. Riboflavin and the pteridine, isolated from cultures which were fed C14-adenine, had
very similar molar specific activities, a fact which suggests a close biochemical
relationship between these two substances. Though attempts have been made to
show that this pteridine serves as an intermediate in the biogenesis of riboflavin,
we have been unable to obtain any evidence in support of this view. The most
which may be made of the evidence we have is that riboflavin and the pteridine arise
from a common biochemical intermediate.

This work was done in collaboration with Dr. Hugh S. Forrest.

Publications:

Walter S. McNutt, "The Incorporation of the Pyrimidine Ring of Adenine

Walter S. McNutt and Hugh S. Forrest, "The Incorporation of the C14 of
Adenine into a 2,4-Dioxypteridine by Eremothecium ashbyii, JACS
(in press).

Hugh S. Forrest and Walter S. McNutt, "Observations on the Structure
of a New Pteridine Isolated from Eremothecium ashbyii, JACS
(in press).

84. SYNTHESIS OF SQUALENE FROM ACETATE AND MEVALONIC ACID

Investigators: Laurence M. Corwin, Guy A. Thompson, Jr.
Support: National Institutes of Health, Public Health Service
Arthur McCallum Foundation
General Funds

Cell-free preparations of yeast possess the ability to synthesize labeled
squalene and labeled ergosterol from labeled acetate. The cell-free extract was
prepared according to the method of Klein in which the material is ground with
sand in powdered dry ice. After three hours of incubation at 30°C squalene was
isolated from the reaction mixture. Carbon of 3-labeled β-methylcrotonate is
incorporated poorly under similar experimental conditions. Carbon of β-methyl-
crotonyl coenzyme A (CoA) although somewhat more effective, is still less effective than acetate itself. It is believed that β-methylcrotonate is incorporated into squalene only after conversion of the β-methylcrotonate to a 6-carbon compound. The 6-carbon compound involved in squalene formation in yeast may be identical with mevalonic acid. In the present experiments authentic mevalonic acid (a) if supplied in unlabeled form to reaction mixtures containing labeled acetate greatly diluted the appearance of radioactive carbon in the final squalene and (b) if supplied as 2-labeled mevalonic acid was incorporated effectively into squalene.

Preliminary purification of the enzyme systems involved in conversion of acetate to squalene indicates that small particles (removable from the homogenate by centrifugation at 80,000 x g for 30 minutes after preliminary removal of large particles by centrifugation at 19,000 x g for 30 minutes) and soluble enzymes are required. That the soluble enzymes are essential for the conversion of acetate to mevalonic acid is indicated by the fact that the small particles alone are able to convert mevalonic acid in high yield into squalene.

85. ENZYMATIC STUDIES OF HEVEA LATEX

Investigator: Roderic B. Park
Support: Woods Hole Oceanographic Associates
          General Funds
          Frasch Foundation

A variety of C\textsuperscript{14}-labeled substrates including acetate and β-methylcrotonate (BMC) were incubated with Hevea (rubber tree) latex in the presence of adenosine-triphosphate (ATP), diphosphopyridine nucleotide (DPN), coenzyme A (CoA), ethylenediaminetetra acetic acid (EDTA), inorganic phosphate, and sucrose, under aerobic and anaerobic conditions. The rubber became most highly labeled under anaerobic conditions. The distribution of labeling in the rubber and other compounds was then investigated. The labeling in the rubber was determined by degradation to levulinic acid. Acetate was found to be the effective precursor of rubber as judged by radioactivity of the derived levulinic acid. BMC was less effective than acetate as a rubber precursor. A compound with the same solubility properties as rubber (soluble in chloroform, insoluble in 10% base) but slightly volatile at room temperature became very highly labeled from acetate under anaerobic conditions. ATP, DPN, and CoA enhanced incorporation of acetate into this material so that up to 15% of the label fed appeared in the volatile fraction. Treatment of the fraction with 3,5-dinitrobenzoyl chloride yielded a derivative which could be purified by chromatography. Incorporation of mevalonic acid into Hevea latex is currently under study.

86. METABOLISM OF METHIONINE IN THE OAT COLEOPTILE

Investigators: Clifford S. Sato, Richard U. Byerrum, Peter Albersheim
Support: Tobacco Industry Research Committee
         Arthur McCallum Foundation
         General Funds

The presence of indoleacetic acid increases the rate of incorporation of the methionine methyl group into the methyl ester groups of pectin in the oat coleoptile. This process may be related to the auxin-induced plasticization of the cell wall. A study of the mechanism of methyl transfer in this tissue is therefore of interest.

In living oat coleoptile sections as well as in homogenates, methionine is in part oxidized to methionine sulfoxide. This oxidation may be peroxidative since it is prevented to some extent by the presence of catalase. Methionine sulfoxide is active as a methylating agent, being about one-third as efficient in this function as methionine. Methionine sulfoxide does not appear to be converted to methionine.
Methyl methionine sulfonium is also found as a product of methionine metabolism in the oat coleoptile and is produced from methionine as well as from methionine sulfoxide both in vitro and in vivo. Experiments with sulfur-labeled materials have shown that the sulfur of methionine alone appears in methyl methionine sulfonium although methyl groups from both methionine and methionine sulfoxide appear in this material. No evidence has yet indicated that methyl methionine sulfonium can itself act as a methyl donor.

An attempt was made to isolate sulfur-labeled S-adenosyl methionine from an appropriate incubation mixture containing adenosinetriphosphate (ATP) and sulfur-labeled methionine. The attempt was unsuccessful, and no evidence for the presence of S-adenosyl methionine has been obtained. Relatively large quantities of sulfur-labeled homocystine appear in such homogenates, however.

**THE ISOLATION OF PECTIN FROM HOMOGENATES**

Investigator: Robert E. Cleland  
Support: Frasch Foundation, Arthur McCallum Fund, General Funds

Previous work has shown that the methyl group of methionine-C-14 is incorporated as the methyl ester group of pectin in intact oat coleoptile tissues. Attempts to study the reaction in homogenates have been hampered by the lack of a suitable method for separating the water soluble pectin from the unreacted methionine-C-14. Such a procedure has now been devised. The homogenates, after incubation, are heated for one hour to solubilize all the pectin. The supernatant is filtered off and centrifuged. The supernatant is then dialyzed for 24 to 36 hours against one liter of water at 4°C, using three changes of water. Reproducible results are obtained by this procedure. Reproducible isolation of pectin by precipitation with ethanol or by column chromatography has not proved possible.

Publications:


**FACTORS INHIBITING THE ANAEROBIC PERSISTENCE OF CELL-WALL LOOSENING**

Investigator: Robert E. Cleland  
Support: Frasch Foundation, Arthur McCallum Fund, General Funds

Treatment of oat coleoptile cells with auxin under aerobic conditions results in a loosening of the cell wall. The potential for expansion thus produced can then persist anaerobically for short periods of time if expansion is inhibited by excessive external diffusion pressure deficit. Some insight on the nature of this conservation of potential has been gained by the use of potassium cyanide (KCN) and ethionine.

Treatment of oat coleoptile sections with 5x10^-4 M KCN under anaerobic conditions, after aerobic treatment with auxin under conditions of non-expansion,
results in a loss of the expansion potential. Removal of the KCN does not result in restoration of the potential. Thus the loss of anaerobic metabolism does not merely suppress the potential but actually reverses the loosening process. Aerobic auxin treatment is then required for reinitiation of the potential.

Ethionine treatment also results in loss of the potential. Thus methylation must be part of the conserving process.

Publication:


89. CONCENTRATION DEPENDENCE OF AUXIN-INDUCED CELL-WALL PLASTICITY

Investigators: Mary Lou Whaling, James Bonner
Support: Frasch Foundation General Funds

It has been established earlier that the increase of growth rate of oat coleoptile sections which results from the supplying of such sections with auxin is due to an auxin-induced increase in cell-wall plasticity. The walls of each cell of the tissue are continuously under tension due to the forces exerted by the osmotic pressure of the cell contents. Decrease in the ability of the cell wall to withstand these tension forces increases growth rate of the tissue. It has also been shown earlier that the growth rate of Avena coleoptile sections rises with increasing auxin concentration to reach a maximum at a concentration of approximately 1 x 10^-5 M. Growth rate then drops off again to become 0 at approximately 10^-3 M.

It is also possible to measure plasticity of plant tissue by determining the resistance of the tissue to deformation under externally imposed load. A device suitable for the quantitative measurement of deformability of tissue under load has been devised by Tagawa. Auxin in low concentrations—those which induce increases in growth rate of tissue—also increases deformability of tissue under such externally imposed load. It has now been found, however, that as auxin concentrations are raised above those which bring about maximum rate of auxin-induced growth deformability of the tissue under external load continues to increase rather than to fall off as does growth rate.

It has been shown earlier that the concentration dependence of auxin-induced growth is quantitatively interpretable on the hypothesis that the auxin molecule must consummate a two-point attachment to some receptor entity within the plant in order to bring about such growth. Decrease in growth rate at high auxin concentrations is, according to this hypothesis, due to the occupation of the receptor site by two auxin molecules each attached through one point. The present results are not in agreement with this hypothesis. They indicate that the metabolic reaction in which auxin participates to bring about increased tissue deformability, as measured by response to external load, does not decrease at high auxin concentration and does not demand therefore interpretation as a two-point attachment mechanism.

Publications:


Several workers have shown that 2, 3, 5-triiodobenzoic acid (TIBA), 2, 4-dinitrophenol (DNP), iodoacetate, para-chloromercuribenzoic acid, phenyl mercuric chloride and certain maleimides inhibit the transport of auxins through stem sections. Such transport is of a special nature. It is polar and is from parenchymatous cell to cell. In the present experiments it has been shown that one of the compounds, TIBA, does not inhibit the transport of auxin in the phloem of intact bean plants. Transport of C\textsuperscript{14}-labeled 2, 4-dichlorophenoxyacetic acid (2, 4-D) from leaf to stem was followed by methods described earlier (see Biology 1956). In one group of plants, TIBA (100 mg/plant) was applied in alcoholic solution to the two petioles of each seedling 11 hours prior to 2, 4-D treatment. In another group of plants TIBA was applied at the same time as 2, 4-D treatment to the leaf. Pre-treatment with TIBA resulted in a two-thirds reduction in the amount of 2, 4-D (identified by paper chromatography) reaching the epicotyl in 5 hours. No reduction in the amount of transport occurred when TIBA was given at the same time as 2, 4-D. TIBA does not therefore compete with 2, 4-D in transport, but does interfere (after a lag period) with the transport mechanism itself.

The effect of TIBA on the transport of sucrose from the leaf was also investigated. Leaves of TIBA treated and non-treated were allowed to take up C\textsuperscript{14}-labeled carbon dioxide and the course of appearance of label in the rest of the plant was determined over a period of 4 hours. The rate of loss of label from the leaf was the same in both groups of plants. Sucrose appears to be the major, perhaps the only, compound transported from the leaf. When labeled sucrose was applied to the leaf, the amount transported to the epicotyl was negligible, due presumably to low absorption.

It is concluded that TIBA appears to be specific in its inhibition of polar auxin transport and has no effect on the transport of 2, 4-D or of sucrose in the phloem.

Studies on the Organ of Production of the Natural Gibberellic Factor in Pea Seedlings

Removal of the cotyledons, the roots or the stem tip from dark-grown pea seedlings results in rapid termination of stem growth, even though an adequate energy source is supplied. These various organs of the plant must therefore supply specific stem growth factors (hormones). Experiments were undertaken to determine whether gibberellic acid, a recently discovered hormone of higher plants, is effective in replacing any of these organs.

Gibberellic acid failed to restore stem growth rate in plants from which either
the cotyledons or the roots were removed, but when applied to plants from which the stem tip had been removed, it increased stem growth rate to a value almost equal to that of the intact plant. It is concluded that the natural gibberellic hormone of the pea plant is produced in the tip and moves to the elongating region of the stem where it promotes stem elongation.

Publication:


92. INTERACTION OF LIGHT AND GIBBERELLIC ACID ON THE STEM GROWTH OF NORMAL GARDEN BEANS

Investigator: James A. Lockhart
Support: Frasch Foundation
Rockefeller Foundation

It has been shown previously (Biology, 1956) that the application of gibberellic acid reverses the light-induced inhibition of pea stem growth. In an attempt to repeat these results with garden beans (Phaseolus vulgarus) it was established that normal (non-dwarf) beans do not respond to gibberellic acid when grown in darkness. When the plants were grown in light, however, the gibberellic acid-treated plants were taller than the dark-grown plants, while the untreated plants were, of course, inhibited by light.

With beans, as with peas, gibberellic acid treatment reverses the light-induced inhibition of stem growth. Light appears to have a further growth-promoting effect in the presence of gibberellic acid, which is normally masked by light inhibition of stem elongation.

Publication:


93. INTERACTION OF LIGHT AND GIBBERELLIC ACID ON THE STEM GROWTH OF DWARF BEANS

Investigator: James A. Lockhart
Support: Frasch Foundation
Rockefeller Foundation

Greenhouse-grown garden beans (Phaseolus vulgarus) of the bush (dwarf) varieties may be changed to the pole (normal) growth habit by the application of small amounts of gibberellic acid. During the course of work on the interaction of light and gibberellic acid it was found that dwarf bean seedlings do not respond to gibberellic acid treatment when grown in complete darkness. Dark-grown dwarf pea seedlings had previously been found to respond markedly to gibberellic acid treatment.

Since the dwarf beans responded fully to gibberellic acid treatment when grown in light, the minimum quantity of light necessary to elicit a response was determined. It was found that irradiation with red light (2,000 ergs/cm²/second) for 2 to 5 minutes results in a marked response of the plants to gibberellic acid. This red light effect could be reversed by far-red radiation, indicating that the light control of the gibberellic acid response is through the same red/far-red pigment system which controls many other morphological characters in plants.
Light-grown dwarf beans are changed to the normal habit by gibberellic acid, and like normal plants, do not respond to treatment when grown in darkness. It may be that these plants are "dwarfs" only when grown in light.

94. THE EFFECT OF TEMPERATURE ON THE RESPONSE OF DARK-GROWN PEAS AND BEANS TO GIBBERELIC ACID

Investigators: James A. Lockhart
Support: Frasch Foundation
Rockefeller Foundation

Peas (Pisum sativum) and bean (Phaseolus vulgaris) seedlings grown in complete darkness at or near their respective temperature optima show no significant response to gibberellic acid treatment. It has already been shown that application of gibberellic acid restores stem growth to its maximum rate following inhibition of stem elongation by exposure to light (Biology, 1956). Experiments were therefore undertaken to determine whether gibberellic acid treatment would also restore the rate of stem elongation in dark-grown seedlings whose growth rate had been repressed by sub- or super-optimal temperatures.

It was found that application of gibberellic acid in no case altered the maximum growth rate in darkness at any temperature.

95. EFFECTS OF GIBBERELIC ACID ON THE PHOTOPERIOD-CONTROLLED GROWTH OF WOODY PLANTS

Investigators: James A. Lockhart, James Bonner
Support: Frasch Foundation
Rockefeller Foundation
General Funds

Camellia plants maintained on short-day conditions remain dormant for extended periods of time, while transfer to long-day conditions results in an immediate flush of vegetative growth. Periodic applications of gibberellic acid to dormant plants maintained on short-days causes the plants to break dormancy and initiate a flush of growth completely comparable to that produced by plants maintained on long-days. Gibberellic acid application to plants growing in long-day conditions speeds the initiation of growth but has no effect on the final length of the new shoots.

Following the flush of growth under long-day conditions, the plants form a terminal bud and resume dormancy. Gibberellic acid has no effect on the onset of this dormancy.

The growth of Coulter pine (Pinus Coulteri) and big cone spruce (Pseudotsuga macrocarpa) is also promoted by long-day conditions and stopped by short-day conditions. Gibberellic acid treatments had no effect on these two species.

Publication:


96. ATTEMPTS TO INHIBIT VERNALIZATION

Investigators: C. I. Davern
Support: Commonwealth Scientific and Industrial Research Organization
Rockefeller Foundation
General Funds
Seedlings of subtropical clover (Trifolium subterraneum L.) were vernalized for eight weeks at 4°C in the presence of the following substances: chloramphenicol, aminopterin, dinitrophenol and 5-bromouracil. In no instance could a significant depression in the vernalization response be detected. Other seedlings, on the completion of their vernalization treatment, were immersed for various times in ribonuclease solution (1 mg/ml). Prolonged exposure (6 hours) killed all of the plants. Shorter exposures (1 hour, 3 hours) permitted some survival. Among the survivors no significant devernalization effect could be detected.

97. DIFFUSION KINETICS AND FREE SPACE IN POTATO TUBER SLICES

Investigator: George G. Laties  
Support: Rockefeller Foundation

In order to properly interpret kinetic studies of salt transport into cells of potato disks in experimental periods of short duration it has been necessary to ascertain the time required for diffusion equilibrium to be reached in those parts of the potato disk readily accessible by diffusion. It was also of interest to determine what fraction of the total cell volume offers no apparent barrier to diffusion of salts from the environment; i.e. what fraction of the total volume can be designated as free space.

To this end potato disks were gently shaken in solutions of potassium chloride (KCl) labeled with radioactive chloride (Cl\(^{36}\)). Temperature was maintained at 0°C to minimize metabolically mediated absorption. At the end of the experimental period groups of disks were gently blotted, dried on planchets, and their radioactivity determined; comparable groups of disks were placed into relatively large volumes of water at 0°C until whatever salt could freely diffuse from the disks had done so. The radioactivity of these disks was also determined. The freely diffusible chloride was determined by subtracting the activity of the second set of disks from the activity of the first set.

The half-time for diffusion equilibrium was found to be approximately 1.5 minutes, whether diffusion was measured into or out of disks 1 mm in thickness. As anticipated, the half-time was much the same at room temperature. The calculated free space was from 5 to 6% of total tissue volume and was essentially the same at concentrations of KCl from 0.01 to 0.07 M. This value for the free space proved independent of temperature, and was the same whether disks were rinsed in distilled water or in unlabeled 0.01 M KCl. From microscopic sections of potato prepared in the freezing microtome cell-wall thickness and cell diameter were determined. From these measurements cell-wall volume was calculated to be approximately 4% of the total volume.

98. THE DISTRIBUTION OF THE INTERCELLULAR GAS SPACE IN POTATO

Investigator: George G. Laties  
Support: National Science Foundation  
Rockefeller Foundation

Potato tubers have been found by others to be adequately supplied with oxygen throughout their bulk. On the other hand thin slices of incubated potato slices exhibit an inverse relation between thickness and respiration rate, and it has been commonly considered that the diminution in rate with increasing thickness reflects a decreasing oxygen supply to the deeper-seated tissue. The possibility was therefore investigated that the act of preparing slices resulted in the plugging with water of intercellular air passages and that the apparent coefficient of diffusion of air in potato tissue would thereby be markedly altered (diffusion of oxygen in air is almost \(10^6\) times as rapid as diffusion in water).
Slices and cores of potato tissue were purposely vacuum infiltrated in the hope of replacing air with water in the intercellular spaces. Absolutely no effect on the respiration as a consequence of this procedure was subsequently perceived. The ability to vacuum-infiltrate dyes into potato slices was studied, and it was observed that dyes failed to penetrate any more rapidly than could be expected by diffusion. An attempt was thereupon made to force dye into a potato slice 5 mm thick by replacing the slice for the filter pad in a Seitz filter, and applying a pressure of 5 atmospheres to the dye solution. Once again, dye moved into the tissue only in accordance with expectations for a diffusing system. Furthermore no water was forced through the disk at high pressures.

The picture which arises of the distribution of air spaces in potatoes is that of Jamin chains in which pockets of air are separated from each other by water barriers held in pores of very small diameter. Water is retained by capillarity in such pores with forces in excess of 5 atmospheres. Thus, under reduced pressure air can escape from the tissue, but the water barriers are not dislodged, and by the same token, water is not forced into the intercellular spaces when the vacuum is released. The foregoing concept of the nature of air spaces in potato slices seems to adequately explain why tissue slices fail to become water-injected after many days in water solution.

99. A NEW BASIS FOR THE INVERSE RELATION BETWEEN SLICE THICKNESS AND RESPIRATION RATE IN SLICES OF POTATO TUBER

Investigators: George G. Laties, Kenneth Fleshman
Support: National Science Foundation
Rockefeller Foundation

It has long been recognized that the respiration rate of freshly prepared potato slices considerably exceeds that of the intact tuber on a unit weight basis, and that the respiration of such slices rises several fold during the course of a 24-hour incubation period in air. These observations, together with the observed inverse relation between thickness and respiration rate in incubated potato disks, have led to the long accepted presumption that the metabolic events which are elicited by the preparation of slices are due to an increase in oxygen availability. The foregoing hypothesis must now be abandoned.

The respiration rate of freshly prepared potato slices proved to be independent of thickness on a unit weight basis. The inverse relation between thickness and respiration rate was however found to hold with slices which had been incubated for 24 hours in air. It can be readily calculated that the relation between thickness and respiratory activity in aged slices is not the consequence of the high respiration rate typical of incubated tissue. Experimental affirmation of this conclusion was obtained by study of the respiration of incubated slices in pure oxygen. Precisely the same inverse relation between thickness and respiration rate was observed in pure oxygen as in air. Thus, there is no evidence that the respiration rate in air was controlled by the oxygen tension.

The effect of slice thickness on the respiration rate of incubated disks was found to be upon the development of the age-induced respiratory increment, and not upon the manifestation of the developed respiration. Thus, if 1 mm slices were removed from the faces of incubated potato cores (12 mm in diameter, 20 mm long), only the surface slice from each face showed the development of a respiratory increment. Conversely, when aged slices 2 mm thick were split into three, the combined respiration of the three thinner slices was precisely equal to that of the parent 2-mm slice. Taken together the above experiments suggest that an endogenous volatile metabolite inhibits the development of the respiratory increment which arise in slices upon aging. It is considered that the metabolic system which arises during incubation produces the inhibitor of its own development. Only at the very surface of a slice is the inhibitor concentration deemed sufficiently low to allow the considerable
development of the new respiration system which previous experiments have shown to be qualitatively different from that found in fresh tissue.

100. THE UPTAKE AND UTILIZATION OF PHOSPHATE ASSOCIATED WITH RESPIRATION CHANGES IN POTATO SLICES

Investigator: B. C. Loughman
Support: National Science Foundation

The respiration of fresh potato slices increases 5-fold during a 24-hour incubation in water at 25°C. The phosphate content of the slices decreases during the incubation, only inorganic phosphate being released to the medium. An exponential increase in the ability to absorb phosphate from dilute solution occurs during the incubation; in 24 hours this increase is 200-fold. Approximately 80% of the phosphate absorbed by fresh slices in short periods is released during subsequent periods in water at +1°C, whereas phosphate absorbed by aged slices is retained under these conditions.

The capacity to incorporate the absorbed phosphate into organic fractions increases at a rate similar to the respiration rise. During a five-minute absorption period fresh slices esterify approximately 20% of the phosphate while up to 80% is esterified by aged slices. Phosphate incorporated by aged slices under these conditions can be recovered as 30% nucleotide, 50% hexose phosphate and 20% inorganic phosphate. It appears that the ability to absorb phosphate depends on the prior development of a respiratory and phosphorylative capacity which may be different from that existing in the whole tuber.

101. INHIBITION OF POTATO SLICE RESPIRATION BY LOW CONCENTRATIONS OF AMYL ALCOHOL

Investigator: George G. Laties
Support: National Science Foundation
Rockefeller Foundation

Amyl alcohol, a natural metabolite of potato tubers, has been shown by others to repress sprouting of potato buds at low concentrations. Since it has been deduced from experiments in this laboratory that the development of one or more new respiration systems in potato slices during incubation is controlled by an endogenous volatile metabolite, the effect of amyl alcohol on the rise in respiration in slices was studied. Complete suppression of any change in respiratory metabolism was observed when potato slices were incubated for 24 hours in 0.024 M amyl alcohol. When slices were removed from the alcohol solution the normally observed respiration rise took place during the subsequent 24-hour incubation period. The range in which amyl alcohol proved effective was small, little or no effect being manifest at 0.01 M concentration and irreversible injury taking place above 0.03 M. Although amyl alcohol satisfies the requirements for the hypothetical natural inhibitor in terms of its effect, the concentrations of amyl alcohol required to bring about the effect experimentally are very much higher than those found naturally in potato tubers. However, the effectiveness of this type of compound may provide a clue to the nature of the natural inhibition.

102. EVIDENCE FOR AN ENDOGENOUSLY PRODUCED VOLATILE INHIBITOR OF POTATO RESPIRATION

Investigator: George G. Laties
Support: National Science Foundation
Rockefeller Foundation
An analysis of the relation between respiration rate and thickness in incubated potato disks has shown that the development of the respiratory increment which occurs in potato slices upon aging is inversely related to thickness. It was deduced that an endogenous volatile metabolite of the age-induced respiration represses further development of this self-same respiration system. Attempts have therefore been made to collect the material.

Six to eight pounds of diced peeled potatoes were placed in a large desiccator which served as a respirometer chamber.

Air was circulated through the system at 3.5 liters/minute by a small diaphragm pump. The gas stream passed over a bed of Ascarite to remove carbon dioxide, and back into the respiration chamber. Oxygen was replaced from an oxygen reservoir, which in turn was kept at one atmosphere by water from an aspirator bottle operated on the Mariotte principle. The rate of oxygen displacement by water in the calibrated oxygen reservoir was taken as the respiration rate.

The respiration rate rose for some 20 hours in the closed system after which the rate diminished during the next 40 hours to well below the initial value. The respiration rise is presumably confined to the surface millimeter of tissue which, in a cube 1 centimeter to a side, constitutes half the total cube volume. If the desiccator was now opened and flushed with air for several hours the respiration rate, upon re-establishing the closed system, was soon equal to the maximal observed rate. However the rate now dropped to a minimal value in a matter of a few hours. If cubes were aged in a stream of air in an open system for two days, the onset of respiratory inhibition was immediate when the system was closed.

From the time-course of respiration and the inhibition thereof, it has been tentatively decided that the volatile inhibitory metabolite which presumably accumulates in a closed system is a product of the respiratory metabolism which develops when tissue is aged. Thus not only does this inhibitor affect the development of the respiratory increment, as shown with millimeter slices, but it also appears to inhibit the respiration when the concentration is built up in a closed system. An analysis in the mass spectrometer of the gas phase in a closed system 60 hours after the beginning of an experiment showed carbon monoxide as the only aberrant constituent. Although carbon monoxide was present to the extent of 0.4%, an effect of this concentration of carbon monoxide on the respiration would not be expected. Carbon monoxide may prove to be a break-down product of the time inhibitor. Alternatively inhibition may be accomplished by an entirely different component existing in a concentration too low to be detected in the mass spectrometer.

103. RACE-SPECIFIC INHIBITION OF RUST UREDOSPORES BY WHEAT SEEDLING EXUDATES

Investigator: Vernon D. Burrows
Support: Lucy Mason Clark Fund

In the breeding of wheat, emphasis has been placed on the development of varieties resistant to prevalent races of the rust Puccinia graminis. The mechanisms of resistance remain obscure. Studies of the mechanism of resistance are complicated due to the obligate nature of the pathogen and the failure to find in the past any direct inhibitory effect of plant extracts correlated with pathogenicity. A new approach to this problem has been developed. The guttation fluids from resistant and susceptible varieties of wheat have been tested for possible differential effects on the germination and growth of the uredospores of different physiological races of rust.

This was done by germinating uredospores of races 34, 56, 48-A and 39 in water and in the guttation fluid collected in the morning from the wheat varieties
Lee, resistant to all these races, and Marquis, which is susceptible to races 34 and 56 and moderately resistant to races 48-A and 39. The spores of each race are inhibited in germination and in growth by guttation fluid collected from Lee seedlings. The spores of races 34 and 56 are only slightly inhibited in the guttation fluid form Marquis. Spores of races 48-A and 39 are markedly inhibited in the same exudate.

Guttation fluid collected from either wheat variety at the end of the day did not show any inhibitory effect on spore germination. It has been found that this is due to the fact that the substances inhibiting spore germination only appear in guttation fluid of plants grown on short days and long nights (16 hours). Apparently the inhibitory substances are synthesized or released when the plants are in darkness.

104. INDUCTION OF EARLY FLOWERING IN PINE TREES

Investigators: James Bonner, Henry Hellmers
Support: U. S. Forest Service
National Science Foundation
General Funds

Forest tree breeding programs and forest management practices would be facilitated by knowledge of how to hasten and control flower production of timber species. The present study is being conducted to test on forest trees the methods known to hasten and control the flowering of other species. These methods involve varying temperature and photoperiod regimes.

Catkins were formed on one loblolly pine tree and female cones on three two-year-old trees. All flowers, male and female, were produced on the eighth node. While the eighth node appears to be significant it is not the only factor involved. The trees under moderate day temperature (23°C) and warm night temperature (26°C) have made over 15 flushes of growth but have not produced flowers. Present indications are that the length of photoperiod and its duration as well as the length of the cold treatment (17°C day with 7°C night) may be two of the more important factors.

105. STUDIES OF SEED GERMINATION AND SEEDLING GROWTH OF WESTERN CONIFERS

Investigator: Henry Hellmers
Support: U. S. Forest Service

Each year in California more and more timber land is dropping out of tree production. Most of this loss is due to unsuccessful regeneration of the logged or burned areas. The present studies, started during the last year, are being conducted to increase the fund of basic information on the germination of seeds and the growth of seedlings.

Two experiments now under way in the Earhart Laboratory will furnish data on the temperature requirements for optimum seed germination and seedling growth. The first species being tested are the coast redwood and Douglas fir.

106. GERMINATION PHYSIOLOGY OF FOREST PINE SEED

Investigator: Dennis Richardson
Support: U. S. Forest Service

A study of the effects of light, temperature and gibberellic acid on the germination of Douglas fir seed demonstrated that germination of non-stratified seed can
be accelerated by exposure to long (16 hours and above) photoperiods at temperatures below 20°C. At higher temperatures no such effect is apparent. This response appears to be a photoperiodic one since it is elicited by low light intensities of sufficient duration and by treatment with light of higher intensity but of shorter duration. Gibberellic acid at 5 parts per million accelerates germination but there is no evidence of any interaction of light and gibberellic acid. Investigation of the wavelength dependence of the light reaction revealed maxima in the red and blue regions.

An investigation of the influence of the above factors on radicle elongation in the same species was also made. Exposure to light markedly inhibits root growth at all temperatures between 14° and 26°C. This inhibition can be partially overcome by the application of gibberellic acid in concentrations ranging from three to ten parts per million according to the development stage of the radicle.
PLANT ECOLOGY AND PHYSIOLOGY -- EARHART LABORATORY

STAFF:
Professor: F. W. Went
Entomologist: William Hovanitz
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Research Assistant: Lillian Overland
Student Assistant: Robert M. Thornton
Laboratory Assistants: Josephine Arriola, Luiz C. Franco, Michael St. Marie
Earhart Laboratory Superintendent: G. P. Keyes; Assistant Superintendent, James E. Cunningham

107. EARHART LABORATORY INVESTIGATIONS

Investigator: F. W. Went
Support: General Funds

Work on a book describing the Earhart Laboratory and the results obtained in it has been completed this year. The book has just appeared in print under the title, "The Experimental Control of Plant Growth," and is published by the Chronica Botanica Company of Waltham, Massachusetts.

108. THE PHYSICAL BASIS FOR THE TEMPERATURE RESPONSE OF PLANTS

Investigators: F. W. Went, Lillian Overland
Support: General Funds

A physiological analysis of the temperature response of tomato plants and of peas had indicated that only at low temperatures (below 12 to 17°C) is their growth controlled by chemical processes. In line with this it had been found that auxin and other plant growth factors were effective largely when the plants were grown under cool conditions. At intermediate temperatures and at higher temperatures in the case of tomatoes, a physical, rather than a chemical process seems to control growth. It has been suggested that this rate limiting step may be translocation or some other diffusion process. A specific search for physical processes which might explain the temperature responses of plants has been made. Through work carried out by Drs. Hillman, Paton and Mr. Kristoffersen, it has become clear that one of the most important factors in the normal development of plants is the coincidence of an internal autonomous 24-hour rhythm with the external daily cycle of temperature and light. Some published work suggests that the internal autonomous cycle (the so-called Bünning cycle) is slightly temperature dependent, that is to say, the length of the cycle is increased somewhat at low temperatures. This matter has been reinvestigated, using the sleep movement of bean leaves. These movements were recorded at different temperatures with a lapse-time movie camera under weak infrared light. Increase in cycle lengths from 23-1/2 hours at 23°C, to 30 hours at 14°C, was found. This agrees with Bünning's earlier measurements.

Tomatoes grown at any cycle length other than 24 hours become abnormal. A plant normally adapted to a 24-hour daily cycle at a high temperature might not therefore be able to grow at low temperature because of lack of correspondence between the lengths of the external and the internal cycles. This has been tested in
a number of experiments with the following results: Saintpaulia (the African violet) dies when grown at 10°C on a 24-hour cycle. These plants grow normally at 10°C on a 32-hour cycle, however. For tomatoes, the optimal cycle length was found to be 27 to 30 hours at 10°C. It is therefore likely that in many plants, poor growth at higher and lower temperatures is primarily due to lack of coincidence between external and internal cycles. Work is now being initiated to identify the nature of the internal cycle.

Experiments have been carried out with pea plants grown either in continuous light and constant temperature, or under conditions in which either temperature or light, or both, were fluctuated over a 24-hour cycle. It was found that under constant conditions the plants soon decreased their growth rate, and became highly irregular, producing populations having a high coefficient of variability. This coefficient of variability dropped to a low level whenever either temperature or light was fluctuated. Variability did not further decrease when both light and temperature alternated on a 24-hour cycle. This is another indication of the importance of the Bünnning cycle for the normal development of the plant.

109. LEAF INJURY, GROWTH AND FLOWERING OF TOMATOES UNDER VARIOUS CONDITIONS OF TEMPERATURE AND DAYLENGTH

Investigator: Trygve Kristoffersen
Support: Norwegian Agricultural Research Council
National Science Foundation
General Funds

The injury to tomatoes which occurs in continuous light and constant temperature has been studied.

Leaf Injury: Plants grow and develop normally in continuous light provided the temperature is varied sufficiently over a 24-hour period. Cooling of the stem has little influence on preventing injury. Cooling of the root is as successful as cooling of the whole plant. Darkness followed by lower temperature with light is more effective in preventing leaf injury than lower temperature followed by darkness. Temperature and daylength cycles of 12, 48, and 72 hours have always produced injury. The plants are injured earlier and more heavily when they grow rapidly than when they grow slowly. Kinetin has so far been found to be the only chemical whose application reduces severity of the injury.

Growth: The highest dry matter production has been found in continuous light with fluctuating temperature, although under these conditions stem growth is reduced as compared with constant temperature and variation in light intensity.

Flowering: At 26° or 23°C the first flower cluster is formed after fewer leaves, possesses more flowers, and develops more rapidly than is the case with plants grown with variation in temperature (e.g. between 26° and 10°C) at constant light.

110. A STUDY OF THE EFFECT OF TEMPERATURE UPON A BIOLOGICAL RHYTHM

Investigators: J. Woodland Hastings, Beatrice M. Sweeney
Support: National Science Foundation
General Funds

Many animals and plants have been found to exhibit persistent rhythmic activity. It has been observed that some cellular activity fluctuates regularly with a period of approximately one day, and that this fluctuation will continue unabated even if the organism is kept under rigorously constant conditions. In several
cases, such as the bee and the fruit fly (Drosophila), it seems clear that the rhythm has an adaptive value and perhaps serves as a chronometer or clock for the organism. Previous work has shown that in many rhythmic organisms the temperature has little or no effect upon the period, i.e., the organism continues to mark off approximately a 24-hour period irrespective of the temperature. This fact is remarkable in itself, since we do not know how such a temperature-regulated physiological process works. In addition, it supports the speculation that rhythms function as biological clocks.

This study has been concerned with a rhythm of luminescence in a unicellular marine organism (Gonyaulax polyedra), which is responsible for a portion of the bright phosphorescence of the ocean. Under normal day-night conditions, the light emitted by the organisms is much brighter (30 to 60 times) at night than during the day. This variation continues when the cells are kept under constant conditions. The experiment was performed by placing cells in rooms at nine different temperatures and determining the character of the rhythmicity. It was found that in fact the duration of a period did change slightly with temperature, but in precisely the opposite way from what was expected. At higher temperatures (27°C) the length of a period was about 27 hours, and at lower temperatures (16°C) the period was 22-1/4 hours. These results suggest that temperature independence in biological clocks or rhythms is achieved by a temperature compensation or feedback mechanism, and that the case studied is one in which there exists overcompensation.

111. NORMAL AND CHLOROPHYLL-DEFICIENT PLANTS

Investigator: Arnulf R. Persson
Support: National Academy of Sciences
General Funds

The growth of two tomato strains has been compared under controlled conditions. The two strains used were derived from the variety Selandia and differed in a dominant gene A which affects chlorophyll concentration. The strain, heterozygous for dominant gene A, contained approximately one-third of the chlorophyll a concentration of the normal variety and one-eighth of the chlorophyll b content of the normal variety.

In general the two varieties were found to respond similarly to changes in temperatures over the range 17°C day and 11°C night to 30°C day and 22°C night. Over the entire range the chlorophyll-deficient plants produced approximately one-sixth of the dry weight of normal plants. The chlorophyll-deficient plants were also more responsive to changes in daylength than normal plants. Thus, dry weight accumulation in chlorophyll-deficient plants was decreased 25-fold as daylength decreased from continuous light to 6 hours of light per day, whereas in the normal plants dry weight production decreased only 12-1/2-fold under the same conditions.

112. THE INFLUENCE OF TEMPERATURE ON THE REACTION OF PLANTS TO ULTRAVIOLET RADIATION

Investigator: Ursula Brodführer
Support: American Association of University Women
National Science Foundation

The reported reactions of plant growth to ultraviolet radiation are mostly inhibitions; however stimulations had been observed by several investigators, especially when using the middlewave ultraviolet with wavelength not below 280 mµ. Open air investigations in the Swiss Alps at 1,500 meters altitude showed a different reaction of plants according to the amount of sun ultraviolet they received and the time of the season. In spring and fall only inhibition of plant growth was observed, whereas in summer stimulations up to 40 to 50% occurred. The aim of the investigations
carried out in the Earhart Laboratory and the High Sierra Station of the Carnegie Institute for Plant Biology in Stanford was to find out if the seasonal differences of the reaction of the plants are due to an interaction between growing temperature and ultraviolet radiation.

In the experiments at the High Sierra Station Timberline at 3,000 meters altitude the plants were grown in a warm and a cold series with a temperature difference of 6°C. Ultraviolet transmitting polyethylene and ultraviolet absorbing plexiglass were used as filters. In each series the plants received relative amounts of 100%, 66%, 33%, 16% and 0% ultraviolet in the region from 280 to 290 mμ to 340 to 360 mμ.

In the Earhart Laboratory the plants were grown at three different day temperatures in 17°, 23°, and 30°C greenhouses, and received daily 0, 3, 6, and 12 minutes ultraviolet radiation from a Westinghouse sun lamp with a continuous spectrum very similar to that of the natural sun. The plants used were Arabidopsis thaliana and the following alpine plants from the High Sierra: two clones of Mimulus tilingi, one clone of Mimulus tilingi x guttatus, Muehlenbergia filiformis (Gramineae), Achillea borealis, and Antennaria alpina var. media. All plants grew under long-day conditions. Records were kept of stem length, fresh weight, and dry weight.

Both in the open air investigations and in the Earhart Laboratory, the plants were predominantly inhibited by ultraviolet radiation at low growing temperatures. The highest stimulation occurred either with small, medium, or with the largest amount of ultraviolet used. With some plants it was as high as 50%. Different parts of the plant reacted somewhat differently.

The behavior of the plants under different temperatures while receiving ultraviolet radiation corresponds to that observed in Switzerland. The average growing temperatures during springtime in the Alps and of the cold series in the High Sierra were almost the same, and caused only inhibitions. The 6°C higher temperatures during summertime in the Alps and in the warm series in the High Sierra stimulated most of the plants noticeably.

Seasonal differences in the reaction of plants to ultraviolet radiation can be interpreted as different responses to ultraviolet at different growing temperatures.

113. THE EFFECTS OF CONSTANT AND FLUCTUATING AIR TEMPERATURE ON APPARENT PHOTOSYNTHESIS IN THE LEMON

Investigator: Frode E. Eckardt
Support: Rockefeller Foundation

Under constant conditions of light and temperature the apparent photosynthesis of lemon saplings growing in moist soil decreased slowly after an initial maximum value was reached shortly after the end of a dark period. The rapidity of this decrease varied among the different individuals, with the fastest response being found in saplings which underwent a previous brief complete drying out of the soil. In the drought-treated plants, one-tenth of the maximum value was reached after six hours in a constant environment, whereas a similar reduction in non-treated plants was obtained only after about two days. At first, it seemed likely that the reduction of photosynthesis was, to a certain extent, due to inability of the roots to absorb sufficient water. This was substantiated by the fact that transpiration, as well as the stomatal aperture, decreased simultaneously with the photosynthesis. However, it could be noticed that a drying out of the soil during the time of reduced photosynthesis caused, in a short time, an increase in the CO₂ uptake, thus indicating a rather high water content. Furthermore, an increase in the humidity of the air never produced any significant change in the slope of the gas uptake curve. When, in plants which strongly decrease their photosynthesis rate under constant conditions, the light was turned off and on every six hours, the average uptake of CO₂ increased.
by nearly 100%. In order to determine whether this was due to recovery from water loss or from the mere effect of changes in temperature, the aerial part of a lemon sapling was placed in constant light in a double-walled container. By circulating hot and cold water, the temperature was made to fluctuate every three hours between 10° and 20°C, and 20° and 30°C respectively. Under these conditions the average photosynthesis increased, after an initial drop, over a period of several days, finally reaching and remaining at the maximum value found immediately after the end of the dark period in previous experiments where the plant was kept at a constant temperature.

114. THE EFFECT OF DRYING OUT OF SOIL ON TRANSPIRATION AND APPARENT PHOTOSYNTHESIS

Investigator: Frode E. Eckardt
Support: Rockefeller Foundation

Tests of the rates of transpiration and apparent photosynthesis were carried out simultaneously on lemon and tomato plants during the time the soil was being dried out. The curves of CO₂ uptake and transpiration were found to be almost parallel in the lemon, both curves being characterized by a definite increase preceding the final drop. In the tomato, on the contrary, the transpiration decreased from the beginning of the drying process, even when photosynthesis was increasing. Simultaneous porometer measurements with the lemon showed clearly that increase of transpiration and photosynthesis was accompanied by opening of the stomata. In the tomato no really consistent results could be obtained, but it seemed likely that in both species the maximum aperture of the stomata was obtained with some degree of water saturation deficit. It could be shown that the cutting of the petiole of the leaf of fully turgid plants was always followed by a definite increase of transpiration, photosynthesis and stomatal aperture. In wilting plants the opposite was true. Other external changes applied to the roots, such as a lowering of the temperature or even a decrease of the atmospheric pressure (to 30 mm of mercury) could produce effects similar to those caused by drying out of the soil. The addition of a narcotic, sodium diethyldithiocarbamate, to the soil water did not speed up the rate of increase of photosynthesis as it would have done if active water uptake were involved. It could be noticed, however, that the decrease of CO₂ uptake following the reestablishment of normal pressure was somewhat slowed down. As a rule in wilting plants the stomata were closed when the plants were in the light and were open when in the dark.

115. THE EFFECTS OF PHYSICAL STIMULATION ON APPARENT PHOTOSYNTHESIS IN MIMOSA

Investigator: Frode E. Eckardt
Support: Rockefeller Foundation

When Mimosa pudica branches were irritated by shaking, the collapse of the leaves was followed immediately by a strong decrease of apparent photosynthesis. This was the case even when the light intensity was much higher than the optimal value for CO₂ uptake; for example 3,000 foot-candles produced a decrease of about 30% of the initial value. A similar effect was also observed in leaves fixed between nylon nets so that they could not move, although the reduction in photosynthesis then was less significant. In no case was it possible to detect any shock-induced decrease in CO₂ output after the light was turned off. At the time of the closing of the leaves the transpiration dropped as much as 10%, preceded occasionally by a slight initial increase. Similar effects could be brought about by increasing the root temperature. It seems likely that the observed changes in photosynthetic rate are to be attributed to changes in internal pressure. Alterations in internal pressure connected with the closure of the leaves are, however, on a much smaller scale than those induced by alterations of root temperature.
116. THE EFFECTS OF SOIL TEMPERATURE FLUCTUATIONS ON APPARENT PHOTOSYNTHESIS OF LEMON PLANTS

Investigator: Frode E. Eckardt
Support: Rockefeller Foundation

The effect of soil temperature changes on apparent photosynthesis has been studied in a series of experiments on lemon seedlings. It was found that in all cases when the soil was moistened to its field capacity, a decrease of temperature around the roots was followed by a significant increase of photosynthesis, whereas an increase in temperature was followed by a decreased CO₂ uptake. When the soil was dry, on the contrary, apparent photosynthesis increased with increasing temperature and decreased with decreasing temperature. These changes in CO₂ uptake were generally accompanied by similar and parallel changes in transpiration. This behavior can probably be explained by assuming that maximum CO₂ uptake is always associated with some water saturation deficit and that the amount of water taken up by the roots increases and decreases rapidly with the temperature. Changes in the temperature of the soil in the field may, therefore, have some effect on the rate of photosynthesis and transpiration. During the daytime the temperature increases at the upper parts of the soil and decreases during the night. As a matter of fact, it was possible by heating the soil during the latter half of the light periods and cooling during the latter half of the dark periods to obtain curves of CO₂ uptake which looked very similar to those characteristic of many plants under field conditions, i.e. with strong photosynthesis in the morning and with a sharp afternoon depression. On the other hand, as should be expected when the soil was drying out, photosynthesis was lower just after the light was turned on. Furthermore, the maximum value was reached at the same time of day that the depression had occurred before.

117. THE EFFECT OF TEMPERATURE GRADIENTS IN THE ROOT AREA ON THE AVAILABILITY OF SOIL WATER

Investigator: Frode E. Eckardt
Support: Rockefeller Foundation

Previous experiments carried out in the arid areas of North Africa showed that cooling of the upper parts of the soil by radiation at night caused considerable condensation of water vapors which originated principally in deeper soil layers and to some extent in dew. This condensation, even in the dry season, amounted to at least twice the total transpiration of the entire vegetation in an area. In spite of this large accumulation of water, nevertheless, the average suction force of the soil in the root area rarely decreases during the summer below 25 atmospheres, which means that normally under constant temperature conditions this water should not be available to the plants. In order to determine whether or not large soil temperature changes as they occur in nature can increase the availability of this water, and to what extent, the following experiment was performed. A tomato plant was grown in vermiculite in a shallow container, the bottom of which had been replaced by a nylon net. By placing this container one centimeter above the surface of one normal sodium chloride solution, so that a layer of air separated the solution from the vermiculite, and by varying the temperature of both the surface of the soil and of the solution by means of appropriate water jackets, it was possible to simulate conditions prevailing in the field. At the beginning of an experiment in which the temperature was the same throughout the set-up described above, the soil slowly dried out. When, due to this treatment, the apparent photosynthesis had been reduced to about one-third of the initial value the solution was then heated to 30°C at the same time as the soil surface was cooled to about 10°C. In a very short time, faster than would have occurred due to a mere watering, the transpiration and photosynthesis increased. A subsequent warming of the surface of the soil and cooling of the solution, after the complete recovery of the plant, induced only a decrease of photosynthesis and transpiration.
118. NEW EQUIPMENT FOR THE STUDY OF WATER RELATIONS IN PLANTS

Investigator: Frode E. Eckardt
Support: Rockefeller Foundation

A complete set of equipment has been built up for the study of water relations in plants. By means of double-walled plexiglass containers which can be cooled or heated by water, it is possible to maintain the temperature of the soil, as well as the air around the shoot, at any desired level. The temperature of the water used for this purpose is controlled by mixing hot and cold water by means of electric valves connected to electric time clocks; air and soil temperature can be made to fluctuate likewise at any given time intervals. The humidity and CO₂ content of the air around the shoot can be varied by mixing dry and humid air, and CO₂-free and CO₂-enriched air. Humidity around the roots is controlled by passing dry or humid air horizontally through the soil. By keeping this air at a temperature three to four degrees higher than that of the soil, it is possible to obtain a rapid moisture increase around the roots when desired, due to consequent condensation of water vapor. The air flow, controlled by a pressure regulator, can be measured by a rotameter. Transpiration is measured by comparison of the humidity of the air before and after passing through the container housing the plant. This is done by means of Aminco sensing elements placed directly in the air stream. Uptake and release of CO₂ are measured by a Liston Becker infrared gas analyzer connected to a Honeywell recorder. By means of electric switches, the CO₂ content of the incoming and of the outgoing air is recorded. Stomatal aperture can be measured by a porometer built into the container housing the plant. The essential part of the equipment is built on one of the high trucks used regularly in the Earhart Laboratory, so that it can be moved easily between the different temperature rooms in the building.

119. DESERT RESEARCH

Investigators: F. W. Went, Lloyd P. Tevis, Jr., Lillian Overland
Support: Mrs. Austin McManus Fund for Desert Research
National Science Foundation
General Funds

A truck and trailer has been developed as a unit and is now a self-contained laboratory which can be taken out into the desert to do observations and physiological research. Work along two lines was initiated.

1) Through transpiration measurements of desert plants with the transpiration meter developed by Dr. Grieve the daily march of transpiration of a number of desert plants was determined. It was found that under extreme drought conditions transpiration of some herbs like Cucurbita palmata was very rapid in the early morning hours, and then decreased to a low rate later in the morning. Desert shrubs like Larrea also decreased their transpiration but to a much lesser extent.

2) The structure of a number of leafless desert plants with green stems was investigated. It was found that whereas in a number of plants such as Beloperone californica, Salazaria, Ephedra, and Stephanomeria, the green structure of the stem was obtained through a great variety of arrangements of epidermal and palisade cells, in another group of plants such as Cercidium, Hoffmanseggia, Dalea spinosa and Asclepias subulata the cortical structures of the stem turned out to be almost identical. In other species of the same genera such as in Dalea emoryi a completely different stem structure was found. Such findings constitute splendid examples of parallel development.

3) Soil and seed samples from desert areas studied by Lloyd Tevis were investigated in the Earhart Laboratory. Good agreement between field observations and laboratory response was found.
4) The feeding habits of the black harvester ant, *Veromessor pergandei*, were investigated in a sandy area where the vegetation is chiefly ephemeral. The ants survived a long drought, during which there was no replenishment of food in the sands, by collecting seeds of *Plantago* and *Pectocarya*. These were the only kinds that were not rare. But when rains in January ultimately produced a new crop, the ants showed a decided preference for seeds of *Oenothera clavaeformis*, *Malvastrum*, and *Mentzelia*; then they avoided those of *Plantago* and *Pectocarya*. Selective feeding of this sort undoubtedly influences the composition of the ephemeral flora from year to year.

5) A study of a population of desert ephemerals germinated by less than an inch of real rain showed that certain species were better adapted to the particular conditions prevailing in the spring of 1957 than were others. Between germination and maturity, *Plantago*, *Malvastrum*, and *Geraea* increased in relative numbers while plants that required more moisture, such as *Abronia* and *Oenothera deltoides*, decreased.

6) Each month a different but comparable area of sandy desert was sprinkled once with a known amount of water to simulate rain and germinate whatever seeds might be in the sands. During the summer, only *Abronia*, *Bouteloua*, and *Euphorbia* germinated. In the winter, 15 species were obtained, not including *Bouteloua* and *Euphorbia*. Study of growth and mortality indicated that some species did better if they germinated early in the season while others favored a late start.

120. INHERITANCE OF HEAT TOLERANCE IN SEVERAL TOMATO CROSSES

Investigator: Lester W. Schaible
Support: Campbell Soup Company

Data collected from a previous series of experiments conducted at Earhart conclusively indicated that within the tomato species *Lycopersicon esculentum* L. there are relatively high inherent levels of heat tolerance. Certain small-fruited, semi-wild varieties of tomatoes were able to set a relatively high percent of fruit when grown at night temperatures of 23° and 26°C whereas commercial varieties such as *Rutgers*, *Sioux*, and *Improved Garden State* set no or very few fruit. A number of other varieties manifested an intermediate level of heat tolerance.

In order to determine the inheritance pattern of heat tolerance in the tomato, first and second generation progenies of the following crosses were grown at night temperatures of 17°, 23° and 26°C:

- (Narcarlang high tolerance) X (Rutgers)
- (Porter high tolerance) X (Rutgers)
- (Texas 966 intermediate tolerance) X (Porter)

In addition the following first generation progenies were evaluated:

- (Rutgers low tolerance) X (Sioux)
- (Sioux low tolerance) X (C17B-54)
- (Rutgers low tolerance) X (C17B-54)

Approximately 18 F3 progenies from the (Narcarlang X Rutgers) cross were
also evaluated for heat tolerance.

The objectives of this study are to determine the inheritance pattern of heat tolerance and whether or not small fruit size is linked to high heat tolerance. The data collected to date indicate that both heat tolerance and fruit size are quantitative characters, determined by many genes. The genes which govern heat tolerance are not closely linked to those which govern fruit size. A wide range of recombinations has been obtained and a number of segregates have been found which combine the setting ability of the small-fruited heat-tolerant parent with 50 to 75% of the fruit size of the large-fruited, non-heat-tolerant parent.

121. TEMPERATURE-INDUCED VARIABILITY IN THE GROWTH AND DEVELOPMENT OF PEAS

Investigator:  Harry R. Highkin
Support: National Science Foundation
General Funds

The studies reported in Biology 1956 on quantitative inheritance in peas have been continued. As previously reported the environment has an effect on the growth and development of plants. That this effect is more profound than is generally realized is shown by the fact that apparently the immediate environment not only affects the growth and development of the plant, but also affects the growth and development of its offspring. The offspring are affected to the extent that their reaction to a new environment seems to be dependent somewhat on the environment under which they were developed.

Two homozygous strains of peas—Unica, which has been developed and cultivated in Holland, and L5, a genetic strain from Sweden—were grown in three different controlled-environment greenhouses. Third generation seeds from each environment were planted in a common environment, together with samples of the original Holland-grown seed.

The results have shown that the groups of seeds from different environments react differently to the same constant environment. Not only is the difference between the means highly significant, but the coefficients of variability are also significantly different.

These strains of peas have been raised at the constant day and night temperatures of 10°, 14°, 17°, 20°, 23°, and 26°C for as many as 9 generations. In each group, regardless of the temperature, it has been found that from the 1st to the 5th or 6th generations the general vigor of the plants decreased gradually, but from the 6th to the 9th generations the vigor maintained approximately the same low level.

When seeds that had developed at any given constant temperature after 5 to 7 generations were returned to the normal control environment (20°C during the day and 14°C during the night) they behaved as though they were still in the previous constant temperature. The seeds formed on these plants when (1) grown in the normal control environment, reverted to a normal type; (2) grown in the original constant temperature, reacted to this environment as though they had never been removed.

122. THE EFFECT OF TEMPERATURE AND LIGHT INTENSITY ON THE EXPRESSION OF A GENETIC CHARACTER IN MAIZE

Investigator:  Harry R. Highkin
Support: National Science Foundation
Frasch Foundation
General Funds
The expression of a necrotic mutant in corn has been shown to be dependent on the environment under which it has been grown (Biology 1955). Under ordinary greenhouse conditions this mutant segregates in a ratio of 3 normal to 1 necrotic. The ratio, however, can be reversed by proper manipulation of the environment. Under high temperatures and high light intensities the necrotic character can become dominant.

The character is expressed by the guttation of a reddish-brown liquid in the seedling followed by a systemic water-logged appearance of the leaves and shortly thereafter death of the plant. It has been shown that the guttation fluid contains a large amount of sugars. Since high sugar content is characteristic of the fluid found in phloem and not that found in the xylems, the high sugar content of the guttation fluid indicates a breakdown of the phloem tissue. Anatomical studies of this mutant are being carried on by Dr. Katherine Esau of the University of California (Davis) to determine whether the phloem is in fact involved.

123. THE INHERITANCE OF VERNALIZABILITY IN PEAS

Investigator: Harry R. Highkin
Support: National Science Foundation
Frasch Foundation
General Funds

The inheritance of vernalizability in peas indicates that this character is not generally inherited in a simple manner. Reports of similar studies of Petkus winter rye have indicated that there is a single gene difference between the spring and winter habits, i.e. for the requirement of a cold treatment in order for flowering to occur. Our studies indicate that in peas the character is not a simply inherited one but is quantitative rather than qualitative in nature.

124. VERNALIZATION INDUCED BY NUCLEOSIDES AND DIFFUSATES

Support: National Science Foundation
Frasch Foundation
Rockefeller Foundation
General Funds

The work on the effect of diffusates and nucleosides in replacing vernalization of winter rye as reported in Biology 1955 is being continued. The diffusates have proved to contain more than one active principle as demonstrated by paper chromatography of the active fractions.

125. VERNALIZATION OF RADISH

Investigator: Harry R. Highkin
Support: Frasch Foundation
Rockefeller Foundation
General Funds

An important difference between winter annuals and biennials is the fact that while the winter annuals can be vernalized during germination, the biennials cannot; the latter require that development to a certain stage of maturity take place before cold treatment becomes effective. That this difference between the two groups is not general is however shown by the fact that three varieties of radish (Raphanus satirum), classified as biennials, have been found which can be vernalized during germination. More important the requirement for cold is absolute in two of the varieties, making them excellent test organisms for activity of vernalization-inducing materials.
STUDIES ON THE INITIATION OF SPORANGIA IN FERNS

Investigator: L. G. Labouriau
Support: Brazilian Government
Rio de Janeiro Botanical Gardens
National Science Foundation
General Funds

Studies on the initiation of sporangia in ferns are required: 1) as an extension of studies on flower initiation to homologous reproductive structures of lower vascular plants; 2) as a means of understanding the physiological causes involved in the determination of the alternating generations (since sporangia are the characteristic organs of the sporphyte).

Initiation of sporangia is essentially a differentiation of mass meristems, which, in many ferns, arise from a single cell initial. The place where these meristems are established and developed is not rigidly determined in the life cycle and this differentiation is a chain process that can be altered at many steps. The establishment of primordia of sporangia in leaves of adult plants follows different patterns in different species of ferns, both as regards the relative time of differentiation (simultaneous, successive or intermittent for sporangia of the same group) and spatial distribution. Establishment of primordia of sporangia is invariably correlated with leaf veins, as was established by experiments with Asplenium bulbiferum.

Environmental factors were found to affect the initiation of sporangia in some species of ferns.

The quantitative long-day behavior of Asplenium bulbiferum, previously reported, was confirmed and the critical nightlength was determined to be 23 hours, at 20°C, by experiments in 48-hour cycles.

A quantitative long-day behavior was found for Osmunda Claytoniana at 17°C. Experimental results indicate that, for this species, the effects of an increment in the length of day and in temperature are additive. The reverse effects prevail for the determination of an undifferentiated leaf as a scale-leaf. These scale-leaves were found to arise by necrosis of the pinnae, which may strike at leaves of different ages and degrees of previous differentiation.

The long-day behavior of Salvinia rotundifolia at 17°C was confirmed.

Regnellidium diphyllum was found to have a thermoperiodical requirement for the initiation of sporangia. This requirement can be met by daily fluctuations of temperature, or by a continuous exposure to low temperatures followed by a period at higher temperature. In both cases the fundamental requirement seems to be an exposure of the axillary buds to low temperature.

THERMOPERIODIC AND PHOTOPERIODIC CONTROL OF FLOWER INITIATION

Investigator: D. M. Paton
Support: Gosney Fund
National Science Foundation

The environmental control of time of initiation and node position of the first flower was studied in the late pea variety, Greenfeast. The environmental variables included photoperiod (8, 16, and 24 hours) combined with a range of either constant or varied temperatures (7°C to 26°C). Both photoperiod and temperature treatments were based on a 24-hour cycle.

The results show that:
1) Growing temperature affects the rate of node formation but photoperiod has very little effect. Since variation in one of the experimental conditions produces different rates of node formation, the node number of the first initiated flower is not a reliable quantitative measure of flowering response. Thus, comparisons of treatments grown at different temperatures are best made between mean number of days from soaking of the seed to flower initiation.

2) The temperature conditions during vegetative growth modify the photoperiodic response. In the absence of temperature variation during the dark period, plants grown in 8 hours' photoperiod (P_8) flower 25 to 60 days later than plants grown in 16 hours' photoperiod (P_{16}). A temperature change of 10°C to 19°C during the dark period almost eliminates the photoperiodic response, P_8 plants initiating the first flower only 6 to 10 days later than P_{16} plants.

3) Variation of temperature during the dark periods is the major factor causing acceleration of flowering in P_8 plants grown between 7°C and 26°C. The greater the difference between the two temperatures given during the night in two 8-hour periods, the more flower initiation is accelerated. This thermoperiodic response is relatively unaffected by a) the temperature of the 8-hour photoperiod; b) the magnitude of the temperatures used for the temperature change; e.g. a change from 7°C to 17°C has the same accelerating effect as a change from 17°C to 26°C; and c) the order in which the two temperatures are given: 7°C followed by 26°C has the same accelerating effect as 26°C followed by 7°C.

These results show that the thermoperiodicity described by Went for vegetative growth is also very important in the control of flowering behavior. Such thermoperiodicity provides the explanation of the differing results obtained from field grown plants and plants grown under controlled conditions and needs to be taken into account in any experiments concerned with flowering.

128. STUDIES ON TUBER FORMATION IN POTATOES

Investigators: F. W. Went, Lillian Overland
Support: General Funds

For a long time it has been a problem whether, when potato plants are grown in too warm a climate, a so-called degeneration of the plants sets in. This has often been attributed to diseases which gradually invade the potato crop, since it is reproduced vegetatively. Because it is possible to keep all disease organisms out of the potatoes in the Earhart Laboratory, one clone of potato (Kennebec) has been grown for a long series of generations. The tubers were produced in the warm or in the cool greenhouses, and track was kept of the effect which the growing conditions of the parent tubers had on the growth and tuber formation of their offspring. It was found that there are very pronounced aftereffects of the various growing conditions. When the parent potatoes were grown in a cool greenhouse (17°C day and 12°C night temperature) their offspring produced more than twice the weight of tubers than when the parent tubers were grown at 26°C during day and 20°C during night.

Effects of the environment of the grandparent were still discernible in these experiments. This work forms a counterpart to Highkin's and shows that under influence of the growing condition changes occur in plant material which can be carried over for several generations.

129. INSECT STIMULATION OF PLANT GALLS

Investigator: William Hovanitz
Support: American Cancer Society
The work on this subject started last year has been continued. Gall development by saw flies in the leaves of willows has been seen to proceed in the following manner: The female saw fly inserts an egg sideways into the under side of the midrib of the young leaf and pushes it into the blade of the leaf alongside the midrib, together with considerable fluids of ejaculation. The color of the leaf at that point begins to change almost immediately from a normal green to a pale yellow surrounding the bulge of the egg. A reddish color may also appear in this area. Growth and differentiation proceed both before and after the hatching of the insect egg, but there is no indication that growth will continue if the insect egg does not hatch. On the contrary, growth of the gall seems to be inhibited and incomplete if the egg ceases its growth. This fact is indicated also by the early cessation of growth of galls in those cases where an insect from one variety of willow lays on another variety to which it is not adapted. Cessation of growth in this case is probably due to the death of the larva on the wrong foodplant.

With the aid of Drs. Weigle, Klostergaard and McNutt, a preliminary analysis of the contents of an accessory sac of the female reproductive system has been made. With the phase microscope, the contents appear to be spiral or non-spiral rods of various lengths and widths. Ultraviolet absorption indicates the presence of nucleic acids. Other tests indicate the presence of ribonucleic acid in quantity, but little if any desoxyribonucleic acid.

This material increases growth rate of young plant tissue cultures but has no effect on older ones.

Host-parasite specificity is great in the saw-fly willow adaptation. Of the many strains of willows in the laboratory, all have been identified by taxonomists as of the same species; the same is true of the many saw flies grown and tested in the laboratory. Yet the specificity is almost uniquely a matter of a single population of flies on a single plant or at least on plants growing along a single stream. This high degree of specificity is paralleled by different tolerances possessed by both the various strains of willows and the saw flies to temperature and moisture conditions in the laboratory and in the field. In addition, the insects may or may not be possessed of the ability to go into diapause in their larval or pupal stages. The willows vary greatly in their ability to withstand dry root conditions, low humidity, etc., and to recover from wilt. The various strains of willows differ too in the vigor of the tissue cultures which are derived from them.

130. FACTORS AFFECTING TUBERIZATION OF CARROT ROOTS

Investigator: Samuel M. Caplin
Support: National Institutes of Health, Public Health Service General Funds

The ability of carrot root tissue to proliferate rapidly in the presence of coconut milk and of tobacco but not of carrot seedlings to develop tumorous proliferations in medium containing coconut milk prompted a study of (a) the environmental factors involved in normal tuberization of carrot roots, and (b) why intact carrot seedlings will not respond to coconut milk.

Four varieties of carrots, a commercial and three inbred strains, were grown in the Earhart Laboratory under different environmental conditions: (a) ranging from 30° to 17°C under constant temperature and with a lower night temperature, (b) under greenhouse vs. fluorescent light, (c) increasing time under fluorescent light, and (d) different intensities of fluorescent light. Growth responses were produced which varied from tiny plants with spindly, untuberized roots to fairly tall plants with large roots. The results indicate that the lowest constant temperature used, highest light intensity and greatest light duration produced the largest roots. A subsequent experiment has been set up with temperatures ranging from 30° to 4°C with two previously tested and two additional varieties.
131. SOURCES OF COCONUT MILK GROWTH FACTOR

Investigator: Samuel M. Caplin
Support: National Institutes of Health, Public Health Service
        General Funds

Following discovery of the marked growth-promoting effect of coconut milk on
carrot root and other tissues a search for other sources of similar activity (CMF)
has uncovered like potent effects in endosperm of corn, English and black walnut
and horse chestnut.

These substances appear to be present in large-seeded fruits in which a de­
layed embryo development permits the accumulation of endosperm. Additional
plants tested were peach, avocado and onion. A preliminary experiment indicates
that peach endosperm is almost as potent as coconut milk in promoting the growth
of carrot root explants.

CMF activity has also been reported for crown-gall tumors. Because such
tumors are known to contain a relatively large amount of auxin, assays are being
repeated with carrots of known low responsiveness to indole-3-acetic acid.

132. INHIBITION OF THE GROWTH-PROMOTING EFFECT OF COCONUT MILK
        BY INDOLE-3-ACETIC ACID

Investigator: Samuel M. Caplin
Support: National Institutes of Health, Public Health Service
        General Funds

Optimal concentrations of coconut milk and indole-3-acetic acid (IAA) together
induced less growth of standard explants from carrot roots than did coconut milk
(CM) alone. Experiments were performed using different concentrations of both
CM and IAA. When a suboptimal concentration of CM was added to different con­
centrations of IAA growth was decreased regularly with increasing concentrations
of IAA. A complementary experiment with suboptimal IAA added to increasing
concentrations of CM produced decreased growth at all concentrations of CM--the
lower the concentration the greater proportionately the amount of growth depression.
The data are suggestive that IAA may act together with coconut milk type factors
in the plant in the control of certain aspects of plant growth.

Publication:


133. SINGLE CELLS FROM PLANT TISSUE CULTURES

Investigator: Samuel M. Caplin
Support: National Institutes of Health, Public Health Service
        General Funds

A piece of tissue in culture medium in a modified florence flask rotating on its
side will in the course of a few weeks fragment and multiply, forming a soup with
culture pieces ranging from several millimeters to single cells. Sedimentation in
test tubes permits easy separation of the single cells from the larger pieces. Cells
are generally spherical or sausage-shaped, containing a nucleus suspended by cyto­
plasmic strands and sprinklings of bright green chloroplasts. Because leaching
from cells may be a factor affecting their ability to grow, slide cultures containing
small amounts of liquid or agar culture medium with different proportions of old
and fresh nutrient are being prepared for observation of selected isolated cells.
134. IAA AND GIBBERELLIC ACID

Investigator: Samuel M. Caplin
Support: National Institutes of Health, Public Health Service
General Funds

Experiments with kinetin and gibberellic acid (GA) have shown little effect on the growth of carrot root explants. A combination of indole-3-acetic acid (IAA) with GA, however, produced a marked response over the effect of either; IAA with kinetin at the concentrations used produced no great effect. In a more extensive experiment being prepared a careful examination of the cells will be made.

135. TISSUE CULTURES FROM TOBACCO SPECIES AND THEIR HYBRIDS

Investigator: Samuel M. Caplin
Support: National Institutes of Health, Public Health Service
General Funds

Tissue cultures from coconut milk-induced tumors on seedlings of tobacco have shown characteristic differences when obtained from different species or their combinations. Of the 28 now being maintained in the laboratory some are white, others yellowish in appearance, with a few producing chlorophyll and becoming pale green. Many cultures are symmetrical and compact, others irregular and somewhat friable. Most cultures remain undifferentiated. Of particular interest is the development of small leafy shoots on cultures from certain species when infrequently subcultured. Tests for the accumulation of a diffusible morphogenetic substance in the culture medium by growing these together with cultures from a closely related species produced no change in the latter.

Publication:

136. MINERAL OIL FOR CONSERVATION OF PLANT TISSUE CULTURES

Investigator: Samuel M. Caplin
Support: National Institutes of Health, Public Health Service
General Funds

An overlay of sterile mineral oil, used previously by other workers for conservation of fungus and bacterial cultures, may also be used to conserve plant tissue cultures growing on agar media. Advantages consist in slowing down growth and in eliminating water loss by evaporation from culture media. Less frequent subculture is therefore necessary. Tissue cultures from many species are now being grown in this way. In experiments with uniform carrot root explants the amount of growth was reduced as the depth of oil increased. In light the ability to synthesize chlorophyll was unaffected.

Publication:

137. STUDIES ON THE DEVELOPMENT OF Vicia graminea

Investigator: L. G. Labouriau
Support: Brazilian Government
Rio de Janeiro Botanical Gardens
National Science Foundation
General Funds
F. Ottensooser has found that the seeds of Vicia graminea (native to Southern Brazil) contain materials that specifically agglutinate human erythrocytes of the N group. Since the culture of the plant has never been achieved outside of its native habitat, the physiological requirements for its development were investigated in the Earhart Laboratory.

Seed germination was found to be delayed by low water permeability of seed coats and this can be overcome by scarification of seeds. Germination is not light dependent and has an optimum temperature of 14°C. Germination does not take place at 30°C and at this temperature this germination cannot be caused by charcoal or by periodical washing of the imbibed seeds with water. Such seeds do germinate, although with a delay, when transferred to 14°C.

Good vegetative development takes place at temperatures of 7°, 10°, 14°, 17° and 20°C. Flowering does not take place in short days. Under long-day conditions 60 days were required from germination to flower initiation at 10°C and at 14°C. The same time is required for flowering under long days and with day temperatures of 20°C and night temperatures of 14°C. Under these conditions seed production is abundant. The seeds were found by Professor Owen to have the anti-N activity described for plants grown in their native habitat. The seeds are dormant for 30 days after harvest.

138. A HYPOTHESIS CONCERNING THE ORIGIN OF PETROLEUM

Investigator: F. W. Went
Support: General Funds

A paper has been written suggesting that although most petroleum source rocks are marine deposits, a considerable portion of the petroleum is derived from land plants. The volatile plant products, such as terpenes, which evaporate from conifers, sage brush, and other aromatic vegetation are being particularized in the atmosphere under the influence of light in the same way that gasoline vapors become a blue smog cloud, under the influence of light. The blue hazes which are found especially during summer over all vegetated areas of the world must have their origin in such volatile plant products. It is suggested that the particulate material in this blue haze is the condensed and polymerized aromatic content of the air. This is carried down by rain and snow and accumulated in river sediments. The amount of aromatic material produced each year by the land vegetation more than suffices to account for most petroleum now found in the world.

This work was done in collaboration with A. J. Eardley of the University of Utah.

139. COFFEE RESEARCH

Investigator: C. Franco
Support: IBEC Research Institute

Several subjects in connection with growth and flowering of coffee were studied. A number of experiments on flowering were repeated with the same results as Dr. Mes obtained in 1954. Seed storage experiments showed that at a relative humidity of about 50% of the air, coffee seeds will remain viable for at least a year, whereas they normally lose their viability within six months.

Cold and heat injury was produced by subjecting portions of the stem to temperatures just below freezing and to temperatures of 50°C. Typical injury symptoms were produced looking very much like what had been observed in the field after a cold spell.
Much work was carried out on root systems and the effects of temperature on them. It was found that optimal growth was obtained with a fluctuating root temperature, and that any temperatures, either below or above the normal root temperature in the greenhouse, reduced growth. When the roots were kept at 38°C or higher, death occurred in the plants within a few months. Plants grown with all their roots in a nutrient solution transpired less, and also grew somewhat slower than plants with part of the root system developed outside of the nutrient solution.

140. SEED LONGEVITY

Investigator:  F. W. Went
Support: General Funds

The seed longevity experiment, which was started in 1947 when seeds of approximately 100 native California plants were sealed under vacuum, was tested in its tenth year. It was found that the majority of seeds showed the same germination percentage as at the outset, and therefore had not aged. Only the seeds in a few tubes which were cracked and in which the vacuum had disappeared had lost their viability, indicating the importance of the vacuum for preservation of viability.

The five-year germination tests had shown no change in viability at all, but after the tubes were opened viability of the seeds gradually dropped to zero in the course of four years of open storage. In some cases an after-ripening of the seeds (Carpenteria, Sisyrinchium) was observed, but not during vacuum storage.
PSYCHOBIOLOGY

STAFF:
Professor: R. W. Sperry
Research Fellows: John T. Eayrs, Gilbert M. French, Allan M. Schrier, Frederic G. Worden
Graduate Students: Ivan J. Mayfield, Colwyn Trevarthen
Research Aides: Harbans L. Arora, Lois Cooper
Laboratory Assistants: Charlene M. Chapman, Charlotte Hoffman, William Thon, Frieda Van Harreveld

141. ISOLATION OF CEREBRAL MECHANISMS FOR LEARNING AND RECALL OF TACTILE DISCRIMINATION

Investigators: R. W. Sperry, A. M. Schrier
Support: Frank P. Hixon Fund
National Science Foundation

It has been found that a small unilateral island of cerebral cortex including the sensorimotor field is sufficient for the learning and retention of tactile discriminations made with the forepaw in the split-brain cat. Conversely, local removal of the corresponding area on the opposite side abolishes the performance with the affected paw but not with the first paw controlled through the isolated remnant of sensorimotor cortex. Attempts are being made to further delimit the cerebral structures that are necessary by progressive removals of hippocampus, amygdaloid complex, and subcortical centers.

142. DETERMINATION OF MINIMUM CEREBRAL APPARATUS REQUIRED FOR VISUAL LEARNING AND MEMORY

Investigators: R. W. Sperry, R. E. Myers, A. M. Schrier
Support: Frank P. Hixon Fund
National Science Foundation

Isolation of the cortical field for central vision has been found to leave the split-brain cat practically blind on the operated side. If the non-visual cortex is removed in two or in three separate operations starting with the cortex immediately surrounding the visual area to be preserved, it is not until the final removal of the frontal, or temporal lobe as the case may be, that the visual impairment becomes really severe. This approach is being carried further in an effort to determine more exactly the contribution of the different cortical areas to visual functions.

143. TRANSFER STUDIES IN SPLIT-BRAIN MONKEY

Investigator: R. W. Sperry
Support: Frank P. Hixon Fund
National Science Foundation

In the normal and in the chiasma-sectioned monkey visual discriminations for color, form, size, and brightness learned with one eye have been found to transfer readily to the untrained eye. If the callosum also is completely transected along with the chiasma and anterior commissure and hippocampal commissure, such interocular transfer fails and the task has to be relearned completely with the second eye. Under the latter conditions, opposing discriminations can be trained concurrently to the separate eyes without conflict or interference between the habits. Visual learning and memory thus seem to proceed quite independently in the separate hemispheres. These preliminary findings in the rhesus monkey are in accord with
earlier data obtained in the cat.

Unlike visual discriminations, tactile discriminations for weight, form, and hardness learned by touch with one hand and with vision excluded, were found to transfer at a high level to the untrained hand. This result, based so far on one rhesus monkey, is contradictory to that obtained in similar tests in the cat. It suggests that in the monkey the secondary uncrossed cutaneous system is sufficiently developed to permit learning in the ipsilateral as well as in the contra-lateral hemisphere, or that the effects of training in one hemisphere can be utilized for performing tactile discriminations with either hand. A more complicated type of discrimination involving a combination of visual and tactile cues, as described below, has failed to transfer from the trained hand-eye combination to the untrained hand and eye.

144. CEREBRAL ORGANIZATION UNDERLYING INTERMODAL CONDITIONAL DISCRIMINATION

Investigator: R. W. Sperry
Support: Frank P. Hixon Fund
National Science Foundation

Monkeys are trained to employ a combination of sensory cues of different modality in order to select the correct one of two objects. For example, choice of the softer of two plaques is rewarded if the plaques are yellow, whereas choice of the harder of the two is rewarded if the plaques are black. The black and yellow pairs of plaques are otherwise similar and are interchanged in random order. The correct tactile cue is made conditional on the visual color cue. This is a moderately difficult task for the monkey intellect; it has been mastered by the first split-brain rhesus monkey of the series in approximately 900 trials. After stabilization of the performance by overtraining, cortical removals and incisions will be undertaken to separate the cortical areas involved and to learn thereby something about the nature of the cerebral organization utilized in this kind of sensory integration.

145. INTELLECTUAL FUNCTIONS IMPAIRED BY ABLATIONS OF PREFRONTAL CORTEX

Investigator: G. M. French
Support: National Science Foundation

In the classical test for delayed response, an animal is shown a food reward at one of two alternative loci, both positions are covered with similar objects, a period of several seconds or minutes elapses, then the animal is allowed to displace a single object to obtain the hidden reward. Intact monkeys soon learn to remember under which cover the reward was placed.

After bilateral resection of the dorsolateral frontal granular cortex monkeys are unable to perform above a chance level when delays are longer than a very few seconds. The impairment is specific for the prefrontal cortex, in that bilateral ablation of no other block of association cortex will produce it. Although this has long been known, the exact nature of the intellectual impairment remains obscure.

Squirrel monkeys have been trained in classical delayed response and then tested with several variants of the problem to determine the kind of information being used. In the classical problem the monkey is free to attend to the rewarded locus, the non-rewarded locus, or both. The present tests show, however, that attention is directed toward the positive (food) cue to the almost complete neglect of the negative (no food) cue. Only with extensive additional training can a monkey learn to use the absence of food at the negative locus as a cue to correct response. It remains to be determined whether preoperative training to use both positive and
negative cues will tend in any way to overcome postoperative difficulty with delayed response.

146. CENTRAL NERVOUS STRUCTURES INVOLVED IN DELAYED RESPONSE

Investigators: G. M. French, R. W. Sperry
Support: National Science Foundation
Frank P. Hixon Foundation

Previous research has emphasized the role of the dorsolateral frontal granular cortex in ability to perform delayed response with consistent efficiency. It is possible, however, that impairment follows cortical injury only because the cortex provides essential facilitation to neural activities which are primarily organized at a lower level. Present experiments use the split-brain method with appropriate controls to determine if delayed response ability, like visual discriminations, may be transferred from one hemisphere to the other by the pathways of the corpus callosum. Should transfer occur after corpus callosum section, the importance of subcortical structures will be indicated. If it does not occur, it will be possible to undertake more extensive unilateral analysis of the mechanism for delayed response and short term memory.

147. SENSORY-MOTOR INTEGRATION IN SPLIT-BRAIN CATS

Investigators: A. M. Schrier, R. W. Sperry
Support: National Science Foundation
Frank P. Hixon Fund

The major portion of the motor cortex controlling a given forelimb lies in the contralateral hemisphere. If, in the split brain cat, only one hemisphere receives information relevant to solving visual discrimination problems, can this information be utilized by the motor system of the opposite hemisphere as well as by the motor system of the same hemisphere?

Cats were trained to use a forelimb to displace the correct one of two wood blocks painted to differ in brightness or in geometric pattern. A mask restricted vision to one eye and a leather band restrained one or the other forelimb. The cats responded more quickly with one forelimb than with the other, the difference in latency diminishing with practice. Despite the difference in latency, there was little difference between forelimbs with regard to performance on discrimination problems. This was true when vision was either monocular or binocular.

Early results on monocularly trained split-brain cats are similar to the results on normal animals. This supports the view that each hemisphere is able to govern the motor activity of both forelimbs. The nature of the visuomotor integration involved is being investigated further through cortical removals and incisions in the receiving hemisphere.

148. TESTS FOR INTEROCULAR TRANSFER OF A CONDITIONED AVOIDANCE RESPONSE

Investigators: A. M. Schrier, R. W. Sperry
Support: National Science Foundation
Frank P. Hixon Fund

Interocular transfer of visual pattern discrimination training has been shown to be absent in split-brain cats and monkeys despite the presence of intact diencephalic and mesencephalic crossed pathways. It remains possible, however, that such transfer might take place for simpler types of visual functions.
Earlier attempts to test for interocular transfer in split-brain cats that were trained to avoid the lighted compartment in a two-compartment shuttle box yielded ambiguous results. A major problem was the prolonged training required to establish the conditioned response. However, changes in the construction of the apparatus and in the training procedure appear to have remedied a great deal of the difficulty. A new series of operations has been performed and preparations are now under way to test for interocular transfer.

149. CEREBRAL MECHANISMS IN SENSORY INTEGRATION

Investigator: J. T. Eayrs
Support: Frank P. Hixon Fund

By training the split-brain cat to use a given eye and a given forepaw to learn a visuo-tactile discrimination, one can predetermine the cortical receptor areas used for the visual and tactile components of the sensory input. The effects of suitably placed lesions on the learning and retention of such discriminations can then be used to determine the integrative structures involved in establishing the sensory association.

Cats have been trained to press spring-loaded pedals, discriminating those which are heavily loaded and have a studded surface (hard) from lightly loaded pedals having a spongy surface (soft). When pairs of pedals were presented with a black covering in dim illumination, the hard pedal provided the positive stimulus, the soft pedal being positive when presented in bright light with a white covering. In this situation the cats showed a strong tendency to respond independently to either the visual or the tactual components. Only with considerable difficulty and after many thousands of trials did they eventually learn to respond to visuo-tactile configuration. Although there is good reason to believe that training time can be considerably reduced as a result of experience gained during this study, it is by no means certain that the cat, with its limited powers of abstraction, is the best subject for pursuing this investigation. It may be necessary either to change the mode of presenting the problem to the cat or to examine the capacities of other species for solving such conditional discriminations.

150. NEURAL SPECIFICITIES INVOLVED IN LACTATION

Investigator: J. T. Eayrs
Support: Frank P. Hixon Fund

The peripheral nerves serving selected pairs of nipples have been interrupted in the rat and the functional effects of orderly and of abnormal forms of reinnervation of the nipples have been studied. Observations have shown that in the rat the nipples are arranged in an orderly manner in relation to the segmental nerves, each nipple being positioned immediately over a cutaneous nerve trunk which provides its main nerve supply. The innervation provided by this nerve alone is found adequate to maintain lactation which continues after adjacent nerves have been transected. On the other hand, if the main nerve supply to the nipple is interrupted, lactation persists in about 50% of cases provided that the immediately adjacent nerves are left intact. Lactation invariably ceases if these nerves are cut also. Hence, the intersegmental overlap of the peripheral nerves concerned in maintaining lactation, although to some extent variable, is small, not extending beyond one segment on either side of the nipple.

If a nipple is denervated by crushing the principal nerve trunk, the capacity to lactate returns after the regenerating nerve fibers reestablish their former connections with the periphery. When, however, the nerve trunk was transected, followed by reunion of the two cut ends or by anastomosis with adjacent or distant "foreign" nerves, under which conditions regeneration is characterized by the forma-
tion of atypical connections at the periphery, the capacity to lactate was never re-
stored. It remains to be determined whether an adequate amount of sensory rein-
nervation is obtained after a nerve transection and also whether additional recovery
time may bring any reorganization of central connections. Work on the problem is
continuing.

151. STUDIES ON COLOR DISCRIMINATION AND OPTIC NERVE REGENERATION
IN THE CICHLID FISH

Investigators: R. W. Sperry, H. L. Arora
Support: Eli Lilly and Company
Frank P. Hixon Fund

The ability of the completely divided optic nerve to regenerate and to restore
accurate direction sense is well established in many fishes and amphibians. The
present investigation was undertaken to find out if the perception of color also is
restored in normal form by optic nerve regeneration.

It was reported earlier that by use of a jumping technique in which fish
were taught to jump out of water at the correct one of two differently colored feeders,
a species of cichlid fish, Astronotus ocellatus, could be trained to distinguish colors.
The colors used involved light reflected from the painted surfaces of the feeder
plaques. The findings have now been rechecked and confirmed with a new type of
feeder in which the colors are presented with light transmitted through color filters
and through neutral filters of various densities. It has been confirmed that these
fish distinguish such colors as red, blue, yellow and green from each other and
from various shades of gray; also that these color discriminations can be trained
in fish with regenerated optic nerves; and further, if the training is prior to section
of the optic nerve, the color discriminations are reinstated without training upon
the completion of regeneration and the recovery of vision. The results suggest
that reconnection of optic axons in the brain is governed by fiber specificities for
color as well as by the topical specificities for direction.

152. FUNCTIONAL RECOVERY FOLLOWING SECTION AND REGENERATION
OF MOTOR OCULAR NERVES

Investigators: H. L. Arora, R. W. Sperry
Support: Eli Lilly and Company
Frank P. Hixon Fund

The oculomotor nerve supplying four of the six external muscles of the eyeball
has been sectioned intracranially in the cichlid fish, Astronotus ocellatus and
allowed to regenerate. Although the cut nerve ends were frayed to encourage hap-
 hazard redistribution of fibers among the four muscles, regeneration resulted in
complete functional recovery with normal coordination. This result stands in
marked contrast to the undifferentiated mass contraction and contracture that
follows regeneration of this same nerve in man and other mammals. It is inferred
that each of the eye muscles has an individual specificity and that there exists a
corresponding specificity among the motor nerves to these muscles.

Further operations have been carried out in which nerves of the individual
ocular muscles have been sectioned in the orbit and cross-connected to antagonistic
or other foreign ocular muscles. The aim was to find out if the crossed nerves
give up their original discharge pattern and take on a new incidence of firing
appropriate to the new muscle connection. Because of the complexity and inter-
dependence of muscle action in eyeball movement, along with other complications
in the procedure, it has been extremely difficult to obtain a clear cut answer to
this latter question in spite of several repetitions with different nerve-muscle
combinations. Whether the normal function following regeneration of the whole
oculomotor trunk can be ascribed to respecification of misregenerated fibers or to factors that favor normal over abnormal reconnections in the musculature is still a problem.

153. INTEROCULAR TRANSFER OF TRAINED VISUAL DISCRIMINATIONS IN FISHES

Investigators: R. W. Sperry, H. L. Arora
Support: Eli Lilly and Company
Frank P. Hixon Fund

In conducting experiments on color discrimination and visual acuity following optic nerve transection and regeneration in the cichlid fish, Astronotus ocellatus, it became desirable to know whether discrimination habits learned with one eye would transfer without additional training to the other eye. Tantalum foil caps were carefully molded to the curvature of the eye and inserted under the outer cornea to exclude vision from one eye. In one series the fish were trained to discriminate colors with one eye and in another series they were trained to discriminate polka dots from various shades of gray. The eye caps were then shifted to cover the trained eye. In both series, the visual discrimination habits transferred readily at a high level from the trained to the untrained eye.

Publications:

Virology

Part A. -- Animal Viruses

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154. Quantitative Studies on Mutability in Poliovirus

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

The discovery in poliovirus of d mutants having reduced efficiency of plating in overlay, with low bicarbonate and reduced neuropathogenicity, has already been described. Reversions for the d character can be counted in a d stock by a selective procedure.

The d mutants occur in two main classes: some having an efficiency of plating under low bicarbonate of $10^{-4}$ to $10^{-3}$; and some having an efficiency of around $10^{-6}$. They are called "high efficiency" and "low efficiency" d mutants, respectively.

Reversions of "high efficiency" mutants are similar to wild-type strains both in d character and neuropathogenicity; reversions of "low efficiency" mutants are intermediate in d character; by a successive selection procedure several phenotypically intermediate types having decreasing d properties can be isolated. None of them is equal to wild type in d character. All these intermediate types are attenuated.

A statistical analysis of the occurrence of reversions in the two types of d lines suggests that reversions from "high efficiency" d lines are mostly contained in the virus stock, whereas reversions from "low efficiency" d lines are mostly produced after growth on the plate.

Reversions from "high efficiency" d lines were used to perform a Luria-Delbrück fluctuation test. This test showed that the mutants are produced during the growth of the d lines randomly in time and are not induced by the low bicarbonate medium in which the reversions are detected. The test also allowed the accurate determination of the mutation frequency, which is of the order of $10^{-5}$ per particle per duplication.

The genetic situation involved in the d mutants can be represented as follows: The "high efficiency" mutants can be attributed to mutations affecting a locus controlling both d character and neuropathogenicity. The reversions may be due to back mutations. The "low efficiency" mutants can be attributed to a deficiency; the reversions affecting the d character only may be due to mutations at suppressor genes.
155. INDUCTION OF MUTATIONS IN POLIOVIRUS

Investigators: R. Dulbecco, M. Vogt
Support: National Foundation for Infantile Paralysis
General Funds

Experiments on mutagenesis could be carried out by using the mutations from \( d \) to \( d^+ \) of a high efficiency \( d \) mutant of poliovirus. Three agents were investigated: X-rays, ultraviolet light and proflavin. Each type of radiation was used to irradiate virus-infected cells rather than free virus, since previous work with bacteriophages had shown that this was the most suitable way for inducing viral mutations. Although a large variety of doses of radiation was used and the irradiation was given at various times during the latent period, no mutagenic effect was observed with either type of irradiation. Proflavin, on the contrary, was found to be effective. Infected cells were exposed to different concentrations of the drug during the whole period of virus multiplication (about 24 hours). At adequate concentrations of proflavin the total virus yield from these cells was depressed and the proportion of mutants in the yield was increased, the two effects being correlated. The maximum effect obtained was a nearly tenfold increase in the proportion of mutants over background. A selective effect of proflavin on the mutants was excluded. It is not yet known whether proflavin is equally effective in increasing the proportion of other mutations. However, since mutagenic effects are generally non-specific, it may be suggested that a proflavin treatment should precede any attempt to isolate mutants of poliomyelitis virus endowed with special genetic properties.

156. INTERFERENCE AND MULTIPLETY REACTIVATION WITH POLIOVIRUS

Investigator: John W. Drake
Support: National Science Foundation
Arthur McCallum Fund
General Funds

Polioviruses infecting a variety of animal or human cell strains are rapidly able to block infection by any other antigenically different poliovirus (interference). In contrast to other animal virus systems, however, polioviruses killed by ultraviolet irradiation (UV) do not prevent superinfection by any live poliovirus. In addition, infecting a cell with two or more killed viruses sometimes results in the production of live progeny. This process, termed multiplicity reactivation, is well known for bacteriophages. The weak reactivation is being studied with poliovirus grown in a strain of human cells. Virus killed to a certain level is adsorbed to cells, and the fraction of cells releasing virus is determined (corrected for infection by residual live virus). The interesting parameters of such a system are the UV dose to the virus, and the number of viruses infecting each cell. The fraction of cells releasing poliovirus falls off at the same rate as the virus itself is killed. Up to about five times the number of "yielders" are found as can be accounted for by input residual live virus. Since the small fraction of cells showing viral reactivation increases even with very large numbers of viruses per cell, probably only a small fraction of the killed virus can participate in a reactivation attempt.

157. THE MULTIPLICATION OF INDIVIDUAL HeLa CELLS IN DROPS UNDER PARAFFIN OIL

Investigators: M. Vogt, R. Dulbecco
Support: National Foundation for Infantile Paralysis
General Funds

Individual HeLa cells will multiply in drops of medium under paraffin oil provided the drops are large (2 to 3 mm in diameter) and the layer of oil is deep (around 5 mm). The cells were dispersed by trypsin and transferred individually
to the drops under a stereoscopic microscope. The multiplication of cells of two lines under these conditions was followed. It was found that the cells have a lag period shorter than one generation time, followed by a regular geometric growth with a doubling time of about 20 hours.

This method is important for virological studies and for isolating cell populations derived with certainty from a single cell.

158. VARIANTS OF HeLa CELLS SELECTED BY VIRUS ACTION

Investigators: M. Vogt, R. Dulbecco
Support: National Foundation for Infantile Paralysis
General Funds

Variants were isolated by exposing HeLa cells to poliovirus several times serially. The cells that formed the surviving population were different morphologically—fusiform—from those of the original HeLa culture—polygonal—and were more resistant to virus infection. The selected culture was grown in the presence of antiviral serum; in its absence the culture degenerated. It has been pointed out in the past that such a culture may be "virogenic." This point has been analyzed extensively and the following results were obtained.

1) By cultivating serially the selected culture under antiviral serum, one "cures" it of the virus. A culture that did not degenerate without antiserum was obtained. This culture had the same type of fusiform cells.

2) Fusiform cells can be found in small proportion in the original HeLa populations not exposed to virus.

3) Clonal lines of cells from the selected culture did not produce virus; their cells showed an increased resistance to virus infection, similar to that shown by the "virogenic" selected culture. This resistance is due to a lesser probability for a virus particle attached to the cell to give rise to an infective center. There is no real immunity.

4) Cells of a virus-resistant clonal line were infected with virus, distributed in drops of oil under paraffin oil; the medium was sampled at time intervals and tested for virus. Most cells degenerated and released virus within 48 hours. The others grew and gave rise to "non-virogenic" clones. Three cells that divided degenerated, with virus release, after a few divisions.

All these results show that by virus treatment one selects for preexistent resistant cells, which are of different morphological type and differ also physiologically (less requiring nutritionally, in an undefined way). It is likely that the "virogenicity" of the selected culture was of a trivial nature (interference between virus multiplication in cells of increased resistance and virus inactivation by antiserum). No evidence for permanent virogenicity was obtained. However, it appears that about 1% of the infected cells may divide once or twice before degeneration and virus release. The state of the virus in these cells is not known.

159. CHANGES IN CELLULAR NUCLEIC ACIDS DURING INFECTION WITH POLIOMYELITIS VIRUS AS STUDIED BY FLUORESCENCE MICROSCOPY

Investigator: E. Tenenbaum
Support: National Foundation for Infantile Paralysis
Abbott Laboratories

It has been previously shown that in cells treated with acriflavin and examined in a fluorescence microscope, the ribonucleic acid (RNA) fluoresces red and the
desoxyribonucleic acid (DNA) green. This property was utilized to study the changes of the two acids occurring during infection of monkey kidney cells with poliomyelitis virus. These experiments were carried out in monolayer cultures the cells of which were simultaneously infected. The first change of the infected cells is an increase of green fluorescent material in the nucleus; later this material seems to spread to the cytoplasm, whereas the red fluorescent material of the cytoplasm is pushed to the periphery of the cell and finally discarded in lumps. The green fluorescence was found sensitive to DNAase and the red fluorescence to RNAase.

These observations suggest that an essential and early step of virus multiplication involves the nuclear DNA. The subsequent migration of the fluorescent material to the cytoplasm may either indicate the migration of virus precursors, or may be a consequence of the alteration of the nuclear membrane—for which exists independent evidence—and not primarily connected with the reproduction of the virus.

Since poliovirus contains RNA and no DNA, the findings may suggest a DNA participation in the multiplication of an RNA virus.

160. THERMOSTABILIZATION OF POLIOVIRUS BY CYSTINE

Investigator: P. Pohjanpelto
Support: Emil Aaltonen Foundation
National Foundation for Infantile Paralysis

Incubation overnight at 37°C of a temperature-sensitive line of poliovirus in cystine makes it temperature resistant. The inactivation curve at 50°C shows a seven-fold decrease in slope as a consequence of the treatment. The stabilization is slow, requiring several hours to be completed. The degree of stabilization acquired persists for at least 20 hours at 37°C after diluting the virus-cystine mixture to a point where cystine would be ineffective. When intermediate degrees of stabilization occur, the shape of the inactivation curve indicates that the stabilization of the individual particles varies, the majority of them having an intermediate degree of stabilization.

Cystine is not effective at pH 4; the acquired stabilization is stable at this pH. The effect of cystine cannot be imitated by reducing agents (thioglycolate, glutathione); thioglycolate added to the virus-cystine mixture decreases markedly the stabilization.

The stabilization appears due to the carboxyl-ionized cystine, not to a reduction by cysteine simultaneously present; perhaps the amino acid forms interhelix bridges which tend to prevent the thermal denaturation of the virus protein.

161. KINETICS OF THE EARLY STEPS OF NEWCASTLE DISEASE VIRUS INFECTION

Investigator: Harry Rubin
Support: American Cancer Society
American Cancer Society, California Division
National Institutes of Health, Public Health Service

The first step of infection is adsorption of virus to the susceptible cell. The kinetics of this step have been studied here with Newcastle disease virus (NDV). It was found that the rate constant for this reaction was about $6 \times 10^{-9}$ cell$^{-1}$ min$^{-1}$. There is no measurable variation in this rate between 0° and 37°, nor between pH 6.0 and 8.0.

Following adsorption, NDV acquires a resistance to serum inactivation. A precise method for studying this step has been worked out. In this method, virus is adsorbed on monolayers of cells in a brief pulse, and the unadsorbed portion
quickly washed away. The amount of virus used is such as to give a countable number of plaques directly on the plate. The cells are incubated under appropriate conditions and antiserum added after varying periods of time. Any virus which has not reached the serum-resistant stage will be inactivated, as indicated by the reduction of plaque number.

In contrast to adsorption, the second step proves to be highly temperature dependent. A first approximation of the activation energy gives a value similar to that found for the enzyme activity of the virus, suggesting that the enzyme plays a role in the second step. At 37°C, the average particle has undergone the change to serum resistance within five minutes after adsorption.

An experiment was devised to determine whether the cell plays an active role (such as engulfment) in producing the serum-resistant stage. Virus was adsorbed to cells in the presence of cyanide; then serum was added, still in the presence of cyanide, and the number of infected cells determined. It was found that the cyanide, which blocks cellular activity, had no influence on the acquisition of serum resistance by the virus. Furthermore, it was found that the lung epithelial cells, which are highly susceptible to NDV infection, cannot actively phagocytize such particles as carmine at all. These observations, when taken together with positive evidence for participation of the viral enzyme in penetration, suggest that the cell plays no major active role in the early steps of virus invasion.

162. INTERACTIONS BETWEEN NEWCASTLE DISEASE VIRUS, ANTIBODY AND CELL

Investigator: Harry Rubin
Support: American Cancer Society
National Institutes of Health, Public Health Service

The first part of this investigation was carried out to learn whether immune serum neutralized Newcastle disease virus (NDV) by inactivating the viral enzyme, as suggested by previous work in this laboratory. The enzymatic activity of the virus was measured by its ability to elute from red blood cells after adsorption. In an antibody-treated preparation, the amount of eluting virus could be easily determined if it had been previously labeled with radiophosphorus (P32). It was found that the logarithm of the virus eluting was inversely proportional to the concentration of antibody with which the virus had been treated before adsorption.

Curves for the survival of enzyme activity and of infectivity as a function of antibody concentration had parallel slopes, thus supporting their suggested relationship. However the infectivity curves were displaced slightly to the right due to a slight shoulder at the origin. This displacement was shown to be due to secondary phenomena of reactivation.

The number of enzyme sites on the virus could be determined by repeating the adsorption-elution cycle several times with virus which had been treated with varying antibody concentrations. The amount eluted at each successive cycle was compared with that of an untreated control. If there were a large number of sites, the fraction of antibody-treated virus eluting in each successive cycle will be only slightly larger than that of the preceding; if the number of sites is small, or if all the sites are critical, the fraction eluting during the same cycle will approach one. The results indicated that there are about four enzyme sites per virus particle.

Even in the case of untreated virus, however, only about one half of the virus which adsorbs to red cells can elute. If, after elution, the cells are disrupted by physical methods, only about 5% of the infective virus which should be present, according to P32 measurement, can be detected by infectivity test; the remainder loses its infectivity soon after invasion of the cell. However, a peculiar paradox arises when virus treated with small amounts of antibody is adsorbed to cells. After
the antibody treatment a fraction of the virus retains its infectivity when directly assayed. In contrast to the untreated control, however, this surviving fraction is only slightly reduced if the virus is first adsorbed to cells which are then disrupted and assayed. It is clear then that the surviving fraction has been in some way affected by antibody, since it does not break down after adsorbing to the cell.

It is concluded from these findings that the virus has a number of sites at which it can initiate infection. When it adsorsbs to a cell through a site occupied by an antibody molecule it cannot initiate infection, i.e., it is not broken down. If it adsorbs through a free site, it can initiate infection, even though some of its remaining sites are inactive.

The number of infectivity-initiating sites on the virus can be determined by quantitative treatment of the above experiment. This number is in good agreement with the number of enzyme sites determined by an independent technique (see above), providing further support for the role of the enzyme in starting infection.

The failure of antibody-treated virus to penetrate the cell could be demonstrated by a more direct method. After adsorption of various antibody-virus mixtures to cells, additional antibody was added. If, as suggested, the virus remained at the cell surface it could be further inactivated by adding more antibody; if it had penetrated the cell it would no longer be accessible to neutralization. It was found that the surviving virus could be further inactivated after adsorption, showing that this virus is largely retained at the cell surface.

Evidence was obtained for a limited amount of reactivation of neutralized virus at the cell surface. This could be seen with virus exposed to doses of antiserum large enough to reduce the number of survivors below 1%. In such cases, more virus could be obtained by disrupting cells after adsorption of the antibody-virus complex than by direct assay.

All the experiments described above could be carried out on susceptible tissue cells and red blood cells.

163. AN ATTEMPT TO DEMONSTRATE GENETIC INTERACTION IN NEWCASTLE DISEASE VIRUS

Investigator: William A. Strohl
Support: Arthur McCallum Fund
           General Funds

In an effort to gain information about the organization of the genetic material of an animal virus, preliminary work was done with Newcastle disease virus to see whether genetic interaction could be demonstrated conveniently and reproducibly in a tissue culture system. Up to the present, most of the work has been mainly concerned with the isolation of stable mutants. A heat-resistant mutant and a small plaque-forming mutant have been isolated thus far. Other types of mutants were looked for unsuccessfully. Although small plaque formers are easily isolated, most of these are too unstable genetically for genetic work; that is, a stock of one of these mutants may contain 1 to 5% large plaque formers. The relatively stable plaque mutant which is being used has been shown to have a three- to four-hour longer lag period, and a slightly smaller burst size.

Several rescue experiments were attempted, in which the heat-resistant mutant was killed with ultraviolet irradiation, and cells were mixedly infected with the killed virus and with live heat-sensitive virus. An increase over the expected in the number of heat-resistant progeny would show that the heat-resistant marker was incorporated into particles which otherwise would have been heat sensitive, and therefore would be indicative of some sort of genetic interaction between the two types of virus. No real rescue of the heat-resistant marker could
be shown by this means. Some further work will be done using the small plaque former also, in hopes of increasing the efficiency of detection of any recombinant progeny.

164. STUDIES ON CELLS INFECTED WITH ROUS SARCOMA VIRUS: I. ASSAY; VIRUS TRANSMISSION AND GROWTH; CHROMOSOME NUMBER

Investigator: Howard Temin
Support: Arthur McCallum Fund
General Funds

Although the chicken sarcoma No. 1 (Rous sarcoma) was shown in 1911 to be virus induced, no detailed, quantitative analysis of the cell-virus relationships was carried out until recently. Then Rubin (1955), applying modern virological concepts, made certain quantitative observations about the nature of the cell-virus relationships. More recently Manaker and Groupe have found that the virus could transform the appearance of normal chicken embryo cells in vitro. An effort has been concentrated on the adaptation of this finding to the development of a precise and practicable assay for the Rous virus, based on the formation of discrete foci of transformed cells, analogous to plaques.

Early efforts in this laboratory have been directed toward developing the assay to the point where it is precise, practicable and reliable. The virus is adsorbed to relatively sparse culture of chicken embryo cells (chiefly fibroblasts) and these are overlaid with soft agar-containing nutrients and chicken serum containing neutralizing antibodies against the virus. The cells are incubated seven days under conditions favorable to their multiplication, when foci of about 50 round, highly refractile cells can be seen at a low magnification. Frequently a hole develops in the continuous monolayer at the center of each of the foci. The number of foci is linearly related to the virus concentration; the assay appears to be at least as efficient as the various in vivo assays commonly used.

The high antiviral activity of the serum, combined with the slow rate of virus production would, in all likelihood, lead to neutralization of any virus released before reinfection of surrounding cells can occur. Therefore, it is likely that the foci are due to multiplication of the cells infected by the original inoculum, rather than to reinfection of surrounding cells.

The transformed cells can be grown in mass culture; clonal cultures can also be obtained. However, it has not yet been possible to maintain them in culture indefinitely. After 20 to 40 generations the cells become enlarged, multinucleated and highly vacuolated and stop dividing. Virus is released at the rate of about one infectious unit per cell every 10 hours. A culture of cells which had been cloned and continuously grown for over 20 generations in the presence of antiserum still released virus at the maximal rate when the serum was removed, suggesting that the virus is transmitted from parent to progeny cell in some serum-resistant state. Presumably the transmission of the capacity to produce virus is intracellular, occurring at the time of cell division.

The recent prominence of theories associating cancer with changes in chromosome number has prompted studies of the chromosome complement in the Rous cell; preliminary studies have revealed no major changes in the large chromosomes of the chicken following transformation of the cells.
165. STUDIES ON CELLS INFECTED WITH ROUS SARCOMA VIRUS: II. VIRUS STOCKS; GROWTH OF NORMAL CELLS; SUPERINFECTION

Investigator: Harry Rubin
Support: National Institutes of Health, Public Health Service
American Cancer Society

One of the prerequisites for studying the course of virus infection is to have virus stocks of high enough titer to infect all the cells simultaneously. The most reliable source of relatively high-titered Rous sarcoma virus in this laboratory has proved to be the chorioallantoic membrane of the developing chick embryo, where 2 to 5 x 10^6 infective particles can be obtained per membrane. Efforts are now being directed toward building up a large supply of this material for concentration by centrifugation, in order to assure infection of all cells in future experiments.

An investigation is also being carried out to determine the most favorable conditions for growth of normal fibroblasts. Allied with this is an attempt to obtain a permanent culture of fibroblasts. Chicken serum at a concentration of 10% in Eagle's medium has proved the most efficient medium to date. It has also been possible to clone and subclone the fibroblasts without a feeder layer of X-rayed cells, if the proper medium and techniques are employed. This permits isolation of clonal lines and enables an unequivocal study of the cell types which can be infected in vitro, and of how the individual cell types respond to infection.

Rous cells transformed in vitro were tested for their ability to support superinfection by a group of unrelated viruses. These all multiplied in the Rous sarcoma cells and killed them. However, detailed study with one of them (Western equine encephalomyelitis) showed the latent period to be prolonged, the rate of multiplication decreased and the death of the cell delayed in transformed as contrasted with normal cells.

166. STUDIES ON VARIATION OF MAMMALIAN CELLS CULTIVATED IN VITRO

Investigator: E. C. Weiler
Support: Deutsche Forschungsgemeinschaft
American Cancer Society
National Institutes of Health, Public Health Service

The tubular epithelium of the hamster kidney contains an organ-specific antigen, the presence of which can be detected by examining, with fluorescence microscopy, cells exposed to a properly adsorbed antiserum labeled with fluorescein isocyanate. It was found that during one passage in vitro, which involves a nearly eight-fold increase in cell number, the kidney cells lost the specific antigen. At the same time also the complement-fixing activity of the specific antigen, which is present in the cells of the kidney, was lost in the cultivated progeny. These results may derive from two different phenomena: (a) the cells lose the antigen during their multiplication; (b) only cells which did not have the antigen in the first place multiply in the culture. These possibilities are now being investigated by radioautographs of cultures of various ages treated with antiserum labeled with radioactive iodine (^131).

An antiserum against hamster kidney kills in the presence of complement the kidney cells cultivated in vitro. This killing is not due to the antiserum against the organ-specific antigen, because cells of other organs (lung, for example) are also killed by the same antiserum under similar conditions.

In examining the kidney cultures exposed to the antiserum, it was found that 3 to 4% of the cells survived, and grew in the presence of the antibody. To assess the nature of the surviving cells, clones of kidney cells were obtained from primary
cultures; only 3 to 5% of these cells produced clones. When the clones were exposed to the antiserum the following results were observed. Some clones survived completely, others were completely killed; the majority of the clones showed destruction of most of the cells, except a few, mostly arranged in small patches. The distribution of the number of resistant cells in the patches was clonal. This shows that during the growth of the serum-sensitive kidney cells, genetic events occur, randomly in time, which cause a change from serum sensitivity to serum resistance. The nature of these events will be further investigated.

It is interesting to note that the sera employed, although cytotoxic for lung cells, did not fix complement with the lung cells; thus cytotoxicity appears to be a more sensitive test for antibody than complement fixation.
PART B. -- BACTERIAL VIRUSES

STAFF:
Professor: Max Delbrück
Research Associate: J. J. Weigle
Senior Research Fellow: Giuseppe Bertani
Research Fellows: Denise Cohen, Walter Harm, Erich Six, Franklin W. Stahl, Roger Weinberg
Graduate Students: L. Elizabeth Bertani, Charles M. Steinberg
Research Aides: Giuseppina Dulbecco, Ilga Lielausis
Laboratory Assistants: Lois Edgar, Eva Leach

167. THE PREPARATION AND CHARACTERIZATION OF STOCKS OF T4 BACTERIOPHAGE CONTAINING 5-BROMOURACIL

Investigators: Franklin W. Stahl, Matthew Meselson *
Support: National Research Council
National Science Foundation
National Foundation for Infantile Paralysis
General Funds

It is known that the thymine in the desoxyribonucleic acid (DNA) of bacteriophage T2 grown in the presence of 5-bromouracil on bacteria with deficient thymine metabolism is partially replaced by the 5-bromouracil. Sulfanilamide has commonly been used to produce the thymine deficiency. Chromatographic analysis of the DNA from phage T4 grown under these conditions has revealed substitution of thymine by 5-bromouracil. The sensitivity of such T4 preparations to both X-rays and ultraviolet treatment is greater than that of control preparations. This increased sensitivity is maintained following infection.

It appears that either aminopterin or methylaminopterin may substitute for sulfanilamide or 5-bromodeoxyuridine for 5-bromouracil since T4 stocks prepared in the presence of these materials show X-ray sensitivity greater than that of control stocks.

It has been reported that T2 stocks prepared under conditions which permit the incorporation of 5-bromouracil contain a high frequency of mutant phage. We have confirmed this observation for T4 and have mapped two mutants so produced with respect to each other and to a third well known marker.

The presence of inactive phage particles in substituted stocks is revealed by the observation that substituted stocks contain a greater number of particles able to contribute genetic markers to the progeny of a mixed infection than can be accounted for by the number of particles able to form plaques on single infection.

168. THE SEPARATION BY EQUILIBRIUM SEDIMENTATION OF NORMAL FROM 5-BROMOURACIL-SUBSTITUTED PHAGE DNA

Investigators: Matthew Meselson *, Franklin W. Stahl, Jerome R. Vinograd *
Support: National Science Foundation
National Research Council
National Institutes of Health, Public Health Service
National Foundation for Infantile Paralysis
General Funds

* Gates and Crellin Laboratories of Chemistry, C.I.T.
Models of desoxyribonucleic acid (DNA) replication may be classified according to the predictions which they make concerning the distribution among progeny molecules of atoms derived from the parental molecule. The use of radioactive tracer atoms has so far given only partial answers to this problem. It was anticipated that a label which gave to the DNA molecule an increased density might permit an analysis of the distribution of parental atoms among progeny molecules by sedimentation techniques. To this end a new technique for the detection of small density differences between macromolecules was developed.

A solution of a low molecular weight solute is centrifuged until equilibrium is closely approached. The opposing tendencies of sedimentation and diffusion have then produced a stable concentration gradient of the low molecular weight solute. The concentration gradient and compression of the liquid result in a continuously increasing density along the direction of centrifugal force. Macromolecules present in this density gradient are driven by the centrifugal field into the region where the density is equal to the effective density of the macromolecules. The density distribution of the macromolecular material may then be observed through the optical apparatus of the analytical ultracentrifuge.

Normal phage DNA in a cesium chloride density gradient has an effective density of 1.70. Phage DNA fully substituted by 5-bromouracil has been found to have an effective density of 1.80. Mixtures of the two types of DNA are well resolved by this technique. The resolution is sufficient to permit the detection of partially substituted molecules in the experiments proposed to determine the distribution of parental atoms (5-bromouracil) in progeny DNA.

169. THE MOLECULAR WEIGHT OF DESOXYRIBONUCLEIC ACID FROM BACTERIOPHAGE T4

Investigators: Matthew Meselson,* Franklin W. Stahl, Jerome R. Vinograd*
Support: National Science Foundation
National Research Council
National Institutes of Health, Public Health Service
National Foundation for Infantile Paralysis
General Funds

It was indicated above that macromolecules centrifuged in a stable density gradient are driven by the centrifugal field to the region where the density is equal to the effective density of the macromolecules. This concentrating tendency is, however, opposed by Brownian motion, with the result that at equilibrium the macromolecules are distributed with respect to concentration in a band of width inversely related to their molecular weight. It may be shown that in a constant density gradient under certain attainable conditions, the equilibrium concentration distribution of a single macromolecular species is Gaussian. The standard deviation of this Gaussian band is inversely proportional to the square root of the molecular weight.

DNA freed from phage T4 by osmotic shock has been shown to form a Gaussian concentration band when centrifuged to equilibrium in a density gradient of cesium chloride. This result is evidence that the DNA so obtained is uniform with respect to density and molecular weight. The width of the band corresponds to a molecular weight of $1.4 \times 10^7$ for the sodium salt. DNA isolated by the detergent method from a number of other sources has been examined. All have been found to be heterogeneous with respect to effective density.

* Gates and Crellin Laboratories of Chemistry, C.I.T.
170. MULTIPLICITY REACTIVATION, MARKER RESCUE, AND GENETIC RECOMBINATION IN BACTERIOPHAGE T4 FOLLOWING X-RAY INACTIVATION

Investigator: Walter Harm
Support: International Cooperation Administration
General Funds

The soft X-ray survival of bacteriophage T4 in an early intracellular stage is an exponential dose-function with a slope 1.6 times that of the free phage survival curve. This difference has also been found with T2 and T6, but not with T3. The gamma-ray survival of T4 is identical for both extracellular and intracellular phage. It is concluded that the higher survival of free phage treated with soft X-rays is due to the geometry of the target and the close spacing of the ionizations, which results in bunching of lethal hits.

Multiplicity reactivation of T4 under conditions of intracellular X-irradiation is about as extensive as that of ultraviolet-irradiated T4, the log of the multicomplex survival vs. the dose becoming linear at higher doses with a slope of about 0.25 of that of the monocomplex survival function. Very little multiplicity reactivation occurs in extracellularly X-ray inactivated T4.

Cross-reactivation of markers of intracellularly X-rayed T4 is as high as that of ultraviolet irradiated T4, whereas it is considerably lower with extracellularly X-rayed phage.

The frequency of genetic recombination increases as a result of intracellular X-irradiation, but decreases with extracellular X-irradiation.

All these effects can be explained by the hypothesis that the extracellularly X-rayed phage population injects only a dose-dependent fraction of its desoxyribonucleic acid. This fraction can be roughly estimated from the results of cross-reactivation experiments.

171. THE SENSITIVITY OF SUB-UNITS OF PHAGE T4 TO THE DECAY OF INCORPORATED P\(^{32}\) AS A FUNCTION OF THE DEVELOPMENTAL STATE OF THE PHAGE-HOST CELL COMPLEX

Investigator: Charles M. Steinberg
Support: National Science Foundation
National Foundation for Infantile Paralysis
General Funds

Bacteriophage T4 containing radioactive phosphorus (P\(^{32}\)) incorporated in their DNA are known to lose their infectivity as a result of the decay of the P\(^{32}\). The fraction of decays which lead to the loss of plaque-forming ability is 1 in 20 at the temperature of liquid nitrogen. The development of bacteria infected with radioactive phage may be arrested at various times following infection by rapid freezing in liquid nitrogen. Under our conditions, for the first 6 minutes following infection the loss of plaque-forming ability of these phage-cell complexes occurs at the same rate as the inactivation of the free phage. Between 6 and 15 minutes the complexes become progressively resistant to the decay of the incorporated P\(^{32}\). An explanation of this phenomenon will be looked for by examining the nature of the attainment by subunits of the phage of resistance as a function of time after infection.

The ability of radioactive phage to contribute a genetic marker to the progeny of a mixed infection and the ability of the region around this marker to perform a function essential for phage development have been examined. Both have sensitivities at time zero after infection smaller than that of the free phage; both achieve essentially maximum stability within the first six minutes following infection.
172. LYSOGENIZATION BY BACTERIOPHAGE P2

Investigator: L. Elizabeth Bertani
Support: National Science Foundation
General Funds

It was previously reported that the proportion of cells of Escherichia coli strain C infected with the temperate bacteriophage P2 that becomes lysogenic can be increased by treating the infected cells with chloromycetin or by starving them for a required amino acid. It has now been found that the proportion that becomes lysogenic can also be increased by treatment with amino acid analogues or 5-hydroxyuridine, but not by treatment with cyanide, azide, dinitrophenol, or pretreatment before infection with ultraviolet light. Chloromycetin treatment also increases the proportion of cells of Salmonella typhimurium infected with the temperate bacteriophage P22 that becomes lysogenic. It can be inferred from the above that the selective inhibition of protein synthesis in infected cells tends to favor the lysogenic response over the lytic one.

The proportion of cells of strain C infected with P2 that becomes lysogenic can also be increased (up to 100%) by treatment with proflavine. Since chloromycetin and proflavine are most effective if they are added to the infected cells about halfway through the latent period, it is possible that their action is to block processes, beginning about that time, that lead to the formation of mature, infective phage particles.

Publications:


173. A DISMUNE VARIANT OF TEMPERATE BACTERIOPHAGE P2

Investigator: G. Bertani
Support: National Foundation for Infantile Paralysis

From a virulent (i.e. unable to establish lysogeny) mutant of phage P2, a temperate dismune variant has been isolated. This variant has the following properties. It is serologically indistinguishable from wild-type P2; it forms plaques slightly smaller than wild-type P2; it lyses cells lysogenic for wild-type P2, and, conversely, wild-type P2 lyses cells which are lysogenic for the dismune variant. The important feature of this variant is thus that of not giving cross-immunity with the wild-type P2. This does not occur with most other temperate mutants of temperate phages, which are as a rule coimmune with the wild type. The location of the dismune prophage on the bacterial chromosome can be shown by appropriate bacterial crosses (see reports of previous years) to be allelic to the preferential location of P2 prophage. One has thus succeeded here to separate immunity-specificity from specificity of site of attachment of a prophage to the bacterial chromosome—a strong support for the notion that immunity to superinfection of lysogenic bacteria is a physiological property of the cells, controlled by the prophage, rather than a case of 'steric hindrance' caused by the specific chromosomal site being occupied by the prophage. For the origin and other properties of the dismune variant, see report by Denise Cohen.
174. ORIGIN OF THE DISMUNE VARIANT OF TEMPERATE BACTERIOPHAGE P2

Investigator: Denise Cohen  
Support: French National Center of Scientific Research  
General Funds

The dismune variant of bacteriophage P2 (see report by G. Bertani) is obtained each time wild-type P2 is grown on Escherichia coli B (its normal host is Shigella). In one-step growth experiments, the dismune appears chiefly in late-lysing cells; whereas only 1% of the cells lysing within 1 hour after infection liberate dismune P2, a maximum of 30% do so within 4 hours. Late liberation of a new type of phage has already been observed by D. K. Fraser with a semi-temperate mutant of T3. The dismune variant adsorbs, but is unable to give plaques on E. coli B. In other words, E. coli B is naturally immune to infection by P2 dismune. These facts suggest that E. coli B carries a defective dismune prophage sufficiently related to P2, and that when P2 infects strain B, it can recombine with such a prophage to give origin to the dismune variant. Besides wild-type P2, five mutants of P2 were tested for the ability to give origin to the dismune variant upon infecting E. coli B. All did so. Only two of them, however, in which the mutant property did not affect their ability to lysogenize, appeared in the dismune form with their original mutant character; the others lost it. This result confirms the conclusion obtained from other experiments in several laboratories that the same region of the phage chromosome that controls immunity specificity also controls the ability to lysogenize. An attempt is now being made to obtain a derivative of E. coli B sensitive to P2 dismune and thus presumably unable to contribute to the formation of dismune phage.

175. MEASUREMENT OF THE RATE OF PHAGE LIBERATION BY LYSOGENIC BACTERIA

Investigator: Erich Six  
Support: Deutsche Forschungsgemeinschaft  
General Funds

In a culture of lysogenic cells, spontaneous lysis with release of free phage occurs as a rare event (in 1 out of about 10,000 cells each generation). The study of the frequency of spontaneous lysis (FSL) should yield some information about the interaction between prophage and bacterium. The ratio of free phage to bacteria in a growing culture is an indirect measure of the FSL, depending strongly on the experimental conditions. Therefore, a new technique to measure directly the FSL of Escherichia coli C and Shigella Sh strains lysogenic for bacteriophage P2 was developed. The cells are plated with a streptomycin-resistant indicator and then are killed at various times after plating by spraying with streptomycin. The number of plaques that develop, therefore, is equal to the number of cells that lysed between the time of plating and the time of spraying.

The preliminary results are as follows. Different strains singly lysogenic for various mutants of P2, including P2 dismune (see reports by Bertani and Cohen) show about the same FSL; doubly lysogenic strains generally show a slightly higher FSL, but in every case tested the increase was less than by a factor of 2; for the one triply lysogenic strain tested, the FSL was decreased by a factor of 2 as compared with singly lysogenic strains. It can be concluded that the FSL is certainly not proportional to the number of P2 prophages present in the cell.

It is known that the P2 prophages are attached in at least three different locations on the bacterial chromosome, the prophage of singly lysogenic strains being normally in the so-called "location I" (as in the experiments mentioned above). One singly lysogenic strain was obtained that carries P2 in location II (usually occupied only in multiply-lysogenic strains). The FSL of this strain was significantly lower.
than the FSL of singly lysogenic strains with P2 in location I, thus indicating an
influence of the prophage location on the FSL. Even taking into account this influ­
ence of the prophage location on the FSL, the experimental results suggest that the
prophages do not act independently in determining the FSL of the multiply-lysogenic
strains.

176. STUDY OF PROPHAGE SUBSTITUTION

Investigator: Erich Six
Support: Deutsche Forschungsgemeinschaft
General Funds

Lysogenic cells are immune to phages of the same or a closely related kind
as the prophage carried—that is, upon superinfection with such a phage the majority
of the lysogenic cells remains apparently unchanged, only a small minority shows
some interaction between the lysogenic cell and the superinfecting phage, the most
common type of interaction being the substitution of the incoming phage for the
prophage. In singly lysogenic cells also, double lysogenisation occurs, with a
frequency about 10 times smaller than the frequency of substitution.

A method was developed to study prophage substitution in mass cultures of
Escherichia coli C or Shigella Sh strains lysogenic for P2 and superinfected with
genetically marked P2. The frequency of substitution was found to increase linearly
with the multiplicity of superinfection up to a multiplicity of about 10 (phages/cell)
and then to tend to level off, the probability of substitution per superinfecting phage
being about 10^-3 to 10^-2 for low multiplicities. "Partial substitution" leading to
recombinant prophage types was also found, the frequency of occurrence being
about 10 times smaller than the frequency of "total" substitution. Complementary
recombinants occurred with about equal frequencies.

177. TRANSDUCTION OF THE GALACTOSE MARKER BY PHAGE LAMBDA

Investigator: J. J. Weigle
Support: National Foundation for Infantile Paralysis

Prophage lambda is known to be located in that part of the genetic chromosome
of the Escherichia coli host cell which controls the ability to ferment galactose
(Gal^+). When bacterial mutants which have lost such an ability (Gal^-) are infected
with phage liberated by lysogenic Gal^+ cells, one finds among the cells that survive
infection some which have now become Gal^+ (transduction). It has been shown that
particles with the specificity of phage lambda are the carriers of the Gal^+ allele.
In most cases however the transduced Gal^+ allele does not substitute for the Gal-
allele but is added to it in an unstable way: the phenotypically Gal^+ clones segregate
out Gal^- cells with high frequency. Such strains are called heterogenotes. Phage
lambda produced by a heterogenote has the additional property of being able to
transduce the Gal marker with a very high efficiency (Morse, Lederberg, and
Lederberg).

Lysates obtained from lysogenic heterogenotes have been investigated with
the following results. Electron microscope observation shows that these lysates
contain a majority of "inactive" particles which are unable to form plaques, although
they kill most of the bacteria which they infect. Since the sensitive Gal^- cells which
are transduced by such lysates at low multiplicities of infection become usually
defective rather than normal lysogenic clones (that is, they do not liberate any active
phage, but are immune to superinfection), it seems that it is the "inactive" particles
that carry the Gal^+ allele, transduce, and establish a defective prophage. The
"inactive" particles have a lower probability of establishing lysogeny than the normal,
active particles. They can however go through a complete reproductive cycle in
mixed infection with a normal phage (or prophage), which presumably supplies the functions blocked in the inactive phage. It is likely that non-defective heterogenotes are actually doubly lysogenic cells, carrying a normal prophage, and a defective prophage, to which the heterogenotic Gal⁺ allele is attached.

This work was done in collaboration with W. Arber and G. Kellenberger of the University of Geneva, Switzerland.

Publications:


178. PHENOTYPIC EXPRESSION IN Escherichia coli STRAIN K-12 OF THE TRYPTOPHANE LOCUS OF E. coli STRAIN C

Investigator: Roger Weinberg
Support: National Institutes of Health, Public Health Service

Association of resistance to bacteriophage T1 with requirement for tryptophane is found in T1 resistant mutants obtained from a number of strains of Escherichia coli, including strain C. This association is usually not found in strain K-12, which is the most suitable for genetic studies. Preliminary to fine structure studies of the tryptophane locus in strain K-12, the T1 locus was transduced from strain C into strain K-12 by using temperate phage P1 and selecting for T1 resistance. The C locus for T1 resistance still expresses resistance to T1 and requirement for tryptophane when present in K-12. Since each transduction involves the transfer of a short chromosomal region, this result is consistent with the idea that the same short chromosomal region controls both tryptophane synthesis and resistance to T1.
VISITING LECTURERS

Twenty-three visitors gave lectures or seminars in the Division of Biology during the year. The lecturers and their subjects were:

Dr. Raymond Latarjet, Institut du Radium, Paris, "Radiobiological Studies on a Transforming Agent of 'Pneumococcus'."

Dr. Neville Symonds, London School of Hygiene and Tropical Medicine, "A New Class of Phage Mutants."

Dr. Bernard O. Phinney and Dr. Anton Lang, Botany Department, University of California at Los Angeles, "Genetical and Physiological Studies on Gibberellins."

Dr. W. F. H. M. Mommaerts, University of California at Los Angeles, School of Medicine, "Chemical and Molecular Physiology of Muscular Contraction."

Dr. Alan Garen, Purdue University, "Mechanism of Growth Inhibition of Certain Bacteriophage Mutants."

Dr. Louis N. Katz, Director of Cardiovascular Research, Michael Reese Hospital, Chicago, "Coronary Flow and the Energetic Metabolism of the Heart."

Professor Edgar Zwilling, University of Connecticut and Program Director for Developmental Biology, National Science Foundation, "Tissue Interaction During Limb Development."

Dr. Ludwig von Bertalanffy, Director of Biological Research, Mount Sinai Hospital, Los Angeles, "Nucleic Acids in Normal and Abnormal Growth and Their Study by Fluorescence Microscopy."

Dr. J. Herbert Taylor, Botany Department, Columbia University, "Mode of Replication and Organization of Chromosomes as Revealed by the Distribution of Tritium-labeled DNA."

Dr. Merrill W. Chase, Rockefeller Institute for Medical Research, New York City, "Immunologic Unresponsiveness to Drugs."

Dr. Wiktor W. Nowinski, University of Texas - Medical Branch, "Some Aspects of Metabolic Patterns and Growth."

Professor R. A. Brink, Department of Genetics, University of Wisconsin, "Paramutation of the R² allele in Maize, Conditioning Aleurone and Plant Color."

Dr. H. H. Weber, Director, Max Planck Institut für Physiologie, Heidelberg, Germany, "The Four Mechanisms Involved in the Movements of Cells."

Dr. Aaron Novick, Institute of Radiobiology and Biophysics, University of Chicago, "Enzyme Induction, an All-or-None Phenomenon."

Professor Bruno Huber, Institute of Forestry, University of Munich, "The Daily Course of Photosynthesis and Transpiration."

Dr. Maurice H. Bernstein, Biological Research Laboratory, Mt. Sinai Hospital, Los Angeles, "Cytochemical Studies in Electron Microscopy. Observations on Bacteria and Phage."

Dr. F. Jacob, Institut Pasteur, Paris, "Conjugation and Genetic Recombination in Escherichia coli."

Professor E. Hadorn, University of Zurich, Switzerland, "Gene Action on Protein Metabolism and Pterine Pigments in Drosophila."

Dr. Sam Gross, Stanford University, "Aromatic Synthesis in Neurospora."

Dr. Charles Yanofsky, Department of Microbiology, Western Reserve University, "Studies on Tryptophane Synthetase (desmolase) - a Progress Report."

Professor Rene Wurmsaer and Dr. Filiotti-Wurmsaer, University of Paris, "Thermodynamic Studies of Iso-Agglutination."

Dr. Kenneth J. Richland, White Memorial Hospital, College of Medical Evangelists, Los Angeles, "The Para-aquaductal Grey Matter."

Dr. J. M. Mitchison, University of Edinburgh, Scotland, "The Growth of Single Cells."
GRADUATES

Six men and one woman were awarded B.S. or Ph.D. degrees in Biology in June, 1957. The names, degrees conferred, the titles of doctoral theses, and the plans of the recipients for future work are as follows:

Warren A. Furumoto, B.S. To the Graduate School at the University of California at Los Angeles.
Truman E. Long, B.S. To Stanford University School of Medicine.
Donald P. Nierlich, B.S. To the Graduate School at Harvard University.
Marcel A. Baluda, Ph.D. Thesis: "Homologous Interference by Ultraviolet Inactivated Virus in Newcastle Disease Virus." He is now at the City of Hope Medical Center, Duarte, California.
James H. Berrian, Lt., U.S.N., Ph.D. Thesis: "The Immune Response in Homologous Transplantation." He has returned to the Naval Medical Research Institute, National Naval Medical Center, Bethesda, Maryland.
L. Elizabeth Bertani, Ph.D. Thesis: "Studies on the Establishment of Lysogeny by Bacteriophage P2." She will do postdoctoral research at the University of Southern California.
Robert E. Cleland, Ph.D. Thesis: "The Hormonal Control of Cell Wall Properties." He has received a National Institutes of Health, Public Health Service fellowship and will do postdoctoral research at the University of Lund, Sweden.

RESEARCH TRAINING STIPENDS

The Institute has been awarded a three-year grant by the National Science Foundation for the support of the research of graduate students. Such training stipends are awarded on the basis of two quarters per year, the support for the remaining two quarters being provided through teaching appointments with the title graduate assistantship. Thus, for a given student, four-quarter support is provided at a level of $500 per quarter with teaching duties for only two of the four quarters; in addition a tuition grant may be awarded.

By making financial support of graduate students independent of both the field of thesis research and the professor under whom such research is done, this plan provides for a desirable degree of freedom and independence on the part of the student. The basic plan of thus divorcing support from choice of major field and sponsoring professor has now been in operation in the Division for several years and is generally regarded as in fact having the indicated advantages.

NUTRITION FOUNDATION FELLOWSHIPS

During the past 11 years work in chemical genetics has been supported by grants from the Nutrition Foundation. The use of this grant has recently been modified somewhat and is currently being used in much the same way as described above for the National Science Foundation Research Training Stipends.

ARTHUR McCALLUM FELLOWSHIPS

The present plan for the support of graduate students in the Division was first made possible by the existence of the Arthur McCallum Fellowship Fund. Currently this fund plus the two funds just mentioned have made it possible to offer support to most of the graduate students in the Division not otherwise adequately provided for.
DU PONT FELLOWSHIP

The E. I. du Pont de Nemours and Company Graduate Fellowship in Biology in 1956-57 was awarded to Mr. Ellsworth H. Grell, a graduate student in genetics. This fellowship is usually awarded to a student in the final year of his graduate work. There are no teaching duties involved, which means that the recipient of the Du Pont Fellowship has maximum time to devote to his thesis research.

NATIONAL INSTITUTES OF HEALTH, PUBLIC HEALTH SERVICE FELLOWSHIP

The National Institutes of Health, Public Health Service has awarded to the Institute a predoctoral fellowship for the support of graduate study in biophysics. This grant, which is for a period of four years, is being used in the same way as are other predoctoral fellowships in the Division of Biology. Mr. Jacob Maizel, Jr., who is investigating the synthesis of protein by microsomal ribonucleoprotein particles, is currently supported by this fellowship.

KIRK MACOMBER FELLOWSHIP

Mr. John M. Clark, Jr., was awarded a Kirk Macomber Fellowship for support during one term of the academic year. His thesis work is in the field of plant biochemistry.

GOSNEY POSTDOCTORAL FELLOWSHIPS

For the year 1956-1957, invitations to accept Gosney Fellowship appointments were extended to Doctor Yonosuke Ikeda of the Institute for Applied Microbiology of the University of Tokyo and to Doctor Dugald M. Paton of Hobart University, Tasmania, Australia. Unfortunately Doctor Ikeda was unable to accept. Doctor Paton has spent the year working in the Earhart Laboratory on the interaction between temperature and light in the control of flowering in the garden pea.

SUMMER RESEARCH PROJECTS

In May 1957 the National Science Foundation made a two-year grant in the amount of $20,294 to the Institute for the purpose of encouraging and assisting teachers of science and mathematics in high schools, junior colleges, and some of the smaller liberal arts colleges in acquiring in-laboratory research training at the Institute during the summer months. Four men have now been appointed to work on research projects in the fields of corn genetics, biochemistry, and plant biochemistry during the summer of 1957. An account of their activities will be given in next year's report.

HONORS AND AWARDS

Henry Borsook received the Franz M. Groedel Award of the American College of Cardiology.

Renato Dulbecco and C. A. G. Wiersma were each awarded a John Simon Guggenheim Memorial Fellowship for study abroad.

R. D. Owen was appointed Visiting Professor at Cornell University during the summer of 1956.

A. H. Sturtevant received the Kimber Genetics Award of the National Academy of Sciences.
F. W. Went received an Honoris Causa doctorate from the University of Paris. He was also awarded a Certificate of Merit by the Botanical Society of America.

FINANCIAL SUPPORT

The financial support that made the work of the Division of Biology possible came from many sources: from general Institute endowment and from special endowment funds; from non-endowment funds for broad areas of work; from grants made by individuals, companies and foundations for specific projects; from unrestricted annual gifts; from contracts with various U. S. governmental agencies; and from contributions to general funds provided by Industrial Associates and Institute Associates. During the year additional special endowment funds were set up in support of the Institute's program of research and instruction in chemical biology. Such funds have been matched out of the $1,500,000 contingent grant made to the Institute by the Rockefeller Foundation. In addition to the grants given special mention in the introduction to this report of the Division a grant made by the National Institutes of Health of the United States Public Health Service is particularly noteworthy. It is a substantial grant for work in experimental biology. Subject to appropriation of funds it will continue for five years. A considerable part of the funds of the grant will be used for movable equipment, much of which will finally end up in the proposed new wing connecting Kerckhoff and Church Laboratories. This grant has already been used to good advantage for the expanded program in biophysics.

A second grant, made by the Commonwealth Fund, is especially valuable in that it provides fluid research funds for exploratory research and for filling in the gaps in support that inevitably arise when a large proportion of support for research comes in the form of relatively short-term grants for particular projects.

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<td>Mrs. Henry M. Robinson</td>
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<td>Norman W. Church</td>
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<td>Fredericka B. Williams</td>
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<tr>
<td>Alwilda Harvey</td>
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<td>Tomato fruit set studies</td>
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<td>Research on muscle reinnervation</td>
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<td>U. S. Atomic Energy Commission</td>
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<td>U. S. Department of Agriculture</td>
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<tr>
<td>U. S. Navy, Office of Naval Research</td>
<td>Hemoglobin and red cell studies; immunogenetics research</td>
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<tr>
<td>U. S. Public Health Service</td>
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