BIOLOGY
1955
AT THE CALIFORNIA INSTITUTE
OF TECHNOLOGY
A Report for the Year 1954-1955
on the Research and other Activities of the
Division of Biology
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INTRODUCTION

Each summer, as reports of individual investigators are gathered and edited for inclusion in this review of the year's work, the usefulness of such an annual report is again demonstrated. It serves to disseminate information to interested persons and organizations prior to formal publications. It provides an over-all view of the organization and activities of the Division that is of particular interest to prospective graduate students and research workers. It is also a progress report for individuals and organizations who have helped financially and in other ways to make research and teaching possible.

Within the Division of Biology itself, the report not only encourages research workers to reexamine their objectives and take stock of their accomplishments, but it also informs them as to what their colleagues are doing.

The bits of new knowledge that result from research efforts sometimes seem to individual investigators to be small, insignificant and discouragingly costly in time and effort. When, however, the many small contributions are combined, the total begins to be impressive and even inspiring.

The contributions of the Division of Biology for the year under review are indeed inspiring. To take one segment of them as an example, the increased understanding of protein synthesis and its relation to the nucleic acids is particularly satisfying. The views of geneticists, molecular structure chemists, biochemists, immunologists, virologists and others working in this area are at last beginning to converge and fit together in a way that seems clearly prophetic of a major advance in biological knowledge.

Although research is an important activity of the Division, teaching at the undergraduate, graduate and postdoctoral levels likewise receives strong emphasis. During 1954-55 there were eleven undergraduates in the biology option, twenty-nine graduate students in the Division and seventy-seven postdoctoral research fellows on the staff.

Support for teaching and research has continued to come from many sources: from general Institute endowment; from Industrial Associates and Institute Associates; from designated endowments such as the Mrs. Henry M. Robinson Fund, the Frank P. Hixon Fund for physiological psychology, the Gosney Research Fund; and from gifts, grants or contracts by individuals, companies, foundations and U. S. Government agencies. Sources of support are indicated elsewhere in this report—in abstracts of individual projects.

Progress has been made toward matching the $1,500,000 contingent grant of the Rockefeller Foundation that was announced last year for the support of the Institute's joint Chemistry-Biology research program. The Charles B. Corser bequest of $300,000, designated for biology, and the Arthur McCallum Fund of $200,000 for undergraduate, graduate and postdoctoral scholarships and fellowships in the biological sciences represent the two most significant steps toward the goal.

The Norman W. Church Laboratory for Chemical Biology is nearing completion as this report is being prepared and should be ready for occupancy in the fall of 1955. As previously announced the first group from the Division of Biology to occupy this laboratory will be the virologists.
Construction is under way to the north of Crellin Laboratory on a solvent storage building. This will replace the storage platform at the Biology Experimental Farm. It will be another of several facilities shared by the Divisions of Biology and Chemistry for work in chemical biology.

Much of the research work summarized here is not yet 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.
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7 Commonwealth Fund Fellow
8 American Cancer Society Fellow
9 Foreign Operations Administration Fellow
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2 National Science Foundation Fellow
3 National Foundation for Infantile Paralysis Fellow
4 DuPont Fellow
5 Lucy Mason Clark Fellow

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Administrative Assistant

Gerald Fling

Animal Rooms Staff

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Richard Anderson                                             William A. Moeur
Nick Bogroff                                             Randolph W. Myers
Gordon L. Brennan                                        James Trew
Byron E. Gipson, Jr.                                      Charles von Berg
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John L. Krebs

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Grace Marshall  George Zentmyer
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<th>Name</th>
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<td>Kerckhoff Marine Laboratory</td>
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PART I

RESEARCH SUMMARIES
ANIMAL BIOCHEMISTRY

Much of the work of the animal biochemistry laboratory has centered around the problem of protein synthesis. Hemoglobin synthesis by red blood cells is found to be stimulated by a number of factors including sugar amino acids, iron and a new class of compounds, the fructose amino acids.

The number of red blood cells formed by an animal and their filling with hemoglobin are two processes which are influenced by different factors.

On the basis of the many facts now known about protein synthesis, a general hypothesis has been formulated. It is postulated that the amino acid building blocks of protein are activated by means of adenosine triphosphate (ATP) through their carboxyl groups. Such activated amino acids are then carried to a ribonucleic acid (RNA) template where they are arranged in a specific sequence, corresponding to the specific structure of the RNA template, and joined together through peptide bonds. In a subsequent step the protein molecule separates from the template and in so doing or shortly thereafter undergoes specific coiling or folding as determined by its amino acid sequence. Its coiling pattern is modifiable either in the initial coiling or subsequently by such agents as antigens or inducers of adaptive enzymes.

Other activities of the animal biochemistry laboratory include the following:

- New evidence for the theory that adaptive enzymes are formed in the absence of substrate but normally are rapidly inactivated.
- A new type of peptide bond linkage in proteins involving the ε-amino group of lysine.
- Investigations of the mechanism of metabolism of histidine.
- Studies on the biosynthesis of a number of compounds of biological importance, including the vitamin riboflavin, several aromatic amino acids, purines, pyrimidines and the pyrimidine nucleotides.
- Study of a new class of amino-acid-containing phospholipids.
- Identification of the pteridine pigments of Drosophila, including one not heretofore known.
- Development of methods for separating particulate material from cells by electrophoresis.
- Investigation of methods of fractionating different molecular species by cytochrome c.

ANIMAL PHYSIOLOGY

As in the past, the animal physiology laboratories continue to be concerned mainly with problems of the central nervous system. Further progress has been made in correlating sensory stimuli in the crayfish with specific interneuron fibers. Some seventy different fibers have now been identified with reasonable certainty.
A study was made of action potentials associated with so-called twitch contractions of various claw muscles of the European lobster.

Single muscle fibers of crustacea may be activated with from one to four motor axons and can respond in more than one way depending on which axon carries a stimulus to them. This fact was determined by electrical recording from microelectrodes in single muscle fibers of the California rock lobster.

Evidence continues to indicate that the so-called spreading depression of cortical activity in mammals is a normal mechanism for preventing the spread of localized convulsive activity of the cortex. Evidence has been obtained that a slow potential change normally accompanying spreading depression is not the agent by which spreading depression is propagated.

In animals in which the optic chiasma has been cut, responses in the visual area evoked by light flashes to one eye are largely or completely confined to the same side of the cortex as the stimulated eye. Local application of convulsive drugs results in an increase in transfer to the opposite visual area, presumably by facilitating synaptic connections through the corpus callosum.

BIO-ORGANIC CHEMISTRY

Smog formation is known to involve ozone production as a result of photochemical oxidation of hydrocarbons and other organic compounds in the presence of nitrogen oxides. In tests of fifty substances, butadiene, olefins, alcohols and aldehydes proved to be most effective in ozone production. This result suggests that in the control of smog these and other ozone-producing substances, as well as nitrogen oxides, be kept at the lowest possible level.

In a study of proteins and other constituents of milk, some of the substances responsible for flavor of evaporated milk have been identified. Several fractions of milk are being tested for allergenic properties. Attempts are being made to crystallize $\alpha$-lactalbumin and to determine its physical and chemical properties.

For some years the dicarboxylic acid, traumatic acid, has been known as a plant wound hormone as judged by its induction of cell division of the mesocarp of a bean pod. It has now been shown that higher unsaturated fatty acids, such as linolenic acid, are active in this test and that their activity is augmented by coenzyme A and cytochrome $c$.

EMBRYOLOGY

Several significant additions to our knowledge of the process of fertilization of sea urchins and other marine eggs have been made in the laboratory of embryology. The fertilizin of sea urchin eggs, a glycoprotein, has been shown to have a molecular weight of about 280,000 and to be an elongated molecule with an axial ratio of about 28:1. It is highly acidic with an isoelectric point of about pH 2. Sperm heads have been shown to have of the order of 100,000 receptor spots to which fertilizin molecules can become attached. Methods have been developed by which sea urchin sperm
can be frozen and thawed without losing mobility or fertilizing ability. The plant enzyme papain can be used to prevent formation of the fertilization membrane of the sea urchin egg without interfering with normal development. The processes of fertilization and subsequent mitotic cell division can be inhibited by certain specific antisera, including antisera against fertilizin. The functional life of sea urchin sperm in sea water suspensions is increased by metal-chelating agents such as Versene. It is believed that in this way the supply of energy for sperm metabolism and activity is regulated.

The sperm of several species have been shown by electron microscopy to possess a heretofore undescribed filament, called the acrosomal filament, projecting forward from the head.

A monograph on the subject of fertilization has been prepared and is now in press. Techniques have been developed for using univalent antibodies in specific tests that may have several useful applications.

Auto-antibodies of sponges and sea urchin eggs have been further studied. The antifertilizin of sea urchin eggs is probably a glycoprotein. An auto-antibody found in sponges has been shown to be heat stable and non-dialyzable. The numbers of mitochondria and distribution of mitochondria in sea urchin embryos have been followed during development. There are indications of a relation between these bodies and the process of differentiation. Insulin has been shown to increase respiration in the apical cells of a sponge.

GENETICS--CORN

Work continues on the genetic and cytological location of chromosomal breaks in the collection of stocks carrying chromosomal rearrangements, mostly reciprocal translocations. These rearrangements are being transferred to lines adapted to the corn belt so as to make them of maximum use in corn breeding. An extensive program of mapping genes of corn is being carried on. Cytological studies of pairing translocation involving a normal chromosome 9 and a supernumerary B-type chromosome indicate how a diminutive B-type fragment can arise. These have been found previously but their origin has been obscure.

Anthocyanin production in the aleurone of the corn kernel is under the control of a number of genes. These control the level of hydroxylation of the pigment, its concentration, its formation, and the formation of a leuco precursor that can be converted to pigment in vitro by heating in dilute acid.

Detailed descriptive studies of sixteen mutant types involving tassel structures have been completed. Interaction effects in double mutants are planned as a next step.

GENETICS--DROSOPHILA

Mutant types of Drosophila have been accumulated for forty-five years. The probability that new types will be found is now quite small. Nevertheless, two new mutants of special interest were found during the year. One is a brownish eye color dependent on a sex-linked gene. The other is an autosomal dominant that has the curious property of being lethal to larvae carrying the sex-linked recessive eye color known as prune. It is called "prune-killer."

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An eye-color mutant known as "unstable white" is being investigated to determine the cause of the instability. No gross chromosome aberrations are involved. The gene concerned appears to be slightly to the right of white.

The tyrosinase system has been investigated in a number of mutants in which melanin pigmentation is altered. In no case was tyrosinase activity absent. In the course of these studies it was found that tyrosinase activity may appear in cell-free extracts by a reaction represented as follows:

\[
\text{Protyrosinase} + \text{Activator} \rightarrow \text{Tyrosine} + 2 \text{Activator}
\]

No process of this type has been previously described. Procedures are being devised for the purification of prototyrosinase, the activator and the active enzyme.

A heat-stable, dialyzable substance required for normal development of Drosophila larvae is being purified and isolated.

The osmotic pressure of larval hemolymph has been found by a thermoelectric method to be equal to that of 1.05 percent sodium chloride.

A heat-labile, non-dialyzable, pH-sensitive, tumor-producing agent in Drosophila has been further studied in an attempt to purify and identify it.

GENETICS--NEUROSPORA

An extensive review of the methods of biochemical genetics, especially of Neurospora, yeast, Aspergillus, and bacteria, is in press and will appear in the summer of 1955. A second review is in preparation on genetic mechanisms in microorganisms.

Further studies have been made on ascospore germination in Neurospora and on a mutant unable to grow normally on media of high osmotic concentration.

In a heterocaryon carrying in separate nuclei the alleles for temperature-labile and temperature-stable tyrosinase, both types of enzyme are formed, a result consistent with the template theory of gene action.

Several instances of irregular segregation of alleles in Neurospora have now been found under conditions where mutation and crossing over are highly improbable. Some special type of interaction between alleles, such as occasional miscopying of a small segment of a chromosome, seems to be involved.

Studies have been made of two suppressors of slow-growing mutants that involve the cytochrome system. One suppresses a cytoplasmically inherited defect but does so without apparently altering the cytochrome system. The other suppresses a genic cytochrome defect by restoring the cytochrome system to normal. Both suppressors are nuclear gene mutants.

Nine genes of linkage group VI of Neurospora have been assigned positions relative to one another and to the centromere. Six are in one arm and three are in the other.

Measurements of double crossing over involving regions on opposite sides of
the centromere in Neurospora indicate that crossing over in one region shows no correlation with or influence on that taking place in the region across the centromere.

IMMUNOGENETICS

Studies on the serotypes of thirty-one strains of the ciliated protozoan Tetrahymena pyriformis have been continued and show that a given stock often contains a mixture of serotypes. There is noted a kind of instability in these serotypes like that reported by Sonneborn for Paramecium.

Tissue transplants between animals that are not genetically identical usually fail. There are, however, situations in which grafts that normally would not be tolerated become established and survive. Attempts to understand the basis for this desensitization have been sought in studies of tissue transplants between breeds of chickens. Scale transplants in fish have proved valuable in interpreting tissue graft reactions, and plans are under way for a study of the genetic control of scale transplantability in the goldfish.

The cells of sponges that have been mechanically separated are capable of reaggregation. Such reaggregation is specifically blocked by antisera to the sponge. Data obtained from reaggregation studies are of value in determining taxonomic relations as well as for investigating some of the factors involved in holding the cells together in tissues of higher animals.

MARINE ZOOLOGY

Experiments were conducted on the relation of light to tropic responses and to reproduction of a number of species of sea anemone.

A monograph on the marine invertebrates of Point Barrow, Alaska, has been completed and is now well on its way toward final publication.

During the summer of 1954 Professor Davenport of the University of California, Santa Barbara campus, taught the sophomore marine zoology course and at the same time carried on some research at the Marine Station at Corona del Mar. He investigated the symbiotic relation between a starfish and an annelid worm. These animals presumably make use of chemical recognition in maintaining their partnership.

PLANT BIOCHEMISTRY AND PHYSIOLOGY

A large part of the effort of the plant biochemistry laboratories is devoted to a study of protein synthesis and related problems. The processes involved are studied in intact plants and in cell-free preparations. The mechanism of amide and peptide bond synthesis is being studied, as are factors influencing the incorporation of labeled amino acids into proteins. The precise role of nucleic acids and their building blocks is under investigation.

One of the proteins—myxomyosin—of a slime mold has properties somewhat like myosin of muscle. It has been purified and its chemical and physical properties
studied in detail. Like myosin it undergoes reversible changes in physical properties in response to adenosinetriphosphate.

The biosynthesis of asparagine, caffeine and rubber has been investigated. In connection with the latter substance the metabolism of its presumed precursor, \( \beta \)-methylcrotonate, has been further studied.

Studies of plant hormones have been an important activity in the Division for many years. During the year they have included investigations on the kinetics of auxin action, its uptake by the plant, its role in reducing cell wall pressure, its relation to water uptake, its part in root growth, its interaction with light in controlling flowering, its interaction with anti-auxins and other antimetabolites, and the chemical requirements for its activity.

Several enzymes have been studied in some detail. These include glutamic decarboxylase, and the peroxidases. The pea plant has been shown to contain at least five peroxidases. The characteristics of these, their distribution in the plant and their inactivation by coenzyme A, have been studied. The peroxidases show a close relation to indoleacetic acid oxidase and both show properties of adaptive enzymes in their response to external indoleacetic acid (auxin). Over-all indoleacetic acid destruction appears to be limited and controlled by the rate of peroxide genesis. As cells age, peroxide increases and hence indoleacetic oxidase also increases.

Further studies on the phototropic response of plants containing little or no \( \beta \)-carotene confirms the earlier conclusion that the receptor pigment in this process is riboflavin rather than \( \beta \)-carotene.

Some plants are made to flower earlier than they otherwise would by low temperature treatment of germinating seeds. This process is known as "vernalization." It has been found that vernalizing substances can be extracted from cold-treated pea seeds and effectively transferred to seeds that have not been so treated. The substances responsible appear to be nucleosides. Guanosine is especially active.

Water uptake by oat coleoptiles appears to be largely a simple diffusion process as determined by studies using deuterium-labeled water.

The metabolism of externally applied urea has been investigated. Commercial urea contains biuret as a contaminant and this often causes leaf injury.

Several introduced plants that gave indications in Earhart Laboratory of being well suited to erosion control in local mountains are now being tested in the field.

PLANT ECOLOGY AND PHYSIOLOGY

A book has been prepared describing the operation of the Earhart Laboratory and the work done in it during its first six years of operation. It will be published by the Chronica Botanica Company.

The general growth requirements of a number of plants have been determined in the controlled laboratories of Earhart Laboratory. These studies on the effects of temperature, light and other factors have been made on Eastern forest tree
seedlings, Mimulus (monkey flower), tomatoes, strawberries, the broad bean, the lentil, the cocklebur, various weeds, on the water plants of the family Lemnaceae and on various plant tissue cultures. Root growth in relation to temperature and auxin treatment has been investigated.

Germination and growth of cacti and various other desert plants have been studied in relation to light, temperature and water supply.

Water relations have been examined with respect to transpiration, stomatal movement, and exudation by cut stems.

The flowering of higher plants is influenced in various ways by temperature, by light intensity and duration, and by other factors. The flowering of orchids, peaches, Cestrum (night-flowering jasmine), peas, and coffee has been investigated in some detail. The effect of auxin and other chemical substances in flowering of peas has been determined. In this plant the inheritance of response to low temperature treatment of seeds (vernalization) has been studied as has the resistance to high temperatures by some varieties.

An investigation of the ratio of carbon-13 and carbon-12 in plants has been made in collaboration with members of the Division of Geological Sciences. This ratio is found to vary under different conditions and in different plants.

A theory of petroleum formation through the condensation of "smog" originating from volatile plant organic matter has been proposed.

PSYCHOBIOLOGY

In continued investigations of the central nervous system of higher animals it was found that there is carry-over from one hemisphere of the brain to the other of learned visual discriminations. However, if the corpus callosum is surgically sectioned, there is no such transfer in any tests so far devised.

By means of a special series of operations the degree of functional autonomy of the visual cortex can be investigated by isolating it surgically from all other cortical areas. The result in preliminary studies is severe impairment of visual function.

In fishes and in amphibians there is normal recovery of motor coordination after surgical disarrangement of the normal nerve-muscle connections. It is believed that such recovery is accomplished by chemotropic breakdown of synaptic terminals in the spinal cord, followed by reestablishment of new central hook-ups made in accordance with the new nerve-muscle connections. The readjustment factors involved are thought to be the same as those that may be responsible for the orderly patterning of reflex connections in the developing human brain. Morphological evidence for this is being sought in fishes but so far the results are indecisive.

The results reported previously on the role of the corpus callosum in the contralateral transfer of visual learning and memory have been confirmed in experiments with tactile discriminations. The corpus callosum is found to be necessary for the transfer of learned discriminations when switching from one hand to the other.
VIROLOGY AND BIOPHYSICS

Bacterial viruses (phages) have many advantages for experimental study and they have played an important role in several advances of general significance to biology. Several investigators have continued work on them at the Institute during the last year.

In the phages most used for genetic work it has been shown by special techniques that all genetic traits so far investigated belong to a single linkage group.

Phages may establish a symbiotic relation with host bacteria in what is called a prophage condition. Prophages become linked with genetic traits of the host cell. Presumably the desoxyribonucleic acid (DNA) of the phage attaches to that of the host and in a specific region. Different prophages may occupy different sites.

The transfer of DNA from one generation of phage to another has been studied by labeling the DNA with radioactive phosphorus ($P^{32}$). Refinements of previous experiments of this kind have been made.

The effects of ultraviolet radiation on prophages at various stages of the life cycle have been studied. The cooperation of two X-ray-damaged phage particles in reconstituting viable phages has been re-investigated and the reasons discovered for failure of previous experiments to show the so-called multiplicity reactivation of X-ray-treated phages.

For adsorption to bacterial surfaces some phages require a cofactor such as tryptophane. The related compound indole competes in this process. The protein denaturant urea has been found to activate cofactor-requiring phages. The kinetics of its action in this respect as well as its action in "killing" phages has been examined. Physical changes in activated phage have been demonstrated.

A peculiar dilution effect of antisera on inactivation of phage was discovered a few years ago. The kinetics of the process has been further investigated. Out of this and related studies has come a new theory of antibody-formation that may be widely applicable.

A detailed molecular structure has been proposed for tobacco mosaic virus. It is thought to consist of a hollow ribonucleic acid cylinder inclosed in an outer cylinder made up of helically arranged protein. A new type of X-ray diffraction study suggests that southern bean mosaic virus particles may consist of hollow spheres of ribonucleic acid inclosed in protein coats.

Progress has been made in making a detailed analysis of the growth characteristics of the mold Phycomyces. This work is not related to the virus research work mentioned above but is reported here because it is being done in the same laboratory as is the phage work.

During the year Division virologists have worked with the animal viruses causing the following diseases: poliomyelitis, western equine encephalomyelitis, Newcastle disease, vesicular stomatitis, and Rous sarcoma. Much of this work was done with the monolayer tissue culture technique.

The growth, plaque characteristics, pathogenicity, temperature tolerance and resistance to ultraviolet radiation of normal poliomyelitis virus and several of
its mutants have been investigated. One mutant has been shown to have low pathogenicity for the monkey. The kinetics of release of poliomyelitis virus by single host cells in tissue culture has been investigated. No virus appears for about five hours but one hundred to two hundred particles are released per cell during the next hour. Characteristic morphological changes in infected cells are observed to take place during virus release.

Ectodermal and mesodermal tumors in the chick develop following infection with Rous sarcoma virus. The characteristics of the growth, influence of host cell type, release of virus, time course of infection and other relationships of the virus to the ensuing cancer have been followed.

Similar studies have been made for the virus of vesicular stomatitis and of Newcastle disease. In addition radioactive phosphorous has been used as a means of following phosphorous and nucleic acid metabolism in both these viruses.

Several technical improvements in virus research methods have been made and are reported elsewhere.
PART II

RESEARCH REPORTS
ANIMAL BIOCHEMISTRY

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1. THE BIOLOGICAL SYNTHESIS OF PROTEIN

Investigators: Henry Borsook, Geoffrey Keighley, Peter H. Lowy, Richard S. Schweet
Support: American Cancer Society
U. S. Atomic Energy Commission
Eli Lilly Fund
National Science Foundation
U. S. Public Health Service

Work has been continued on studies of the mechanism of the biological synthesis of protein, and of the biochemical factors that influence hemoglobin synthesis in vitro.

a) Factors Stimulating Hemoglobin Synthesis In Vitro. Hemoglobin synthesis in vitro in rabbit reticulocytes is stimulated by a boiled aqueous extract of liver and by the non-protein filtrate of plasma. The nature of the stimulating systems in both of these extracts has now been established. Amino acids, iron and glucose account, in part, for the stimulating effects of both the liver and plasma extracts.

b) The Stimulating System in Liver: Fructose-amino Acids. In addition we have isolated from liver and identified a new class of compounds. These are fructose-amino acids; their general structure is

\[
\text{H}_2 \text{R} \\
\text{HC.NH.CH.COOh} \\
| \\
\text{C} = \text{O} \\
| \\
(\text{CHOH})_3 \\
| \\
\text{CH}_2\text{OH}
\]
R may correspond to any one of a number of amino acids. We have synthesized the above type of compounds with R groups of fourteen different amino acids.

The mode of action of all the fructose-amino acids appears to be the same; it is to make iron much more effective. Nothing is known as yet regarding the metabolism of these compounds. There are indications that this type of sugar-amino group linkage occurs in glycoproteins. In general they may be viewed as the Amadori rearrangement products of the normal amino N-glycosides.

c) Reconstitution of Stimulating System in Plasma. The total stimulating effect of plasma can be reconstituted, indeed greatly exceeded, by adding to saline solution iron, glucose, histidine, leucine, phenylalanine, valine, tryptophane, lysine, serine, tyrosine and glutamine. The degree to which the rate of synthesis falls short of the maximum when any one of the foregoing amino acids is withdrawn is in the order in which they are given above. The effect of the glutamine, probably, is to provide glutamic acid; it was found with $^{14}$C-labeled glutamic acid that this amino acid does not penetrate the reticulocyte; glutamine does. For maximum effect iron, glucose and amino acids must be present at the same time. Iron and glucose have no effect unless the amino acids are added. The amino acids exert a small stimulating effect alone, but their effect is more than doubled when added with iron and glucose.

d) Inhibition of Calcium and Phosphate Relieved by Citrate and Magnesium Respectively. In addition it was found that calcium, at the concentration in plasma, is inhibitory; this inhibition is relieved by citrate at its normal concentration in plasma. Phosphate, at the concentration in plasma, is inhibitory; this inhibition is relieved by magnesium at the concentration in plasma. Neither citrate nor magnesium was stimulating.

e) Effects of Heavy Metals: High Specificity of Lead as a Poison: Lead Poisoning In Vitro. The effects of the following metals, other than iron, were tested at low concentrations ($10^{-4}$ M or less) for possible stimulating effects: aluminum, cobalt, copper, manganese, molybdenum and zinc. Only cobalt was stimulating; the maximum stimulation attainable with cobalt was about half that with iron. For submaximal effects cobalt was about one-tenth as effective as iron: When cobalt and iron were together only the greater effect of iron was obtained even when this effect was submaximal.

At greater concentrations than $10^{-4}$ M the following metal ions were inhibitory: aluminum, antimony, copper, gold, lead and mercury. Of these the most inhibitory was lead. Even at $10^{-6}$ M lead exerted a twenty percent inhibition; at this low concentration no other metal or metabolic inhibitor had any effect. This is interesting in view of the well known effects of lead poisoning.

f) Effects of Antibiotics and Antimetabolites. A number of antibiotics and antimetabolites were tested. Of these the most inhibitory was aureomycin; it exerted fifty percent inhibition at $5 \times 10^{-4}$ M. Chloromycetin gave thirteen percent inhibition at $10^{-3}$ M. The following had no effect at $10^{-3}$ M: 6-mercaptopurine, 4-amino-5-imidazole carboxamide, 4,6-dihydropyrimidine, benzimidazole, isoguanine $SO_4$, 2-thio cytosine, 5-bromo-uracil, 6-amino uracil, 2,6-diaminopurine $SO_4$, 8-azoguanine, isocytosine, 4-pthalimido-2,6-dimethyl-pyrimidine, o-fluorophenylalanine, 2-thiencylanine, 3-fluoro-L-tyrosine, penicillin G, streptomycin, and bacitracin.
It is noteworthy that plasma does not provide an optimal reaction medium for the synthesis of hemoglobin in reticulocytes. The total iron in plasma—bound to globulin and free—is about 1 µgm. per ml.; the minimum concentration of iron for a maximum rate of synthesis is 2 µgm. per ml. The amino acids in plasma are far from optimal; addition of histidine, leucine, phenylalanine, tryptophane, tyrosine and valine, to plasma more than doubles its effectiveness. There is a suggestion here that some secondary anemias may be due to deficient concentration in the blood of some amino acids.

g) Mode of Hemoglobin Synthesis. In order to get some understanding of the mechanism of hemoglobin synthesis, the two protein fractions of the reticulocytes were isolated: hemoglobin and the water insoluble proteins. The hemoglobin was further fractionated into heme and globin. It was found that iron, amino acids, fructose-amino acids, and glucose all stimulated the synthesis of heme, globin and the water insoluble proteins. The synthesis of heme and of globin was equimolar, i.e., the rates of incorporation of glycine, per equivalent of glycine, were the same. This proved that there are no significant concentrations in the cells of unlabeled intermediates of either heme or globin. Experiments with other labeled amino acids all indicated that their rates of incorporation into globin, per residue, were the same. This finding also argues against any significant concentration of unlabeled peptide intermediates and against unequal labeling. These findings do not exclude intermediates in hemoglobin synthesis, but the concentration of such intermediates must be very low and they must be formed continuously and very quickly from the much larger concentration of free amino acids. The data indicate that the rate of formation of such intermediates is sensitively dependent on the free amino acid concentration. Furthermore the data point to a regulating mechanism whose effect is an equimolar rate of synthesis of heme and of globin.

h) Features of Protein Metabolism in the Whole Animal and In Vitro in Reticulocytes. The synthesis of protein in vitro in reticulocytes was found to exhibit the following characteristics of protein metabolism as seen in whole animals. For synthesis there is a requirement for carbohydrate and the carbohydrate needs to be present at the same time as the amino acids. All the necessary amino acids need to be present at the same time. If the concentration of any one amino acid is suboptimal the rate of the whole process is suboptimal even though everything else needed is abundant. A priori it was possible with suboptimal concentration of any one needed amino acid that the rate at first would be maximal until the amino acid was nearly used up and then the rate would drop nearly to zero; or the rate would be submaximally more or less in proportion to the degree that the amino acid concentration was suboptimal. The latter alternative was the case. A suboptimal concentration of any one needed amino acid is analogous to feeding a partially deficient protein. These findings remove the discrepancy which had existed between, on the one hand, experiments with labeled amino acids in which it was found that all amino acids are rapidly incorporated into proteins under all conditions, and on the other hand, those feeding experiments in which it was found carbohydrate and all necessary amino acids must be fed together within a time interval of a few hours for indispensable amino acids to have their normal effect on growth, maintenance and nitrogen balance.

i) A Theory of Protein Synthesis. A theory of protein synthesis has been proposed, which accounts for all the known facts in animals and microorganisms for the formation of adaptive enzymes and antibodies. The theory is as follows: There are, viewed in the large, three steps in the synthesis of protein. The first step is activation of the carboxyl group of free amino acids by means of ATP. The second
step is transport of the amino acid so activated to a nucleic acid template where
they are arranged in a specific sequence. On this template the amino acids are
linked by peptide bonds and then the protein molecule is peeled off the template;
this is the third step. Either in the course of its peeling off the template or
shortly afterwards the specific coiling of the protein occurs, spontaneously gov­
erned by its amino acid pattern. Or it is modified to another stable pattern by,
for example, an agent such as an inducer in the formation of an adaptive enzyme,
or an antigen in the formation of an antibody with small peptides and quasi-peptides
having, as it were, only a rudimentary amino acid pattern, certain features of
protein synthesis are telescoped or missing in their synthesis.

Publications:

H. Borsook, H. Abrams, and P. H. Lowy, "Fructose-Amino Acids
A. Abrams, P. H. Lowy, and H. Borsook, "Preparation of 1-Desoxy-1-
H. Borsook, "The Biosynthesis of Peptides and Proteins," Atomic
H. Borsook, "The Biosynthesis of Peptides and Proteins," International

2. INCORPORATION OF RADIOACTIVE LYSINE INTO PROTEIN

Investigator: Richard S. Schweet
Support: American Cancer Society
National Science Foundation

The soluble fraction prepared from guinea pig liver which incorporates
C\textsuperscript{14}-labeled lysine into the proteins has been purified thirtyfold. Other amino
acids tested, including D-lysine, are not incorporated. Calcium ion is required
for activity, but is not involved in the lysine-protein bond. The incorporation of
lysine is dependent on reduced sulfydryl groups in the enzyme, but the incorporated
lysine is not bound to protein by sulfydryl groups.

It has been found that the radioactive lysine is bound to the protein by its
\(\varepsilon\)-amino group. This has been confirmed by two independent methods. Enzymatic
and partial acid hydrolyses of the radioactive protein indicate that this bond is
a peptide bond. This is the first report of such a linkage in protein.

The significance of \(\varepsilon\)-bound lysine in protein structure has not yet been de­
termined. The bond may occur in only special proteins or may be of more general
occurrence. A working hypothesis is that \(\varepsilon\)-bound lysine serves as a branch point
linking two peptide chains, or links intra-chain cycles in some proteins.

Publication:

3. FACTORS STIMULATING PRODUCTION OF HEMOGLOBIN AND RED CELLS

Investigators: Henry Borsook, Ashton Graybiel, Geoffrey Keighley
Support: U. S. Navy, Office of Naval Research

The research on erythropoiesis in vivo in collaboration with Capt. Ashton Graybiel, M.C., U.S.N., U. S. Naval School of Aviation Medicine, Pensacola, Florida, was continued along two lines: one, to secure more potent and more abundant crude extracts; the other, to explore the relation between the erythropoietic factor that has been found in this laboratory in the blood of rabbits made anemic by phenylhydrazine and the erythropoietic factor obtained by workers at the University of California at Berkeley (Donner Laboratory and Institute of Experimental Biology) from pituitary. To get more potent extracts, blood was tested obtained at different times after the beginning of phenylhydrazine treatment. One week was found to give the most active extracts. In the preparation of the extracts most of the activity was found in the first filtrate after boiling the plasma; little activity was obtained from subsequent washings. Calves and goats are now being tested to ascertain whether these larger animals can give active extracts after phenylhydrazine treatment. Some of the chemical properties of the plasma and pituitary erythropoietic factors are so similar as to indicate a chemical, and possibly a physiological, relationship between the two factors. Pituitary erythropoietic material supplied by the Berkeley group is being tested at Caltech, whereas the Berkeley group is testing material prepared at Caltech. A collaboration has been arranged to determine the relation, if any, of the two factors.

Studies on hemoglobin synthesis in vitro and on erythropoiesis in vivo have established that erythropoiesis may be separated into two phases. One is formation of the primitive erythroblasts; the other is hemoglobin synthesis once these cells are formed and during their maturation. Erythroblast formation is under the control of a factor which appears to be protein; it is non-dialyzable. Hemoglobin synthesis is governed by substances of small molecular weight, sugar, amino acids, fructose-amino acids, and iron. The erythropoietic factor has no direct effect on hemoglobin synthesis; the factors that stimulate hemoglobin synthesis have no erythropoietic effect.

Publications:


4. IN VITRO INCORPORATION OF AMINO ACIDS INTO PROTEINS

Investigators: Robert L. Lester, Henry Borsook
Support: E. I. du Pont de Nemours & Company
Arthur McCallum Summer Scholarship
General Funds

To explore the mechanism of protein synthesis, a cell-free bacterial system was developed in which the incorporation of radioactive amino acids into protein could be demonstrated.
Lysis of Micrococcus lysodeikticus cells with the enzyme lysozyme in the presence of high sucrose concentrations yielded a particulate system, distinct from intact cells, that carries out the incorporation of amino acids into protein. All amino acids tested have been incorporated; these are leucine, lysine, glycine, histidine, tyrosine, arginine, and glutamic acid.

The system is sedimentable, has a high endogenous respiration, requires the presence of oxygen for activity, and also requires the continued presence of high sucrose or salt concentrations for activity.

Activity is abolished by ribonuclease treatment and stimulated by desoxyribonuclease treatment. Activity is inversely related to the concentration at which the particles are assayed. Incorporation is stimulated by an exogenous supply of amino acids, which indicates that a net synthesis of protein molecules occurred under these conditions.

Publication:

5. ADAPTIVE ENZYME FORMATION

Investigators: J. W. Dubnoff, Elizabeth Bartron
Support: U. S. Public Health Service
Damon Runyon Memorial Fund

Experiments on the reactivation of inactive enzymes by a B(12) system suggested that adaptive enzymes were formed in the absence of their respective substrates but were rapidly inactivated and escaped detection. The reactivation of a number of adaptive enzymes by the B(12) system gave support to this view. Evidence that the substrate stabilizes the enzyme but does not initiate its synthesis was obtained in experiments with the β-galactosidase of Escherichia coli. Under conditions of active general protein synthesis large amounts of β-galactosidase activity could be demonstrated if lactose was present during this period but not if it was absent. However, if chloromycetin was added at the end of a period of active protein synthesis to inhibit further enzyme formation and lactose was added immediately, a high fraction of the active enzyme could be demonstrated. Since enzyme protein synthesis had ceased, the presence of lactose was not necessary to initiate enzyme synthesis. The fact that no activity could be demonstrated unless lactose was added immediately showed that the enzyme was unstable in the absence of lactose.

The activation of enzymes by the B(12) system was correlated with an increase in freely reacting protein sulphydryl groups of the organism. Glutathione was required in this activation. It was observed that the sulphydryl group of this compound did not react readily in vitro but was activated by incubating with various tissues. The possibility that vitamin B(12) activates the sulphydryl group of glutathione is being investigated.
Publications:


6. THE RESPONSE OF RATS EXPOSED TO COLD

Investigators: Seymour Katsh, G. F. Katsh, P. Osher
Support: International Minerals and Chemical Corporation

Many physiological changes occur in animals exposed to cold. Some of these were noted in a previous publication (Am. J. Physiol., 178:457-461). One response important for the survival of cold-stressed animals is an increased production of ACTH by the animal's pituitary, leading to a stimulation of the adrenals and, thus, a greater production of adrenal cortical hormones.

It has been suggested by other authors that the increased production of ACTH during cold exposure is made possible by reducing the synthesis of other pituitary hormones, for example, gonadotrophic and thyrotrophic hormones.

By measuring the responses of the accessory glands of reproduction and the thyroids we have shown that in rats exposed to 4°C for as long as twenty-eight days during which time there is a great increase in pituitary and adrenal activity there is no decrease in the production of thyrotrophic and gonadotrophic hormones by the pituitaries of such animals.

Publication:


7. UNUSUALLY LOW PROTEIN-BOUND IODINE VALUE FOUND FOR THE OPOSSUM

Investigators: Seymour Katsh, E. Windsor
Support: International Minerals and Chemical Corporation

The simplest way to measure thyroid activity is to measure the amount of protein-bound iodine in the serum since protein-bound iodine (PBI) indicates the amount of circulating thyroid hormone. PBI assays were done on monkeys, horses, sheep, rabbits, rats, guinea pigs, hamsters, opossums and chickens.

The opossum was found to have an unusually low PBI value (0.4 µg. percent). The values for the other animals were in the range 2.5 - 6.5 µg. percent. The normal value for man is 4.0 µg. percent. Thus the opossum appears to offer an unusual opportunity to study problems of hypothyroid function, for here is an animal whose activity is seemingly not affected by the low rate of function of its thyroid gland. It is also interesting that the opossum is the only animal which does not fit into the curve established for the body size--body temperature relationship established for animals from the shrew to the elephant. The body temperature of the
opossum is about 95° F. Thus low body temperature and low PBI value in the opossum becomes of interest for further study in problems of thyroid-pituitary physiology.

Publication:


8. A RAPID METHOD FOR ASSAYING ANTI-ACETYLCHOLINESTERASES

Investigator: Seymour Katsh
Support: International Minerals and Chemical Corporation

Acetylcholine is a neurotransmitter which is intimately associated with the transmission of nerve impulses, particularly at the nerve-muscle junctions. Certain neurological disorders (for example, myasthenia gravis) appear to be due to insufficient amounts of acetylcholine or to an abundance of its hydrolytic enzyme, acetylcholinesterase, at the myo-neural junctions. Thus, the treatment for these disorders demands a restoration of the acetylcholine-acetylcholinesterase balance. This is usually done by providing anti-acetylcholinesterase--drugs that inhibit the action of the enzyme and therefore make available a greater amount of acetylcholine.

Heretofore, research relating to anticholinesterases has been made laborious by the method of assaying these drugs. A colorimetric method for assaying such drugs has been developed that is faster than the methods previously in use.

Publication:


9. INTERMEDIARY METABOLISM OF HISTIDINE

Investigator: Alfred G. Knudson, Jr.
Support: National Foundation for Infantile Paralysis Fellowship

When histidine is incubated with a liver homogenate an intermediate, a-formamidinoglutaric acid, accumulates. This compound is degraded by strong alkali to equimolar amounts of glutamic acid, ammonia, and formic acid.

Radioactive a-formamidinoglutaric acid has been shown to be degraded by a centrifuged supernatant of liver homogenate to radioactive glutamic acid. The turnover is low compared to that of histidine in forming the intermediate. The other products of the reaction have not been identified. The preparation progressively loses its activity on incubation at 37° C. for four hours. This loss is largely prevented by the presence of glutathione. Activity is inhibited by p-chloromercuribenzoate. Dialysis in the presence of glutathione does not destroy activity. Folic acid in a wide concentration range is completely inhibitory.
Efforts are being made to identify the number of enzymes involved, the other reaction products, and any coenzymes required.

10. CYTOCHROME C FROM NEUROSPORA MUTANTS

Investigator: H. K. Mitchell
Support: Rockefeller Foundation
General Funds

Earlier preliminary work indicated that several Neurospora mutants that accumulate cytochrome c produce different molecular species of the substance. Current investigations are directed at devising a general method for isolation of cytochrome c in quantity in order to evaluate the differences on a chemical basis. Fractionation on ion-exchange columns and by sponge electrophoresis appears the most promising.

11. CYTOCHROME-DESTROYING SYSTEM IN NEUROSPORA

Investigators: Leonard A. Herzenberg, H. K. Mitchell
Support: National Science Foundation Fellowship
General Funds

Further studies on the cytochromase system (Biology 1953, 1954) have shown that at least one step of the reaction is non-enzymatic. An ether-soluble acid has been isolated in crystalline form from poky and fast poky particles, which combines with cytochrome c in such a manner that, upon reduction of the complex with sodium hydrosulfite, the heme group of the cytochrome is destroyed. Hemin is not affected by this material as determined spectroscopically. The ether-soluble acid, as yet unnamed, has a neutralization equivalent of 290 to 300, a melting point of 58° to 59° C. and reacts with 2,4-dinitrophenyl hydrazine to give the corresponding hydrazone. In a concentration of $10^{-3}$ molar the acid inhibits succinoxidase and succinic dehydrogenase.

The mechanism of the destruction of the heme of cytochrome can, perhaps, be viewed as a specific peroxidation. A maximum of a twentyfold molar excess of hydrogen peroxide over cytochrome c at pH 8.2 will not destroy the cytochrome but in the presence of a tenfold molar excess of the ether-soluble acid, the cytochrome heme is completely destroyed. Catalase will prevent the cytochrome heme from being destroyed by the ether-soluble acid and subsequent reduction.

12. ZONE ELECTROPHORESIS OF PARTICULATE MATERIAL

Investigators: H. K. Mitchell, Carl M. Stevens
Support: Rockefeller Foundation
General Funds
Merck Senior Fellowship

In connection with the problem of the nature of transmission of cytoplasmically inherited characteristics it was considered useful to attempt to devise new methods of separation of cytoplasmic constituents, such as mitochondria and microsomes.
The method of zone electrophoresis on sponge rubber (Mitchell and Herzenberg, submitted to Science) has been applied. It has been found that under the conditions used Neurospora particulates that carry the succinoxidase system do not move in the electrical field. On the other hand most of the ribonucleic acid and a large fraction of phospholipid of a mitochondria plus microsome fraction were found to move rapidly toward the cathode. Some of this material is undoubtedly derived from partial self-digestion of the particulates since it has been found that particulate-contained ribonucleic acid (RNA) is solubilized quite rapidly even at 0° C. It has not yet been possible to determine whether any intact particulate migrates in the system. The system may be of value for examining the action of particulate-bound RNAase on RNA.

13. ATTEMPTS TO INDUCE CYTOPLASMICALLY INHERITED CHARACTERS IN NEUROSPORA

Investigators: Carl M. Stevens, H. K. Mitchell
Support: Merck Senior Fellowship
U. S. Atomic Energy Commission
General Funds

The two cytoplasmically inherited characters that are known in Neurospora (poky or mi-1 and mi-3) are of spontaneous origin and it is considered an important problem to investigate the possible mechanisms for induction of such characteristics. Two general lines of approach have been taken so far.

1. The cytoplasmically inherited characters that have been observed might be caused by transfer of virus-like agents or transforming principles. Wild-type and poky strains have been used as sources of a variety of different kinds of concentrated extracts containing soluble and/or particulate, cell-free materials for use in attempted infections or transformation of characters. Treatments of conidia or germinated conidia with such preparations have failed to produce conversion of wild type to poky or the reverse. Experiments have not yet been carried out using protoperithecia or fertilized protoperithecia as acceptors.

2. Attempts have been made to induce cytoplasmically inherited characters by treatment of conidia, germinating conidia on mycelium with heat, low-dose ultraviolet irradiation and with a variety of chemical compounds. Both shock and long-term treatments have been utilized. Among the chemical compounds tested were acriflavin, phenyl hydrazine, cyanide, azide, fluoride, iodoacetic acid, formaldehyde, phenol and a number of detergents.

14. BIOSYNTHESIS OF PYRIMIDINE NUCLEOTIDES IN NEUROSPORA

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

The riboside of orotic acid, orotidine, was isolated by ion-exchange chromatography from the mycelia of a three-and-one-half-day-old culture of a pyrimidine-requiring mutant of Neurospora. Phosphorylation of the nucleoside was then accomplished by means of a phosphate-transferring reaction employing, as phosphate
donor, phenylphosphate and an enzyme which is active in the seedlings of certain higher plants, a reaction described in recent studies by Kornberg's group. Extracts of young cultures of wild-type Neurospora have also been found to contain the phosphotransferase activity. Fluoride inhibition of the active phosphatase in these preparations has been helpful in permitting a greater accumulation of nucleotide. In future studies the ability of extracts derived from wild type and appropriate mutants of Neurospora to decarboxylate orotidylic acid and yield uridylic acid will be ascertained. It is hoped that biochemical information obtained from a study of this reaction may be correlated with the genetic relationships previously found to exist among the pyrimidine-requiring mutants.

15. PTERIDINES FROM DROSOPHILA

Investigators: Hugh S. Forrest, H. K. Mitchell
Support: U. S. Atomic Energy Commission
Research Corporation (Williams-Waterman Fund)
National Science Foundation
American Cancer Society
General Funds

In continuation of the work on the eye pigments of Drosophila, the field of interest has been broadened to include all pteridines found in Drosophila, since identification of any or all of these will bring additional information on the structure of the colored compounds and on the biosynthetic pathways involved in this synthesis.

As a result of this work, three more pteridines have been isolated from wild-type Drosophila. Two of these, iso-xanthopterin and 2-amino-4-hydroxypteridine, are known compounds and their identity has been established by comparison with synthetic specimens. The third has been proved by degradative experiments to be 2-amino-4-hydroxy-6-(1',2'-dihydroxypropyl)-pteridine—a hitherto unknown compound—and this structure has been confirmed by its synthesis. The significance of these compounds in relation to eye pigment synthesis and their general biological effects are being investigated. Work has begun on the relation between purine and pteridine metabolism in Drosophila.

Publication:


16. BIOSYNTHESIS OF RIBOFLAVIN

Investigators: Hugh S. Forrest, Walter S. McNutt, Jr., H. K. Mitchell
Support: Research Corporation (Williams-Waterman Fund)
National Science Foundation
American Cancer Society
U. S. Army, Office of the Quartermaster General
General Funds
Since the last report, two new riboflavin-requiring mutants of Neurospora have been obtained and one mutant of Aspergillus nidulans has been received through the courtesy of Dr. A. Roper of the University of Glasgow. Thus a total of five riboflavin-requiring mutants are available, but despite intensive search no accumulations of possible biosynthetic intermediates have been found in any of these. Cross-feeding experiments have also given negative results, except that the temperature-dependent mutant, although it requires riboflavin for normal growth, produces considerable amounts of this material, and thus crossfeeds all the other mutants.

However, there are indications that the mold Eremothecium ashbyii produces a compound concerned in some way with riboflavin metabolism, and this lead is now being actively pursued.

17. PHOSPHOLIPIDS CONTAINING AMINO ACIDS OTHER THAN SERINE

Investigators: John L. Westley, H. K. Mitchell
Support: National Science Foundation Fellowship
General Funds

It was shown by Hadorn and Mitchell that in Drosophila at certain stages of development there is material having lipid properties and yielding on hydrolysis several amino acids. The present study has as its aims the isolation and chemical identification of this material and the determination of its physiological functions.

The material has been located in chromatographically purified phospholipid fractions of Drosophila and of rat brain and kidney. Aspartic acid, glutamic acid and cystine in addition to serine and ethanolamine have been identified as the ninhydrin-reacting hydrolysis products of these fractions. Chemical evidence has been obtained indicating that the amino acids are bound in a labile combination involving their amino groups, the carboxyl groups remaining free. Present work is being directed toward the development of methods for isolating the material free of extraneous phospholipids.

18. BIOSYNTHESIS OF AROMATIC AMINO ACIDS

Investigator: Robert L. Metzenberg, Jr.
Support: National Science Foundation Fellowship
General Funds

A number of mutants of Neurospora crassa have been isolated and found to require tryptophan, phenylalanine, tyrosine, and p-aminobenzoic acid. These mutants have been subjected to some genetic analysis and have been found to fall into two groups:

a) Those which accumulate 3,4 dihydroxybenzoic acid; and

b) Those for which no accumulation has been detected.

All in group a that have been studied appear to be alleles. All those in group b are intersterile. Some mutants requiring only one aromatic compound have also been obtained. One which requires tyrosine has been found to accumulate anthranilic acid and phenylpyruvic acid.

Crosses between various strains which require only one amino acid have resulted in isolation of two strains, each of which requires all three of the aromatic
amino acids. One of these strains has been found to accumulate considerable quantities of a labile precursor of phenylpyruvic acid. This material appears to be similar to or identical with a compound studied by Davis and co-workers, and designated as prephenic acid. Studies of the chemical properties of this material are in progress. The other strain containing three mutant genes has been found to accumulate large quantities of 3,4 dihydroxybenzoic acid, and smaller amounts of 3-methoxy,4-hydroxybenzoic acid. It appears that these compounds are detoxification or degradation products of true intermediates in this pathway.

19. PURINE AND PYRIMIDINE BIOSYNTHESIS

Investigator: Paul R. Whitfeld
Support: Gosney Fellowship

A study of eight adenine- and five pyrimidine-requiring mutants of Neurospora crassa is being carried out. By characterizing any compounds which might be accumulated by these mutants it is hoped that a complete pattern for the biosynthesis of adenine and the pyrimidines can be worked out and, furthermore, that it may be possible to determine the exact positions in the metabolic pathway at which the various mutants are blocked. A preliminary examination of the growth medium from these mutants has revealed the presence of a number of interesting compounds and work is now being directed towards the preparation of large amounts of these compounds and to their subsequent identification.
ANIMAL PHYSIOLOGY

STAFF:
Professors: A. Van Harreveld, C. A. G. Wiersma
Research Associate: Esther Bogen Tietz
Research Fellows: Sidney Ochs
Graduate Student: Edwin J. Furshpan
Research Assistants: William F. Agnew, Joseph E. Bogen, Ruth Estey
Laboratory Technicians: Elfriede Scheurenberg

20. CENTRAL NERVOUS SYSTEM OF THE CRAYFISH

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

The investigations on sensory representation in single interneurons of the crayfish have been continued. In order to be able to determine the function of a given well-reacting interneuron in a preparation and to compare its function with that of previously found interneurons, it has been found helpful to use a punch card system. In this system each interneuron whose function and location have been established is given a card that carries its description and on which appropriate holes are punched. This makes it possible to determine readily during the experiments whether or not the interneuron being studied has previously been found, and to see if it reacts in exactly the same manner. This procedure is necessary because so many of the interneurons show a large amount of integration, and different fibers are present which react to almost identical stimulation areas. For example, there exists a fiber which responds to stimulation of hairs on the maxilliped and first four thoracic legs; another, to those on five thoracic legs. However, there are also fibers which respond to all hairs from all over the body.

At present some seventy different fibers have been identified with reasonable certainty. The great majority of these belong to the class of interneurons in which the type of integration just described is present. Relatively few of these have only limited sensory areas as, for example, do three fibers which respond to stimulation specifically of thoracic legs 1, 2, and 3. In this class both interneurons reacting to movement of hairs and of joints are about equally represented. Among those reacting to joint movement there are both phasic and tonically responding ones. The latter are normally active, and their frequency can be increased or decreased by changing the position of the joints. The majority of each of the classes so far determined reacts to a number of joints of different appendages.

From these results it may be concluded that the crayfish "brain" is badly informed about stimulation at specific points on the body surface; instead, the information received is of general relationships of the parts to each other.

21. THE TWITCH CONTRACTIONS AND THEIR POTENTIALS IN THE LOBSTER

Investigator: C. A. G. Wiersma
Support: General Funds
American Philosophical Society
Recently it has become clear that contrary to older views certain crustacean muscles can give spike potentials, which are accompanied by twitches. External leads from muscles have often resulted in monophasic action potentials, even during twitches. However, it was thought probable that conducted spikes would result in diphasic potentials. The lobster was chosen as a study object because it had previously been shown that the two claws of the same animal differ greatly in the requirements for causing a twitch in the muscles that close them. In this investigation, which was carried out at the Laboratorium v. Vergelijkende Physiologie, University of Utrecht, Holland, the action potentials and the twitches were compared and correlated. In the smaller claws of the second and third pairs of thoracic legs, a small twitch results on a single nerve impulse. This was found to be accompanied by small diphasic action potentials. On two impulses in quick succession (five milliseconds) two diphasic deflections, the second considerably larger, result with an increase in the size of the mechanical twitch. In the cutter claw, where a single impulse has at best only a very small mechanical effect, the accompanying electrical effect is almost completely monophasic. On two impulses a very strong twitch results that closes the claw with a snap, and a large, almost maximal, diphasic deflection accompanies this. In the crusher claw even two impulses do not cause a noticeable twitch and the resulting electrical activity is a summation of two monophasic deflections. It is concluded that in this crustacean twitch and spike are closely correlated.

22. PROPAGATION OF SPREADING DEPRESSION

Investigators: A. Van Harreveld, Geronimo Terres, Jr., Ernest A. Dernburg
Support: U. S. Public Health Service
General Funds

Several mechanisms for the propagation of the spreading depression have been proposed. The slow potential change which accompanies this phenomenon has been considered as the agent for propagation. It would act in a manner similar to the nerve action potential and activate adjacent parts of the cortex. The current field set up by the slow potential change should not be impeded seriously by cutting the cortex if the wound edges are well adjusted. It is conceivable that, if the slow potential change were the agent of transmission, the spreading depression would be propagated across such a cut. In about twenty preparations the cortex was cut over a considerable distance. After three weeks to three months the transmissibility of the spreading depression over the scar was investigated. Although the scar was in some instances less than 0.1 mm. wide no crossing was observed. These results do not support the concept that the slow potential change is the agent of propagation.

23. ROLE OF CORPUS CALLOSUM IN CROSSED RESPONSES

Investigators: Ronald E. Myers, Sidney Ochs
Support: F. S. Markham Fund
Frank P. Hixon Foundation Fund

The crossing of impulses from one hemisphere into the other was investigated. Cats were used in which the chiasma had been cut before. One eye was stimulated with light flashes, which caused a response in the homolateral visual area. The response of the heterolateral cortex was very small or absent. Topical application
of convulsive drugs to the homolateral cortex increased the response to light in the heterolateral cortex. The inference is that the elements which produce the homolateral cortical response are not directly connected with the callosal neurones connecting the two visual areas and that the drug facilitates the intervening synaptic connection.

24. ELECTRICAL RESISTIVITY AND CORTICAL VESSELS

Investigators:  A. Van Harreveld, Sidney Ochs
Support:        F. S. Markham Fund
                U. S. Public Health Service
                General Funds

A study was made of impedance changes in the cortex after circulatory arrest. The resistance starts to increase slowly shortly after stopping the blood flow to the brain. After a latency of one to five minutes there is a quick increase in impedance of four hundred to six hundred ohms; then the resistance continues to increase slowly. The quick impedance rise is accompanied by the development of cortical negativity.

The resistance of the cortex has been considered as the equivalent of two resistances in parallel: a high ohm resistance formed by the brain tissue itself, and a low ohm resistance formed by the blood vessels. Emptying of the blood vessels after circulatory arrest may explain the resistance rise observed. This concept is supported by a histological study of vessel filling. During the period of resistance increase the vessels are being emptied. Thus the quick impedance increase may be due to postmortal vasoconstriction which would force a considerable amount of blood out of the cortex in a short time. The thesis that brain resistance is related to vessel filling was also investigated by perfusing a brain with a salt solution after its circulation had been arrested for some time. This could be expected to fill the blood vessels and indeed does cause the expected drop in resistance.

25. CEREBRAL CORTICAL RESPONSES

Investigator:   Sidney Ochs
Support:        F. S. Markham Fund
                U. S. Public Health Service

Brief electrical stimuli applied directly to the cortex produce responses which can be recorded at some distances from the stimulated area. One part of the response—the early wave—has been correlated with a non-synaptic spread along vertically placed neurones and their tangential branches. A later wave has been considered in the literature as a non-synaptic dendritic response. However, the data obtained are more compatible with the view that synaptic activity is involved in this response. Both the early and the later waves were depressed during spreading depression.
The somatic muscles of crustaceans each receive a small number of motor axons, whereas those of vertebrates typically receive a large number. It had been shown a number of years ago in this laboratory that each of the motor axons which a crustacean muscle receives evokes a different type of contraction in that muscle. Questions then arose concerning the distribution of motor axons to individual muscle fibers and it was asked whether a single muscle fiber could respond in more than one way. Evidence was obtained that in muscles innervated by two motor axons the muscle fibers could receive branches from both axons and exhibit two types of contraction. This has been confirmed recently by using the relatively new technique for electrical recording from single muscle fibers with intracellular microelectrodes (Biology 1954, p. 42). In order to study the axon distribution to fibers of muscles receiving more than two motor axons, the main flexor muscle of the carpopodite of the thoracic legs of the California rock lobster was chosen as the experimental object. This muscle is innervated by four motor axons. The experimental procedure consisted of isolating the appropriate axons from the limb nerve by dissection with fine needles and recording the electrical response of individual muscle fibers to stimulation of each of the axons (again using intracellular microelectrodes). It was found that the fibers of the muscle were quite heterogeneous with respect to the number of motor axons that they received. This number could vary from one to four. Only very few fibers received all four axons, however (about five percent). The graded electrical responses evoked by different axons in a single muscle fiber could summate, and, if the summated potential exceeded a certain threshold, an additional membrane process, the spike potential, was called forth.

The spike potential was further studied in the rock lobster muscle, as well as in several other preparations from different crustaceans. It was found that the spike potential differed from the conducted action potentials of nerve and vertebrate muscle in several respects. The crustacean spike could be localized to one part of a muscle fiber; it often did not appear to be an all-or-nothing response; and it seemed to be spread by the motor nerve rather than by self-propagation.
27. AIR POLLUTION STUDIES

Investigators: A. J. Haagen-Smit, Margaret M. Fox
Support: Los Angeles County Air Pollution Control District
          General Funds

In previous years it was established that photochemical oxidation of hydrocarbons in the presence of oxides of nitrogen leads to the formation of ozone. These studies have been continued, and the relative ozone-forming capacity of over fifty substances has been determined. The highest ozone concentrations were obtained with butadiene, olefins, alcohols and aldehydes.

From these studies conclusions were drawn regarding the mechanism of the reaction. The knowledge of the individual contribution of a variety of organic compounds to typical smog effects allows us to make deductions on the relative contributions of sources of air pollution. This work emphasizes again the need for control of organic emissions, but in addition it demonstrates clearly that control of the emission of oxides of nitrogen is imperative in solving our smog problem.

Publication:


28. FLAVOR OF MILK

Investigator: Henry Klostergaard
Support: Carnation Company
          General Funds

Further investigation of the flavor compounds of evaporated milk has shown methylglyoxal and diacetyl to be present in substantial amounts. They originate from the sugar and by a non-fermentation reaction.

It has been shown that reversibility of denaturation is important in processed milk when the length of time for denaturation is short (fifteen seconds).

Twelve different milk fractions are being tested in a hospital at present. Results are not available yet.
Work on β-lactoglobulin is being extended to α-lactalbumin, about which nothing is known at present.

29. PLANT WOUND HORMONES

Investigators: David R. Viglierchio, A. J. Haagen-Smit
Support: Arthur McCallum Fellowship
Arthur McCallum Summer Scholarship
General Funds

Numerous plant products are capable of inducing a response in the bean mesocarp and it was of interest to investigate the nature of wound substances from sources other than Phaseolus. By the modified Wehnelt assay citrus products were found to be very active. On a dry weight basis the activity was greatest in the colored portion of the fruit peels and hence California valencia orange oil was selected as a source material. An enriched active preparation from orange oil was obtained by fractional distillation of the oil, bicarbonate extraction of the high boiling distillate, removal of impurities by crystallization from methanol and petroleum ether and a second fractional distillation. The active fraction was identified as a mixture of the higher unsaturated fatty acids. These findings are in agreement with those of Markley, who observed that linoleic and linolenic acids comprised the bulk of the free fatty acids of the peel oil of the Florida grapefruit, and those of Matlack, who identified palmitic, stearic, oleic, linoleic and linolenic acids as comprising the bulk of the free fatty acids of the peel and pulp of the California valencia orange.

Several water soluble substances (coenzyme A, cytochrome c, etc.) were found to augment the activity of the essential fatty acids. The augmentive effect of accessory factors is in agreement with the observation of English, Bonner and Haagen-Smit that sucrose and glutamic acids enhanced the activity of traumatic acid. The effect of coenzyme A on the growth response to linolenic acid was studied in detail. If the concentration of either coenzyme A or linolenic acid is held constant the growth response is a logarithmic function of the concentration of the other component. Iso-intumescence curves as a function of the concentration of linolenic acid and coenzyme A indicate that at higher intumescences other accessory factors are necessary for the full response of Phaseolus mesocarp.

Several isomers of linolenic acid were found weakly active. Geometrical isomerism is of great importance in activity, for conversion of the all cis 9,12,15-octadecatrienoic acid (linolenic acid) to the all trans form (elaidolinolenic acid) greatly decreases the wound activity. The weak activity of the oleostearic acids may be due to either the trans configuration or to conjugation.

Of the natural saturated fatty acids from acetic to stearic acids, only capric, lauric and myristic acids are active. It is striking that lauric acid is nearly as active as linolenic acid.

Publication:

EMBRYOLOGY

STAFF:
Professor: Albert Tyler
Research Associate: Lord Rothschild
Research Fellows: Gerald S. Bernstein, John R. Shaver, Melvin Spiegel
Graduate Students: James H. Berrian, John W. Brookbank
Research Assistants: Howard E. Hill, Mary Agnes Jones, Edwin A. Lilienborg
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James S. Tyler

30. UNIVALENT ANTIBODIES

Investigator: Albert Tyler
Support: U. S. Public Health Service
General Funds

Earlier work of this laboratory had shown that by means of photo-oxidation (sensitization with a dye such as eosin and exposure to visible light) it is possible to convert precipitating or agglutinating antisera into a non-agglutinating form. The altered antibodies are termed "univalent" (after Heidelberger and Pauling) and while unable to cause precipitation or agglutination of the specific antigen they still react specifically. The present work shows that such antibodies retain also enough of their characteristics as globulins so that they will react with an anti-globulin serum as in the Coombs test. Thus sheep red cells which have been allowed to react with a photo-oxidized rabbit-anti-sheep red cell serum and washed form a stable suspension which can then be agglutinated by a goat-anti-rabbit globulin serum.

It is possible to use such "univalent" antibodies for the preparation of red cell suspensions that will respond by agglutination reaction to antibodies present in allergic individuals. Thus hen's egg albumin can be conjugated (by means of tetrazotized benzidine or by being present during the process of photo-oxidation itself) to photo-oxidized rabbit anti-sheep red cell serum. When sheep red cells are treated with this reagent they subsequently can be agglutinated by the serum of an individual sensitive to egg white. If the antibodies in the serum of the allergic individual are of the non-precipitating (univalent) type they can still be detected by the further addition of an anti-human globulin serum, which then results in agglutination. This, then, provides a sensitive test for the detection of antibodies in various pathological and allergic states and for further investigation of theoretical features of antigen-antibody reactions.

Publications:


42
INHIBITION OF FERTILIZATION AND CELL DIVISION BY SPECIFIC ANTISERA

Investigators: John Brookbank, Mary Jones, Albert Tyler
Support: U. S. Public Health Service
General Funds

Earlier work in this laboratory had shown that fertilization of eggs of various marine animals can be inhibited by treatment of sperm with antisera prepared in rabbits against antifertilizin (a surface constituent of the sperm). The antibodies are effective also when made "univalent" (vide supra). Antisera against fertilizin likewise interfere with fertilization, the sperm being unable to attach to the treated eggs. The antibodies evidently block the receptor sites by which sperm and egg unite. Quantitative aspects of the action are being studied and investigations are continuing on the effect of specific antisera against various egg constituents on the early development of sea urchin eggs.

In the course of investigation of the action of antisera against constituents of eggs and sperm on the development of the fertilized eggs, it was observed that certain of the antisera blocked the early cleavage of the egg very effectively. Nuclear as well as cytoplasmic division was inhibited. In strong antisera the effect was manifest within the period of one division. This effect was obtained with antisera prepared against extracts of unfertilized eggs as well as extracts of fertilized eggs. It was not obtained with antisera prepared against whole sperm or specific extracts (such as antifertilizin) thereof. The blocking action was not removed from blocking antisera by absorption with sperm. Of particular interest was the finding that antisera prepared against purified fertilizin also possessed the mitosis-blocking action. Fertilizin constitutes the gelatinous coat and surface of the unfertilized egg and this is removed after fertilization. On the assumption that the blocking action of the antisera on the fertilized egg is due to initial reaction with its surface, this new surface appears then to be antigenically related to fertilizin. The blocking action could be largely destroyed by reacting the antisera with an excess of fertilizin.

In these antimitotic antisera the fertilized eggs exhibit a temporary marked rise in respiratory rate. Possible disturbance of salt balance was investigated by determinations of the sodium content (normally extremely low inside the cell as compared with sea water) of the antiserum-treated eggs, but no significant change was found. By centrifugation studies it was found that the tension at the surface of the egg was greatly increased by treatment with the blocking antisera. The mitosis-blocking antisera also induce cytolysis of the eggs but this does not become manifest until after several hours' treatment. Thirty minutes in strong antiserum suffices to block cleavage. The antimitotic effect can be reversed only if the eggs are washed after less than fifteen minutes' treatment. Complement is evidently not necessary for the blocking action since heated (56°C for one hour) antisera are effective.

Publication:

Further characterization has been made of the physico-chemical properties of the fertilizins of sea urchins by determinations of sedimentation rates in the analytical ultracentrifuge, intrinsic viscosity, diffusion rates and electrophoretic mobilities. From the sedimentation \(6.73 \times 10^{-13}\) and diffusion \(2.1 \times 10^{-7}\) constants a value of 280,000 is obtained for the molecular weight of the fertilizin of Arbacia punctulata. The corresponding axial ratio of the molecule as a prolate ellipsoid, assuming no water of hydration, is 28:1 and assuming as an upper limit 0.8 grams of water bound per gram of fertilizin it is 15:1. The diffusion measurements represent the least accurate of the measurements involved in the calculation of these values since the gelatinous nature of moderately concentrated solutions of fertilizin necessitates the use of dilute solutions with corresponding decrease in size of the refractive index boundaries. It is estimated that the present values for molecular weight are accurate to within twenty-five percent.

The large axial ratio is consistent with the high intrinsic viscosity and known gel-forming properties of fertilizin, which at relatively low concentration (ca. 0.5 percent) forms the rather firm gelatinous coat of the egg. The electrophoretic data show fertilizin to be a highly acidic substance, the isoelectric point being below pH 2 but the exact value not readily ascertainable because of degradation of the molecule at lower pH. At pH 8 the valence (number of negatively charged groups per molecule) is approximately fifteen.

The data further show that an individual spermatozoon can bind a maximum of 100,000 fertilizin molecules. There are then evidently a minimum of 100,000 receptor sites (antifertilizin) on the head of the sperm, the surface area of which is approximately 9 \(\mu^2\). This, then, provides some basis for understanding the rapidity with which the sperm is able to make effective union with the egg, each being densely covered with their specific interacting substances.

Investigation continues on the sugar and amino acid constituents of the fertilizins of several species of animals. The results show no particular constituent to be responsible for specificity of action.

Earlier work had shown that fertilizin and antifertilizin are the primary receptors, on egg and sperm respectively, by which the union of the gametes is effected. In many species of animals fertilization can be inhibited by treatment of the sperm with a solution of fertilizin. The sperm respond to such treatment by a temporary agglutination, after which they appear intact and remain highly motile but evidently have their receptor sites (antifertilizin) blocked by fragments of the fertilizin molecules. In certain species of sea urchins, such as Arbacia punctulata, the fertilization-inhibiting effect of solutions of fertilizin is ordinarily not very marked. It has been found that if the fertilizin-treated sperm are tested on eggs that have been subjected to a brief treatment with trypsin, there is a very great reduction

*Department of Zoology, The Florida State University

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in fertilizing capacity. The effect of the trypsin is to remove a large portion, but not all, of the fertilizin that constitutes the gelatinous coat and surface of the egg. Such eggs, having fewer receptor sites, require insemination with larger amounts of sperm than untreated eggs require for successful fertilization. Treatment of Arbacia sperm with ordinarily prepared solutions of fertilizin evidently does not neutralize all of the antifertilizin. Such sperm can fertilize untreated eggs. However, on trypsin-treated eggs they are practically ineffective.

Publications:


33. ACROSOMAL FILAMENTS IN SPERMATOZOA

Investigators: Albert Tyler, Lord Rothschild
Support: U. S. Public Health Service
General Funds

By means of the Critical Point Method, invented by T. F. Anderson, for preparing material for electron microscopy with a minimum of disruption of fine structures, it has been possible to demonstrate the presence of a long filament extending forward from the acrosome in the sperm of several species of animals. These include the sea urchins, Strongylocentrotus purpuratus and Echinocardium cordatum, the chitons Lepidochiton cinereus and Mopalia muscosa, and the mussel Mytilus edulis.

Publication:


34. MONOGRAPH ON FERTILIZATION

Investigator: Lord Rothschild
Support: General Funds

Facilities at Caltech, including the Morgan-Tyler reprint collection, permitted the writing of a major portion of a book on fertilization.

Publication:

35. AUTO-ANTIBODIES IN SPONGES AND IN SEA URCHIN EGGS

Investigators: Melvin Spiegel, Albert Tyler
Support: U. S. Public Health Service Fellowship
        U. S. Public Health Service
        General Funds

It has been demonstrated in this laboratory that an antifertilizin is located in the interior of the sea urchin egg as well as on the surface of sea urchin sperm. This has formed part of the basis of an auto-antibody concept of cell structure. The present investigation is concerned with the extraction of an auto-antibody from the marine sponge Microciona prolifera.

Extracts were made of dissociated sponge cells in sea water by boiling for fifteen minutes, centrifuging to remove cell debris and coagulated protein, and adjusting the pH to 7.8. When tested on dissociated sponge cells, agglutination was observed and large macroscopic aggregates were formed. The active principle is non-dialyzable, heat stable, and precipitated out of solution at pH 3.0 or by fifty percent saturated ammonium sulfate. It has no effect on a second marine sponge Cliona celata.

A hetero-agglutinin for Cliona cells can also be extracted from Microciona cells. It is non-dialyzable, precipitated out of solution at pH 2.8 and is inactivated by boiling for fifteen minutes.

Further work on the isolation and characterization of the egg antifertilizin of the sea urchin Strongylocentrotus purpuratus indicates that the active principle is very likely a glycoprotein. The egg antifertilizin of the sand dollar Echinarachnius parma is heat stable, precipitated out of solution at pH's below 4 and by fifty percent saturated ammonium sulfate, and is non-dialyzable.

Publications:


36. FERTILIZATION WITH FROZEN-THAWED SEA URCHIN SPERM

Investigators: Albert Tyler, Melvin Spiegel
Support: U. S. Public Health Service
        U. S. Public Health Service Fellowship
        General Funds

Within the last few years successful fertilizations in mammals have been obtained with sperm frozen in glycerine and thawed rapidly. The present investigation is concerned with fertilization in the sea urchin Strongylocentrotus purpuratus, using frozen-thawed sperm.

Sperm in fifteen percent glycerine, in 5 x 10^-4 M Versene in sea water, are immobilized within ten minutes after addition of the glycerine but one hundred percent motility can be restored by dialysis against sea water. Such a sperm-
glycerine suspension can be frozen slowly in dry ice, thawed rapidly at 26° C., and ninety to one hundred percent motility restored after dialysis against sea water. When tested against normal eggs no fertilization is noted. It is thought that glycerine extracts the antifertilizin from sperm since glycerine-treated sperm, after removal of the glycerine by dialysis, show either a very weak or no agglutination reaction when tested against a fertilizin solution. Varying the concentration of glycerine does not increase fertilization.

Sperm in fourteen percent ethylene glycol, in 5 \times 10^{-4} \text{M Versene} in sea water, remain motile and give normal fertilization when tested against normal eggs. Such sperm, after being frozen slowly in dry ice and thawed rapidly at 26° C., show fifty percent motility and will give ninety percent fertilization with normal development when tested against normal eggs.

37. REMOVAL OF THE FERTILIZATION MEMBRANE OF SEA URCHIN EGGS

Investigators: Melvin Spiegel, Albert Tyler
Support: U. S. Public Health Service Fellowship
U. S. Public Health Service
General Funds

The proteolytic enzymes trypsin and chymotrypsin have been used to suppress fertilization membrane formation in sea urchin eggs by treatment of the unfertilized egg. Urea has been used to remove the fertilization membrane immediately after its formation. However all these substances are variable in their action and may either appreciably lower the fertilization rate or interfere with normal development. We have developed a method in this laboratory using the plant enzyme papain which will give one hundred percent fertilization and normal development in the sea urchin Strongylocentrotus purpuratus.

The technique is to prepare a sperm suspension in 0.02 percent papain in sea water, containing cysteine, HCl and Versene, pH 8.1. The unfertilized eggs are added to the solution. Usually the fertilization membrane at first elevates and then shrinks back to the surface within five to six minutes. There is no further evidence of its presence during later development. The eggs are washed after fifteen minutes' treatment and development is normal.

38. RESPIRATION OF MARINE SPONGES

Investigators: Melvin Spiegel, E. E. Gordon*
Support: U. S. Public Health Service Fellowship
General Funds

It was shown by Krebs and Eggleston (1938), using a pigeon liver suspension, that insulin could effect an increase in oxygen uptake. This action of insulin has been demonstrated in an invertebrate—the sponge Microciona prolifera.

Oxygen consumption was measured by the conventional Warburg technique.

*Harvard University
In the presence of insulin, oxygen uptake is increased by an average of sixteen percent. Neither phenol, in concentrations present in commercial insulin, nor potassium pyruvate, glucose, or sodium citrate had an effect on oxygen utilization. No significant utilization of any of the above-mentioned substrates by either control or insulin-incubated preparations was detected. Determination of keto acids in the pyruvate-incubated preparations revealed a production of these acids.

Preliminary work indicates that this effect of insulin is confined to the apical region of the sponge and is the first evidence that a chemical differentiation exists along the apical-basal axis of a sponge.

Publication:

39. STUDIES ON MITOCHONDRIA IN SEA URCHIN DEVELOPMENT

Investigator: John R. Shaver
Support: U. S. Public Health Service Fellowship

Mitochondria are structural and functional elements of the cytoplasm whose importance in many aspects of cellular life has recently been emphasized. Relatively little attention has been paid, however, to the role of these bodies in normal developmental processes. A cytological study of developmental stages of two species of sea urchins, with the use of methods recommended for the identification of mitochondria, has revealed no apparent localizations of these structures in the embryo until differentiation is under way, at which time there is a rather rapid concentration of mitochondria in gut structures and ciliated cells, suggesting a possible correlation between physiological activity and numbers of these particles. This result is somewhat different from one recently reported by other investigators who, using different species of sea urchins, concluded that differentiation processes were the result of the metabolic activities of mitochondria arranged in a gradient along the principal axis of the undifferentiated embryo. A quantitative method for estimating the number of mitochondria in homogenates has been adapted to sea urchin developmental stages, in an attempt to follow any significant changes in the total quantity of these structures in embryos. Preliminary results indicate that there is no significant change in the numbers of mitochondria per embryo during the first day of development (at 17°C); shortly thereafter, when marked differentiation processes begin, there is a sharp and rapid increase in the numbers of particles identifiable as mitochondria, followed in a few hours by a decrease. Subsequently, there is a slower rise in the number of mitochondria per embryo to a level, somewhat less than the above mentioned sharp peak, which is maintained through the fifth day of development. Further studies will include more accurate determinations of changes in different size classes of mitochondria during development and the relation of change in mitochondrial number to significant biochemical changes.

40. THE EFFECT OF METAL-CHELATING AGENTS ON THE METABOLISM OF SEA URCHIN SPERM

Investigator: Gerald S. Bernstein
Support: National Science Foundation Fellowship General Funds
Sea urchin sperm exhibit a phenomenon known as the "dilution effect." This is characterized by a shortening of the life span of the sperm when a suspension is diluted, and by a large temporary rise in the respiratory rate, followed by a rapid decrease as the spermatosoa die. Work of this laboratory has shown that the "dilution effect" is probably due largely to the action of trace amounts of heavy metals present in sea water; thus the effects of dilution can be practically abolished by treating the sperm with metal-chelating agents such as glycine and Versene (ethylenediamine-tetra-acetic acid). These reagents extend the motile life of diluted sperm; they also prevent the temporary rise in the respiratory rate and maintain respiration at a low, relatively steady level. In addition, glycine supports the anaerobic motility of sea urchin sperm, while under ordinary conditions the sperm cannot remain motile without oxygen.

Further studies have been made of the effects of Versene on sea urchin sperm. Versene, like glycine, supports sperm motility under anaerobic conditions. Versene also prolongs the life of the sperm in the presence of metabolic inhibitors such as cyanide, arsenite, and high concentrations of 2,4-dinitrophenol. Since lack of oxygen and metabolic inhibitors restrict the amount of energy available to the cell, it is suggested that Versene acts by regulating the rate at which the available energy is consumed.

Particular attention was given to the effect of dinitrophenol (DNP) on sea urchin sperm. Low concentrations of DNP have a similar effect on many metabolic systems, increasing respiration while inhibiting energy requiring processes. This occurs because DNP prevents the formation of energy-rich phosphorus compounds, such as adenosine triphosphate, that usually accompany respiration. In addition, DNP sometimes accelerates the breakdown of these high-energy compounds.

Low concentrations of DNP have no appreciable effect on the respiration or motility of sperm suspended in sea water. However, DNP does stimulate the respiration of sperm suspended in Versene. Pretreatment with Versene, which inhibits the "dilution effect" rise in respiratory rate, allows respiration to be stimulated by the DNP.

The effect of DNP on Versene-treated sperm resembles the "dilution effect" in many ways: both cause a temporary increase in the respiratory rate and shorten the motile life of the sperm; both cause a partial dissociation of respiration and motility, because sperm activity is not increased when respiration is stimulated. These similarities raise the possibility that DNP and dilution exert their effects by similar mechanisms, i.e., by inhibiting the formation of high-energy phosphate compounds or by causing these compounds to be used at an abnormally rapid rate.

41. FROG FERTILIZIN

Investigator:      Gerald S. Bernstein
Support:           National Science Foundation Fellowship
                   General Funds

A great deal of work has been done in this laboratory on fertilizin and antifertilizin, the specific interacting substances found in eggs and sperm. So far this work has been largely restricted to marine invertebrate material. A previous investi-
gation, however, has indicated that a fertilizin-like substance is present in the jelly coat of frog eggs. Thus, water that has been in contact with frog eggs will agglutinate frog sperm.

The investigation of "frog fertilizin" continues with a study of its chemical composition and a further study of its effects on frog sperm. The gametes of the frog Rana clamitans are being used in this work.
GENETICS: CORN

STAFF:
Professor Ernest G. Anderson
Research Associate: Albert E. Longley
Research Fellows: Edward H. Coe, Jr., Herbert H. Kramer, Norton H. Nickerson
Laboratory Technicians: Lois Marian McConnell, Elin Thorlund, John S. Willis
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Superintendent, Experimental Farm: John F. Dale

42. TRANSLOCATIONS

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

In connection with studies on the behavior of chromosome abnormalities, the mapping of the chromosomes, and the chromosomal effects of radiation, a large number of translocations and inversions have been accumulated in maize. These are of great potential value both in scientific studies and in the practical improvement of commercial maize.

To prepare these translocations and inversions for future use they are processed as follows: First they are outcrossed to standard lines several times to eliminate other effects of radiation, since the transmission of the chromosomal aberration is followed by the partial sterility of pollen of the heterozygous translocation or inversion. Semi-sterile plants are then self-pollinated, giving rise to F2 progenies from which the homozygous translocation can be isolated. These F2 progenies are composed of semi-steriles and two types of plants with normal pollen, those with only normal chromosomes, and those homozygous for the translocation. These may be distinguished by outcrossing to standard lines. The outcrosses of the homozygous translocations yield plants with semi-sterile pollen. In practice, the normal pollen F2 plants are self-pollinated and also outcrossed to standard stocks. The outcrosses are grown and the pollen examined. If semi-sterile, a duplicate is planted for cytological study of the chromosomes. The self-pollinated seed of the homozygous translocation is grown and crosses made with semi-sterile plants of the same translocation outcrossed several times to standard lines. This builds up the vigor to a satisfactory level. Seed is then ready for placing in cold storage, though in most cases another outcross test is made to insure against errors.

During the past year a special effort has been made to advance the processing of translocations as rapidly as possible.
43. CHROMOSOME MAPPING IN MAIZE

Support: U. S. Atomic Energy Commission, General Funds
U. S. Department of Agriculture, Gosney Fellowship

The map locations of translocations whose cytological positions have been determined give essential data for the mapping of gene locations on the chromosome. Data bearing on the mapping of chromosome 10 were published in 1954. Similar data for chromosomes 4 and 6 are due for publication in the July 1955 issue of Genetics. Tests are under way for mapping the long arm of chromosome 9, with blue fluorescence used as a marker for the distal portion of the arm.

Publications:


44. INDUCED GENE MUTATIONS IN MAIZE

Investigators: E. G. Anderson, James E. Wright
Support: U. S. Atomic Energy Commission, General Funds

The more valuable or interesting mutations obtained from irradiation have been kept in stock and the genes are being located to chromosome by the use of gene-chromosomal techniques based upon a series of translocations. Chief reliance has been placed upon a series of chromosome 9 translocations closely linked to waxy. The work is being done in cooperation with Dr. James E. Wright at Pennsylvania State College. For most of the new genes, crosses to the tester stocks are made here and the F1's are grown at Penn State. Seedling tests are also conducted there.

45. A WILT-SENSITIVE MUTANT IN MAIZE

Investigators: F. D. Petttem, N. H. Nickerson
Support: U. S. Atomic Energy Commission, General Funds

Studies have begun on a wilting sensitivity due to a dominant mutant induced by radiation. Plants carrying the dominant gene wilt badly during hot or dry weather, even though given an abundance of water. There are differences in leaf morphology such as a looser structure of the mesophyll and poor development of the bulliform cells. But these differences may be secondary effects of water shortage in the developing leaves. It has not yet been determined if the effect is due to a deficiency in water uptake, water transport, or excessive transpiration. Linkage tests indicate that the mutant gene responsible for the trait is located in chromosome 5.

*Pennsylvania State College
46. LYCOPENE IN MAIZE

Investigators: B. O. Phinney *, D. S. Robertson
Support: U. S. Atomic Energy Commission

One of the viviparous mutants ps is characterized by a pink color in the scutellum and endosperm, premature germination and pinkish albino seedlings. Studies on the pigments of the scutellum have shown that the normal carotinoid pigments have been largely replaced by lycopene, the characteristic pigment of red tomatoes. Lycopene has not been found in any normal strains of maize.

47. A DIMINUTIVE B-TYPE CHROMOSOME IN MAIZE FROM A B-9a TRANSLOCATION

Investigator: Albert E. Longley
Support: U. S. Department of Agriculture

The heterochromatic areas of a B chromosome and of the abnormal form of chromosome 10, when they are not paired with the homologous areas of a second chromosome, frequently at mid-prophase are folded back on themselves to simulate a paired condition. Similar pairing is found between the distal and proximal sections of the long arm of the B chromosome in B-A translocations. The dicentric pairing of the two sections of a B is such that crossing over occurs which may give a diminutive B-type chromosome or a dycentric chromosome and an acentric fragment. It is also known that the heterochromatic area of a B is associated with the non-disjunction of the B A chromosomes in B-A translocation, during the division of the generative nucleus of the pollen grain, and that the heterochromatic area of ab. 10 is associated with the appearances, during meiosis, of secondary centromeres at the knobs of sister chromosomes. These observations suggest that these two heterochromatic areas are modified by crosses and repeats.

48. ANTHOCYANIN SYNTHESIS IN MAIZE

Investigator: E. H. Coe, Jr.
Support: U. S. Atomic Energy Commission

Anthocyanin pigment production in the aleurone tissue of the corn kernel is under the control of a number of genetic factors. The Pr-pr factor pair, for example, determines the level of hydroxylation of anthocyanin. The In-in factor pair controls the concentration of pigment. A2-a2 and several others determine presence or absence. Colorless a2 a2 kernels are unique in that they contain a leuco-anthocyanin, which can be converted to the colored pigment by mild treatment in vitro (heating a dilute-acid extract). It has now been determined that the anthocyanidin so produced is the same as that normally present in colored kernels and that the Pr-pr factor pair determines the level of hydroxylation of leuco-anthocyanin, just as it determines the level of hydroxylation of anthocyanin itself. Also, the In-in factor pair determines the concentration of leuco-pigment in

*University of California at Los Angeles
a2 a2 kernels, apparently in parallel with the effects on colored kernels.

Sufficiently detailed information of this sort may lead to a clarification of the pathway of anthocyanin synthesis and provide a basis upon which to carry out studies of gene action in the control of this pathway.

49. MORPHOLOGICAL STUDY OF GENETIC ABNORMALITIES IN MAIZE

Investigators: Norton H. Nickerson, Ernest E. Dale
Support: U. S. Atomic Energy Commission

Rapid advances in maize genetics have largely ignored questions concerning the exact morphology of plant parts affected; especially has this action been true of genes involving ear and tassel abnormalities. Structural details of these latter mutations are nearly completely lacking in the literature. The first task has been to assemble as complete descriptions as possible of sixteen separate mutants. The next step, now in progress, is to combine these sixteen genetic forms with each other and then to study the morphological effects manifested in these combinations. The problem provides a unique and thorough method for study of gene interaction, since many of these abnormalities, in addition to being present in otherwise normal plants, may also be obtained in stocks involving translocated chromosome segments.

Publication:

**GENETICS: DROSOPHILA**

**STAFF:**
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Curator of Stocks: Rhoda Mislove  
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Laboratory Technician: Lorrie Sechler  
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**50. TWO NEW MUTANT TYPES IN DROSOPHILA**

**Investigator:** A. H. Sturtevant  
**Support:** General Funds  
U. S. Atomic Energy Commission

Mutant types have been accumulating in Drosophila melanogaster for the past forty-five years, and a very large series of them is maintained in our stock room. It has now become rare to find really new types that condition sharply distinct and easily recognized characters--but every new one of this kind is a welcome addition to the material available for attacking special problems.

Two striking new types have appeared during the year, both of them being of possible interest in connection with the currently revived studies on the development of the eye pigments.

One of these new types is a very distinct new brownish eye color, dependent on a sex-linked gene. This was found independently, from two very different sources, by Dr. J. Weigle in this laboratory and by Dr. M. M. Green at Davis.

The other new type is an autosomal dominant gene, called "prune-killer," that has no known effect except that it kills all flies carrying the sex-linked recessive prune (eye color). The lethal action comes about the middle of larval life before eye pigment has become evident. The locus of the new gene is also interesting, since it is the rightmost one now available for study in the third chromosome. There are also uses for "prune-killer" in the design of efficient experiments.

**51. "UNSTABLE-WHITE" GENE CHANGE IN DROSOPHILA**

**Investigators:** Edward B. Lewis, Helene Fatt  
**Support:** U. S. Atomic Energy Commission  
General Funds

A rare class of mutations is known in which the gene acts as if it were in a permanently unstable state. One of a few such cases in Drosophila has been singled out for study. This example is known as "unstable-white" and its effect is on the eye pigmentation. A male carrying this gene change will show a variegated eye color which results from a mixture of varyingly pigmented facets ranging from yellow to brown. The cause of this unsteady action of the gene is unknown and it
becomes of importance to determine whether it is an intrinsic property of the gene itself, an unstable state of the gene, or whether some extraneous material induces the unstable change in the gene's action. Although a decision between these possibilities has not yet been reached, it has been possible to show that unstable-white is not associated with any gross change in the chromosomes and that it acts as though it occupied a fixed position in the chromosome. Its position has been shown to be very slightly to the right of the gene apricot, which determines a stable orange eye color. This places unstable-white at or very close to the locus of the gene white, whose various alleles are also concerned with determining eye pigmentation. Finally, attempts to see whether unstable-white can be separated by crossing over from the white mutant gene have produced one exceptional type among approximately twelve thousand trials. The results suggest that unstable-white lies extremely close to the right of the white gene. The basis of the instability somehow resides in a cluster of at least three units which act in some ways like a single gene but which can be operationally resolved and treated separately.

52. THE TYROSINASE SYSTEM OF DROSOPHILA MELANOGASTER

Investigators: N. H. Horowitz, M. Fling
Support: U. S. Atomic Energy Commission
John Simon Guggenheim Memorial Foundation Fellowship
Fulbright Fellowship, Nutrition Foundation

The question of whether heritable changes in the melanic pigmentation of animals is correlated with changes in the tyrosinase-dopa oxidase activity in the skin is the oldest problem of biochemical genetics. Attempts to demonstrate a correlation have failed in past instances, but since no other enzyme of animals is known to produce melanin, the failures have been unexplained. Preliminary studies reported last year showed that the tyrosinase system of Drosophila is chemically more complex than had been previously realized. It was found that tyrosinase activity is generated in cell-free extracts of wild-type flies by a reaction thought to be represented by the following equation: Protyrosinase + Activator = Tyrosinase + 2 Activator. Since no process of this type has been described before, this may represent a new general type of biochemical reaction. On the genetical side, further study of the reaction may make possible a more successful attack on the problem of melanic pigmentation. During the past year, work has been continued in Paris and in Pasadena on the genetics and chemistry of the reaction.

Tests of Mutants: A dozen or more genes are known in Drosophila which have major effects on melanic pigmentation. Tests carried out on the mutants yellow, sable, black, ebony, straw, speck and platinum (a new almost colorless semi-lethal mutant obtained from Dr. Hildreth at Berkeley) have shown that all of the mutants produce tyrosinase, even those in which pigmentation is much reduced. This result is consistent with the view held by insect physiologists that tyrosinase plays an essential role in the hardening of the cuticle.

It was reported last year that a thermolabile factor that could be extracted from wild type by saline causes a shift in the pH optimum of tyrosinase activation. The pH optima have now been determined for the mutants. Preliminary results on two of them, yellow and sable, indicated that this factor was lacking in yellow and was ineffective in sable. Other alleles of yellow were then tested, as well as the original yellow in new genetic backgrounds. The pH curves of the saline extracts were normal, indicating that the yellow mutation is not the sufficient cause of the biochemical
abnormality. Since just one stock of sable was available, a new stock was made up which contained less than 2.5 units (but including the sable locus) of the original chromosome carrying sable. Saline extracts of this new "recovered" sable now resemble wild-type extracts, indicating that the sable mutation is also not the cause of the biochemical effect originally associated with it.

Purification of Pro tyrosinase: The analysis of the tyrosinase system has thus far been carried out with crude preparations. It is recognized that purification of the system may reveal new characteristics. Preliminary trials have indicated that purification of the activator, the pH substance and tyrosinase will present no unusual difficulties. Pro tyrosinase is a special problem, however, because of its great instability. A procedure has now been worked out which permits an approximate fifteenfold purification on the basis of total protein. This may be sufficient for present purposes.

53. NUTRITIONAL REQUIREMENTS OF DROSOPHILA MELANOGASTER

Investigator: Michael Begg
Support: Rockefeller Foundation Fellowship
General Funds

The desirability of having available a chemically defined medium for the larval stages of Drosophila in sterile culture needs no emphasis. Media so far devised suffer in greater or lesser degree from the disadvantage of protracting the larval period as compared with media containing whole yeast. Such media are virtually useless for the study of environment-sensitive mutants.

A fifty percent ethanol-soluble fraction can be extracted from autolysed brewers' yeast which, when added to defined media and seeded with larvae rendered aseptic by what is now an efficient technique, leads to normal development. A similar fraction can be obtained from pig pancreas. The activity of such a fraction does not appear to be extractable with common organic solvents (ethanol, n-butanol, pyridine, ether, etc.). It is, however, dialyzable, and can be recovered from the dialyzate; the non-dialyzable portion is quite inactive. Treatment with an anion exchange resin indicates that there may be two active components. One is not adsorbed, the other can be eluted with ammonia. The substance(s) is heat stable. An attempt is being made to concentrate the factor(s) by ion-exchange chromatography.

54. OSMOTIC PRESSURE OF HAEMOLYMPH OF LARVAE OF DROSOPHILA MELANOGASTER

Investigator: Michael Begg
Support: Rockefeller Foundation Fellowship
General Funds

A thermoelectric method has been used to determine the osmotic pressure of larval Drosophila haemolymph as a preliminary to culture in vitro of imaginal discs. The method consists of matching the evaporation rate of a drop of haemolymph against known NaCl concentrations. The difference in rates is measured by thermojunctions mounted in opposition in two polyethylene rings, one containing haemolymph, the other standard NaCl solutions. Experiments carried out in a draught-free,
constant humidity chamber indicate that the osmotic pressure of haemolymph of full grown larvae reared on ordinary media at 25°C is approximately equal to that of 1.05 percent sodium chloride; and that there is little variation of this figure with larval age.

55. THE ISOLATION OF A TUMOR AGENT IN DROSOPHILA MELANOGASTER

Investigators: Lawrence Burton, Frank Friedman
Support: U. S. Public Health Service Fellowships

The activity of an inherited larval tumor agent in Drosophila development and the effects of vitamins and vitamin analogs have been studied.

Larval extracts of the donor (tu-e) strain, purified by centrifugation (high and low speeds), pH alteration, and temperature treatment still induce melanotic tumor formation in over sixty percent of injected Wild 51-52 hosts (a tumor-free strain). Dialysis and subsequent semi-drying indicate that the tumor agent cannot be dialyzed. Nevertheless the non-dialyzed material still is capable of inducing high tumor frequencies when injected into tumor-free host animals. Electrophoresis of this material indicates that the agent may be further separated from other extract components and still maintain high tumor-agent activity.

The activity of the agent can be destroyed by heating and pH alteration. The tumor inductive capacity of the extract may be retained over an extended period by quick-freezing.

Further studies are now being conducted toward the isolation and identification of this tumor agent.

Publications:

56. MONOGRAPH ON METHODS OF BIOCHEMICAL GENETICS

Investigator: Sterling Emerson
Support: General Funds
Rockefeller Foundation

A review of the methods of biochemical genetics, especially referring to Neurospora, Aspergillus, yeast, and bacteria, will be published in the summer of 1955. There is in preparation a review of the genetic mechanisms of microorganisms, covering the relation of life histories of different organisms to the type and extent of genetic information obtainable from them, to the control of genetic experimentation, etc.

Publication:

(Will not be separately available.)

57. CHROMOSOME DISJUNCTION IN THE SILK WORM

Investigator: Sterling Emerson
Support: General Funds

A manuscript prepared in 1933 on chromosome disjunction in the silk worm Bombyx mori containing a treatment of the frequencies of first and second division segregations in the sex chromosome and an autosome of Bombyx with no assumption that the two nuclei in binucleate eggs need be sisters, has been published.

Publication:

S. Emerson, Cytologia, 19, 144-151, 1954.
58. INDUCTION OF GERMINATION OF ASCOSPORES OF NEUROSPORA CRASSA

Investigator: Mary R. Emerson
Support: Damon Runyon Memorial Fund
Rockefeller Foundation

Ascospores germinate following "activation" either by heat or by chemical compounds containing unsaturated rings. Those having furan rings are the most effective, i.e., are active at very low concentrations. Sensitivity to activation by furfural (0.1 parts per million) reaches a maximum within three weeks following shedding of spores, and then declines with increasing age. The loss of sensitivity to activation by heat occurs more slowly. Spores returned to dormancy after activation can be reactivated by furfural as well as heat. Since heat-activation is inhibited by azide whereas furfural-activation is not, it is thought that furfural is effective at a later step in the activation process than that initially effected by heat.

Publication:


59. OSMOTIC MUTANT IN NEUROSPORA

Investigator: Mary R. Emerson
Support: Damon Runyon Memorial Fund
Rockefeller Foundation

Studies have been continued with a mutant of Neurospora which is similar to wild type in growth in media with osmotic concentrations less than about six atmospheres. In media of higher concentration, growth of the mutant is greatly retarded and contorted, whereas growth of the wild type is unaltered up to a number of times the concentration critical to the mutant. Under a number of conditions of growth, the osmotic concentration of the expressed juice of both mutant and wild type is approximately six atmospheres (or 0.2 M).

60. GENETIC DETERMINATION OF ENZYME STABILITY

Investigators: M. Fling, N. H. Horowitz
Support: Nutrition Foundation
U. S. Atomic Energy Commission
John Simon Guggenheim Memorial Foundation Fellowship
Fulbright Fellowship

Previous work has established that the thermostability of the enzyme tyrosinase in Neurospora is determined by a pair of alleles designated T^s (stable) and T^l (labile). In connection with the template theory of gene action, it was of interest to study the thermostability of the tyrosinase produced in a heterocaryon (a strain in which genetically different nuclei are contained in a common cytoplasm). To form such a heterocaryon, it was found necessary to backcross a T^s biochemical (adenine-requiring) mutant to a T^l biochemical (histidine-requiring) mutant for a number of generations to obtain a fairly isogenic background. The adenine-requiring T^s and histidine-requiring T^l strains that were thus obtained readily formed hetero-
caryons. A study of the thermostability of the tyrosinase in extracts prepared from some of these heterocaryons indicates that both forms of the enzyme are produced. Interaction between the two forms, if it occurs at all, is very slight. In some of the heterocaryons there is no evidence of any interaction. These results confirm the previous conclusion that the two tyrosinases are structurally different. The results are consistent with a template theory of gene action although they do not prove it.

61. IRREGULAR SEGREGATIONS IN NEUROSPORA

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

Irregular segregations have been observed occasionally in several organisms in which tetrad analysis (analysis of the four products of a single meiotic division) can be done. For example, from a cross between mutant and normal, or wild type, some tetrads have been found consisting of three wilds to one mutant, or one wild to three mutants, instead of the expected Mendelian ratio of two wilds to two mutants. Recently a few examples of this behavior have been found in Neurospora as a result of an examination of a possible case of pseudoallelism. (Pseudoalleles are genes with similar functions, located close together in the chromosome, but separable by crossing over.) Two mutants, both requiring pyridoxine but differing in that one has a less rigorous requirement than the other, had been found by Dr. T. H. Pittenger and the author to give occasional wild-type offspring when crossed. By introducing two unrelated mutants as markers, located on either side of the pyridoxine locus, it was found that the wild progeny could not all have arisen as a result of single crossovers occurring between two separable pyridoxine mutants and establishing their normal alleles in the same chromosome strand. Such an event would also result in recombination of the marker mutants, whereas it was observed that only about half of the wild progeny or "recombinants" with respect to the pyridoxine mutants showed recombination of the markers. Multiple crossing over between the markers remained a possibility, and, although it would not be expected to occur with so high a frequency in a short region, tetrad analysis (examination of segregants from complete asci) was carried out in order to test this possibility. If the wild "recombinants" resulted from crossing over, a tetrad containing a wild segregant would also contain the complementary crossover type, the double pyridoxine mutant, which could be identified by back-crossing to the parent mutants. Since the double mutant was not present in any of the four tetrads found containing wild segregants, it must be concluded that the latter did not arise as a result of ordinary crossing over. Two of these tetrads consisted of one wild, one of the less extreme pyridoxine mutants and two of the more extreme type. In the third this situation was reversed in that it was the less extreme type which was represented twice, and the fourth tetrad contained two wild segregants and one of each of the pyridoxine mutants. These tetrads represent, therefore, three-to-one segregations with respect to one, or the other, or both pyridoxine mutants, although the nearby marker genes segregated regularly. Since neither pyridoxine mutant, when crossed to itself, has given wild offspring, this behavior cannot easily be accounted for in terms of reverse mutation. Rather, it seems necessary to assume that the wild progeny arise as a result of some interaction taking place between the two pyridoxine mutant genes when the chromosomes are paired at meiosis. The fact that so many of the wild "recombinants" among random spores were also recombinants with respect to the markers suggests that the event producing them is in some way related to crossing over. (Ref: Proc. Nat. Acad. Sci., 41, 215-220, 1955.)
Among the progeny of crosses of either of the pyridoxine mutants to wild type an unexpected type of recombinant has been found with about the same frequency as that with which the wild "recombinants" are detected from the cross between the two mutants. This recombinant is wild with respect to the pyridoxine mutant but carries the marker alleles which entered the cross with the pyridoxine mutant parent and would, therefore, require a double crossover in the short, marked region. The fact that the frequency of this type was greater than that with which double crossovers would be expected to occur suggested that here also some event other than ordinary crossing over was taking place, and an analysis of tetrads was again made. In the two tetrads so far found containing the unexpected recombinant there was, again, three-to-one segregation with respect to the pyridoxine mutant. That is, three spore pairs carried the wild, and only one the mutant allele of pyridoxine; while four nearby markers, one to the left and three to the right, segregated normally and showed no recombination.

Tetrads of this type, described in yeast by Dr. C. C. Lindegren and his collaborators, have been attributed to conversion of the mutant to its wild allele. The behavior of the pyridoxine mutants suggests that the event which produces the "recombinants" involves an interaction between alleles, but just what sort of interaction, or whether it is suitably described by the word "conversion," is not known.

Other data on Neurospora indicate that, although the pyridoxine locus may be more frequently affected by this unknown event than certain other loci, it is by no means necessarily the only locus so affected. There are a number of cases in which occasional wild offspring have been observed from crosses between similar mutants and in which the evidence that these offspring result from crossovers or from reverse mutation is incomplete or unconvincing.

62. SUPPRESSORS OF CYTOCHROME-DEFICIENT MUTANTS OF NEUROSPORA

Investigators: M. B. Mitchell, H. K. Mitchell
Support: U. S. Atomic Energy Commission
General Funds

During the past two years two suppressor mutations of spontaneous origin have been studied with regard to their effect on four mutant strains characterized by slow growth rate and abnormal behavior of the cytochrome system (Biology 1953, p. 63; Biology 1954, p. 71). Both suppressors are inherited as Mendelian factors. One of them increases the growth rate when present with the cytoplasmically inherited character, poky, but appears to have no effect on the cytochrome system of this or other strains with which it was tested. These include wild type and strains showing the mi-3 character, also cytoplasmically inherited, and the two nuclear gene mutations Cl15 and Cl17. The growth rates of strains other than poky were also unaffected. The second suppressor restores to normal both the growth rate and the cytochrome system of mutant Cl15 but does not affect either abnormality in poky, mi-3 and, possibly, Cl17. (No effect was observed on the latter mutant but actual combination of the Cl17 mutant gene with the suppressor was not demonstrated.) The action of this suppressor in restoring Cl15 seems to be through the cytochrome system itself, whereas the suppressor of poky appears to act by increasing the activity of a compensating system. Their responses to the suppressors provide additional evidence of the differences in the nature of the defects introduced by poky, mi-3 and Cl15. This is of particular interest in connection with the two cytoplasmically inherited characters.
Most of the linkage data which have been gathered in Neurospora have been measurements of second division segregation frequency for the mutant under study or observation of crosses of the mutant in question to a linked mutant very close to the centromere. This has resulted in a large amount of data giving a rough measure of the distance between mutant loci and their centromeres but very little reliable information about the relative positions of the mutant loci on the chromosomes.

A cross between a mutant of unknown location and a linked mutant which is very near the centromere provides no suitable point of reference to determine which arm of the linkage group contains the unknown mutant. As a result, there was reliable evidence in only one of the seven linkage groups (up to the present study) that mutant loci were known in both arms, even though there were extensive data on forty-seven mutant loci in the other six linkage groups. The question of which arm of a linkage group contains a mutant locus is best answered by a cross to a linked mutant of known location which is not very close to the centromere.

The current linkage maps of Neurospora place the mutant loci according to the second division segregation frequencies they have exhibited in the crosses studied. This involves the assumption that mutant "a" is closer to the centromere than mutant "b" if "a" had a lower frequency of second division segregation in one cross than "b" had in another. Recent evidence has shown this assumption to be invalid. A given mutant may display markedly different second division segregation frequencies in two different crosses. Studies of an ascospore mutant by the author have yielded second division segregation frequencies ranging from ten percent to sixty percent in different crosses (although the rate is constant for any given cross). Other authors have reported instances in which a given mutant has shown significantly different second division segregation frequencies in two crosses. It becomes evident that the relative positions of mutant loci on the linkage map cannot be reliably determined by measurement of second division segregation frequencies in isolated crosses. The relative positions of neighboring genes can only be established by studying them in the same cross. This requires three-point crosses: three linked mutant genes or two genes and the centromere studied simultaneously.

A map of nine mutant loci in linkage group VI of Neurospora has been prepared in two steps: 1) crosses of all mutants to a reference mutant some distance from the centromere to determine which arm contains each mutant gene; 2) three-point crosses of neighboring mutants to establish the order of arrangement of the mutant loci. Six of the mutants were found to be in one arm of the linkage group and three in the other.

64. MULTIPLE CROSSING OVER IN NEUROSPORA

It is known that a crossover involves exchange of material between only two of the four chromosome strands present at the time. It is unknown whether any particular attributes of these two strands are conducive to the crossing over process.
Are there two strands capable of crossing over and two incapable? Or are the strands which cross over at one level less likely to be involved in a neighboring crossover in the same tetrad of chromosomes? Does the occurrence of a crossover in one region affect the likelihood of a crossover occurring in a neighboring region? Questions of this type can be answered by studies of multiple crossing over. Neurospora is particularly suitable material for this kind of experiment, because all four products of a single meiosis can be recovered together, allowing a complete analysis of crossing over events.

The current study has been concerned with crossing over in the regions adjacent to the centromere. An earlier study with Neurospora (Lindegren and Lindegren, Journal of Heredity, 28, 105, 1937) indicated that the occurrence of a crossover on one side of the centromere increased the likelihood of such an event in the corresponding region in the opposite arm. It was further reported that, in the majority of cases, the same two strands were involved in both crossovers.

An ascospore mutant has been employed in the present work. Asci with crossing over in the interval between this locus and the centromere can be detected by the pattern of segregation for the ascospore character. This allows one to avoid the work of dissection and growth of those asci with no crossover in this region.

Tests have been made for double crossing over between regions on opposite sides of the centromere in 5,625 asci of crosses involving the ascospore mutant. The results show no evidence that crossing over on one side of the centromere has any correlation with or influence on that taking place on the other side.
IMMUNOGENETICS

STAFF:
Professor: Ray D. Owen
Research Fellow: Eleanor Christensen
Graduate Students: John W. Drake, William H. Hildemann
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65. SEROLOGY OF TETRAHYMENA

Investigators: Eleanor Christensen, Lucette Ghekiere, John B. Loefer,*
Ray D. Owen, Harold R. Wolfe†

Support: National Science Foundation
Rockefeller Foundation
General Funds

A distinguished series of investigations of the ciliated protozoa classified as paramecia, primarily by T. M. Sonneborn of Indiana University and his students and co-workers, has demonstrated that these organisms offer material of exceptional interest in fields related to heredity and differentiation. Another group of ciliates, classified as tetrahymena, has been of interest to other workers and in other connections, mainly because these forms can be grown in media free of other organisms and are therefore subject to biochemical study. It was only recently, through the recognition and definition of mating types by Elliott and Nanney at Michigan, that tetrahymena became accessible to genetic study. Specific immobilization of the organisms by antisera provides a convenient basis for the characterization of diverse types, called "serotypes."

Studies of the thirty-one strains of Tetrahymena pyriformis listed in Biology 1954, p. 86, have been continued and extended during the current year. The three mating-type strains, WH6, WH14 and WH52, have been given particular attention. Stock cultures of these three strains form a group of related, but not identical, serotypes, in the sense that antiserum to any one of them will usually immobilize the others, though often only at higher concentrations than are required to immobilize the homologous stock. Early in the year under review it was noted that the kind and amount of such cross-reaction was subject to unpredictable variation. In an attempt to define the basis of this variation, clones were derived from single cell isolates and subjected to treatment with antisera and to different temperatures of culture. The picture to be sketched from the studies is still not clear, but it seems to be similar in many respects to that anticipated from the earlier and more extensive studies of paramecium referred to above. It appears that a given stock often contains a mixture of serotypes, antibodies to each of which may be produced when the culture is injected into rabbits or chickens. A clone of recent derivation from a single cell, cultured under uniform conditions, usually induces a narrower spectrum of antibody specificities, presumably because it contains a more uniform population of serotypes. The population is unstable in this relatively homogeneous condition, however, and the clones may deviate to diverse types under continued

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culture. Transient treatment of cells with antisera seems to favor such deviation, particularly in response to different temperatures of culture.

With this phenotypic instability in mind, the application of serological typing techniques to taxonomic problems in *Tetrahymena* and related forms would seem to be subject to considerable reservation. It is remarkable, however, that over a period of almost two years of rather variable cultural conditions, the serotype relationships of thirty-one strains of *Tetrahymena pyriformis* described in Biology 1954 has shown no essential change. A few additional cross-reactivities have appeared, but these have served almost without exception to reinforce evidences of similarity or difference suspected from the earlier serological studies, rather than to call the previous conclusions into question. In view of the more detailed study of the WH stocks and antisera, it seems likely that many of the other antisera also contain heterogeneous antibodies to mixtures of serotypes. Such antisera may be more useful tools for assigning organisms to broad groups than would be antisera against single serotypes selected from among the several that might be possible for any particular strain.

In addition to the strains of *Tetrahymena pyriformis*, cultures of several other related forms, including *T. vorax*, *T. patula*, Glaucoma scintillans, Colpidium campylum, and Colpidium colpoda, have been examined for their serological specificities. Some of these have shown marked similarities to certain of the *T. pyriformis* strains. The three strains of *T. vorax* studied are serologically different from each other, and one cross-reacts *T. pyriformis* strains PR and GL; another is completely immobilized only by antisera to *T. pyriformis* strains L1 and L2, while the third is not immobilized by the antisera to any of *T. pyriformis* strains. Antiserum to this third strain of *T. vorax* does not react with any of the *T. pyriformis* strains. This kind of result suggests an essential incompatibility between serological classification of this "difficult" group and a taxonomic scheme based on morphological characteristics. Whether a more thorough study may provide a more consistent set of taxonomic tools remains to be seen.

Several other kinds of approach to the serology of *tetrahymena* are too incomplete at present to merit discussion here.

66. MECHANISM OF TOLERANCE TO TISSUE TRANSPLANTS


Support: U. S. Navy, Office of Naval Research, General Funds

Attempts have continued to produce and to understand situations in which the usual host response to tissue transplants from other individuals does not occur, so that the transplants are tolerated and survive (see Biology 1954, pp. 82-84). Several experiments were conducted in the field of skin transplantation, the most critical involving an attempt to desensitize White Leghorn chicks by injecting, over a period of two weeks after hatching, a heavy brei prepared from tissues of newly-hatched

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New Hampshire Red chicks. Skin from the red donors, which had been stored since sacrifice of the chicks at the time of preparation of the brei, was then transplanted to the injected whites, in the hope that an enhancing effect of such treatment, shown primarily by Snell and his coworkers to apply to tumor transplants in mice, together with the use of aggressive and perhaps less antigenic newly-hatched skin, might result in establishment of transplants. A total of one hundred eight sites, including autograft and regeneration controls among treated and untreated hosts, were examined. Although there were early evidences of some modification of host response associated with the treatment, these were of doubtful significance and as judged by the failure of all white hosts to grow red feathers the treatment failed to confer a persistent tolerance. Modifications of this approach and other procedures are under study.

Reagents, animals, and counsel have been provided workers in two other institutions for studies of tolerance to hemopoietic transplants. Related studies will probably be resumed at Caltech in the near future. Preliminary tests in rats of the phenomenon of maternally conferred tolerance, suggested last year in connection with Rh antigens in man (Biology 1954, p. 82) have given promising results.

67. SPECIFICITY IN SPONGES

Investigator: Ray D. Owen
Support: Friday Harbor Laboratories, University of Washington
Rockefeller Foundation
General Funds

The specific reaggregation of sponge cells that have been separated from each other by forcing a sponge fragment through a fine cloth, and the specific blocking of this reaggregation by antisera to the sponges, have provided interesting data for speculation on the factors that hold multicellular tissues together (cf. Spiegel, Biology 1954, p. 54). Antisera were prepared in chickens and rabbits to several sponges native to the Friday Harbor region (kindly collected and shipped to Caltech by Dr. D. L. Ray of the University of Washington), and studies of the reaggregation phenomena were conducted during the summer of 1954 at Friday Harbor. Incidental to these studies, permanent spicule preparations and informal descriptions were made of at least forty-five different species of sponges, a surprisingly large fraction of which do not appear to have been named or described in the taxonomic literature. The chicken antisera were found to function most efficiently in blocking reaggregation in sea water. This behavior was consistent with the fact that such sera showed optimal activity at high salt concentrations in other systems. Microscopic observations of reaggregation in immune as compared to normal serum indicated that the antibody interference is primarily with the dynamic behavior of the isolated cells, preventing their accumulation into aggregates, although there is also evidence that cells "coated" with antibody lose their mutual tendency to adhere even when they are brought into contact, as had been observed by Spiegel. The specificity of reaggregation in mixed suspensions was found to provide a precise technique for deciding whether two given sponges were alike or different, and several taxonomic puzzles were solved by examining mixed suspensions. An experimental design was developed that permitted such decisions to be made even when the cells of the two suspensions to be examined were not microscopically distinguishable. A proteinaceous agent found in extracts of one sponge that killed the cells of other sponges in suspensions was discovered and investigated. Specific interference with
reaggregation by means of sponge extracts was also subjected to preliminary examination, as were a number of other problems in this interesting material.

68. IMMUNOGENETICS OF TYROSINASE IN GLOMERELLA

Investigators: Clement L. Markert, * Ray D. Owen
Support: Rockefeller Foundation
         Merck Fellowship
         General Funds

During the current year, work on the serology and immunogenetics of Glomerella tyrosinase summarized in previous reports of the Biology Division was carried through to publication.

Publications:


69. IMMUNOGENETICS OF GOLDFISH

Investigator: William H. Hildemann
Support: General Funds
         Arthur McCallum Summer Scholarship

Continued study of scale transplantation reactions in the goldfish Carassius auratus has revealed that second-set homografts break down much more rapidly than first-set homografts in all recipient fish. This immune response has been measured by determining the median survival time (MST) and inflammatory reaction of scale homografts under various conditions. A simple and dependable technique of scale grafting has been devised so that reciprocal homografts can be made among ten or more fish at a time; with autograft controls. Tricaine (MS 222 of Sandoz) was found to be a particularly suitable anesthetic for the prolonged operations of multiple scale grafting.

Histological changes in scale grafts have been observed in detail in vivo under the microscope. In all experiments autografts have been normally revascularized without inflammation, whereas homografts are rapidly overgrown with hyperplastic host mesenchyme and elicit capillary leakage in the contact zone with host tissue. The rate of destruction of donor tissues was found to depend upon the constitution of the recipient and the water temperature. Previous grafting from the same donor and higher water temperature both accelerated homograft destruction. In one series of grafts among unrelated fish done at 20°C, ninety first- and second-set homografts had MST's of 7.7 ± 0.1 days and 5.4 ± 0.1 days, respectively. In another group of fish where a comparable series of grafts was made at 10°C, the MST's were 40.4 days and 19.2 days, respectively. Time-mortality curves show that the duration and intensity of the inflammatory reaction are closely associated with the rapidity of donor tissue destruction. Inflammation is much diminished at lower temperatures and did not appear at all in one series of three sets of homografts.

*University of Michigan
where tissue breakdown was studied at 8°C.

In future studies the homograft reaction will be compared among fish of different genetic relationships. Effects of graft dosage, prior immunization with donor tissues (e.g. red cells), and leucocyte infiltration will be investigated. Matings are in progress to determine the mode of inheritance of individual differences in red blood cell antigens, and their possible relationships to scale-transplant specificities.

70. ATTEMPTED SOMATIC TRANSFORMATION IN THE FOWL

Investigator: John W. Drake
Support: General Funds

Washed sperm from two White Leghorn cocks were provided Dr. Norman Simmons, of the U.C.L.A. atomic energy project, who kindly prepared highly polymerized desoxyribonucleic acid (DNA) from each sample. Phosphate-buffered saline dilutions were made, on the assumption of a DNA molecular weight of $7 \times 10^6$, to concentrations of $1.2 \times 10^6$, $1.2 \times 10^9$, and $1 \times 10^{12}$ molecules per 0.01 ml. Penicillin Sodium G was added, 100 units/ml. for operative injections and 267 units/ml. for injection of eggs. Developing ovarian follicles and newly-laid eggs of Rhode Island Red hens, which had previously been inseminated with Rhode Island Red semen, were injected with the DNA solutions. All injections were of 0.01 to 0.03 ml. of one of the DNA solutions, delivered with a screw-driven 1/4-ml. syringe. The chicks hatching from treated eggs, or from eggs that probably derived from treated follicles, failed to show color or red-cell antigenic characteristics of the cocks serving as sources of sperm DNA. The difficulty of accumulating sufficient numbers of cases is a major disadvantage of the technique.
MARINE ZOOLOGY

STAFF:
Associate Professor: George E. MacGinitie
Visiting Associate Professor: Demorest Davenport
Visiting Instructor: Harry K. Fritchman II
Superintendent, Kerckhoff Marine Laboratory: William S. Smith

71. THE REACTION OF SEA ANEMONES TO LIGHT

Investigator: G. E. MacGinitie
Support: General Funds

Most sea anemones either contract or move away from a source of bright light, others may be indifferent, and still others may expand or turn toward the light.

Some experiments have been conducted to determine to what extent light influences the more specialized although normal functions of certain sea anemones. Epiactis prolifera, the small sea anemone that carries its young attached to the side of its column until they reach a diameter of about one-fourth of an inch, is negative to light. Subjection to a strong light will cause the adult to contract and the young to drop off from the mother and roll away to attach to a rock or other hard surface in a darker area. Young anemones that have reached only half the size usually attained before leaving the mother will drop off within a few minutes after being subjected to a bright light, and more mature young may drop off almost immediately. Another sea anemone, Sagartia species, reproduces asexually by expanding its basal disk and then pulling away from the outermost portion. The small portions of the pedal disk thus detached later develop into anemones. This species is highly susceptible to light shining on its pedal disk. It will not expand its base while subjected to strong light and if its base is already expanded and ready to detach a portion, the anemone will not proceed with the process but will contract its base to normal or less than normal size.

72. THE MARINE INVERTEBRATES OF POINT BARROW, ALASKA

Investigator: G. E. MacGinitie
Support: U. S. Navy, Office of Naval Research

The problem was to determine as far as possible the number and kinds of marine invertebrates on the ocean bottom in a small area (no farther than sixteen miles from shore) off Point Barrow base in Alaska, to find how some of these animals lay eggs. In summer animals were collected by dredging, in winter by making holes through the ocean ice and dredging with a dog team, and also by means of screen traps placed on the ocean bottom. Through other holes, vertical plankton tows were taken at regular intervals. It was found that plankton-feeding animals prepare for the winter by storing large quantities of oil in their bodies—often sufficient to provide for the production of eggs during the winter that are ready to be laid as soon as the ice goes out. Some animals, such as certain annelids and certain amphipods, spawn during mid-winter. Others lay eggs in the fall and the
larvae spend the winter without developing beyond a certain stage. Many of the arctic snails lay large eggs (usually in protective capsules, but sometimes they are retained within the body of the mother) that provide nourishment for the larvae until they are young snails and do not have to go through a free-swimming larval stage; others achieve the same end by laying numerous smaller eggs, some of which are for nourishment for the few large larvae that develop. Several of the clams retain the eggs within the body until the larvae develop into young clams. Unusually large eggs were found in other animal groups, such as the echiuroid worms, tunicates, and some of the amphipods. Thus a shortened pelagic life is made possible for the larvae and they have ample time to develop during the summer months. It was also found that light penetrates several feet of ice sufficiently to enable certain diatoms to grow throughout the winter.

Publication:


73. CHEMICAL RECOGNITION IN SYMBIOTIC ORGANISMS

Investigator: Demorest Davenport
Support: General Funds

During the summer of 1954 studies were initiated on the symbiotic partnership between the starfish Patiria miniata and the hesionid annelid Podarke pugettensis which indicated that this partnership is one particularly well suited to the study of chemical recognition in symbiotic organisms. At the same time brief experiments were conducted on luminescence in Pennatulids, which were continued at a later date at the Friday Harbor Laboratories of the University of Washington and which have resulted in two publications in press in the United Kingdom (co-author J. A. G. Nicol of Plymouth).
74. PREPARATION AND PROPERTIES OF AN AMINO ACID-INCORPORATING PARTICULATE SYSTEM FROM PEA ROOTS

Investigators: George C. Webster, John M. Clark, Jr., Mary Perrine Johnson
Support: E. I. du Pont de Nemours and Company
General Funds

Cell-free extracts of pea roots are able to incorporate a number of radioactive amino acids into their protein. This process is promoted by adenosinetriphosphate (ATP) and by magnesium ions, but is inhibited by adenosinediphosphate (ADP) and by many other divalent ions. The rate of incorporation of a single amino acid is generally unaffected by the presence of other individual amino acids. An exception, however, is glutamate incorporation which is inhibited by aspartate, asparagine and citrulline. A mixture of seventeen amino acids significantly increases the incorporation of any one of several radioactive amino acids. This enhanced incorporation is strongly inhibited by various amino acid antagonists.

From the cell-free extracts a system of cytoplasmic particles may be prepared with about eighty percent of the incorporating ability of the cell-free extracts. Optimal conditions for the preparation of this particulate material have been investigated and found to be greatly dependent on hydrogen ion concentration, sucrose concentration, and ionic strength of the medium used in disrupting the cells. The particulate material, prepared under optimal conditions, has been partially purified by differential centrifugation. Material sedimenting between one thousand and twenty thousand times gravity possesses almost all of the specific incorporating activity. As nuclei sediment at about five hundred times gravity under these conditions, the preparation is essentially free of nuclei. Likewise, the fact that mitochondria in these extracts are sedimented at eight thousand times gravity indicates that another form of particle, smaller than mitochondria, is also essential for the highly active incorporation process. Amino acid incorporation by these particles is strongly inhibited by dinitrophenol, hydroxylamine, chloramphenicol, and arsenite. Incorporation is considerably enhanced by arsenate.
ENZYMATIC SYNTHESIS OF PROTEIN

Investigator: George C. Webster
Support: E. I. du Pont de Nemours and Company

In order to study effectively the actual mechanisms of protein synthesis and to relate this synthesis to the phenomenon of amino acid incorporation into protein, it is essential to have a simple cell-free system that will synthesize protein. The development of such a system depends, in turn, on the availability of highly sensitive methods for the determination of total protein. Three different assays for protein have now been adapted to the detection of small changes in total protein content. These are isotope dilution, micro-Kjeldahl, and biuret determinations. By employment of these three methods simultaneously it has been possible to detect increases in the protein content of a purified preparation of pea-root cytoplasmic particles after incubation with a mixture of seventeen amino acids and adenosine-triphosphate. Kinetic studies have shown that the ability of the particles to increase in protein content decreases rapidly and irreversibly after the first two hours of incubation. Studies are continuing on the properties of this system and the nature of the protein being formed.

ROLE OF NUCLEIC ACID AND NUCLEIC ACID COMPONENTS IN PROTEIN SYNTHESIS

Investigators: George C. Webster, Mary Perrine Johnson
Support: E. I. du Pont de Nemours and Company
Frasch Foundation

Various forms of indirect evidence have indicated a relation between nucleic acids and protein synthesis. Several investigators have recently demonstrated that treatment with ribonuclease or deoxyribonuclease inhibits the incorporation of radioactive amino acids into protein by cell-free extracts of various organisms. We have found that amino acid incorporation by purified cytoplasmic particles of pea roots is strongly inhibited by ribonuclease but not by deoxyribonuclease. This inhibition is overcome by ribonucleic acid (RNA) but not by deoxyribonucleic acid. This effect of RNA is not specific for the intact molecule. RNA preparations of decreasing degree of polymerization are increasingly more effective in promotion of amino acid incorporation, and a mixture of the four mononucleotides derived from RNA is more effective than polymerized RNA itself. A mixture of the respective nucleosides is even more effective in increasing amino incorporation, but the purines and pyrimidines derived from the nucleosides are less effective. Certain individual nucleosides enhance amino acid incorporation significantly but always to a lesser extent than the mixture. Which of the individual nucleosides is more effective depends on the amino acid studied. Deoxyribonucleosides and deoxyribonucleotides are completely ineffective either singly or together.

The effectiveness of nucleic acid components in augmenting amino acid incorpo-
ration into protein suggests that the turnover of nucleic acid is important for amino acid incorporation and protein synthesis. This possibility is also suggested by the finding that inhibitors of nucleic acid synthesis (aminopterin, 6-mercaptopurine, etc.) also inhibit amino acid incorporation. Conversely, amino acid antagonists inhibit nucleic acid synthesis. The data, then, indicate a possible direct and reciprocal relation between nucleic acid and protein synthesis.

Publication:


77. THE EFFECT OF PEA RIBOSENUCLEIC ACID FRACTIONS ON THE RATE OF INCORPORATION OF AN AMINO ACID INTO AN ISOLATED CELLULAR FRACTION OF PEA ROOTS

Investigator: A. G. Howard
Support: E. I. du Pont de Nemours and Company
Frasch Foundation

It has been reported that the rate of incorporation of radioactive amino acids into disrupted bacterial cells is enhanced by the addition of nucleic acids from the same bacteria, but that nucleic acids from other sources are not effective. With the pea root particle system, on the contrary, ribosenucleic acid (RNA) from various sources increases the rate of amino acid incorporation. The present experiments were done to discover whether RNA from peas is more effective on the system than RNA from other sources.

Although a number of methods of RNA preparation are employed at the present time, none of them is unambiguously good and it is likely that none of them retains the RNA in a completely unaltered form. The method of Simmons was chosen for this work. The RNA was extracted from blended, frozen peas with a solution of xylene sulfonate. After several purifications the RNA was dissolved in water and fractionated by successive additions of isopropyl alcohol. The RNA fractions which precipitated at isopropanol concentrations of less than thirty-one percent and greater than sixty-seven percent enhanced the rate of incorporation of glutamate into particles from pea roots. Fractions precipitated between thirty-one percent and sixty percent did not increase the rate of incorporation. In fact the RNA which was precipitated by fifty percent isopropanol was somewhat inhibitory. The effects of pea RNA on amino acid incorporation by the system were not markedly different from those caused by yeast nucleic acid, either commercial or prepared by the mild procedure.

78. FACTORS WHICH INFLUENCE THE RATE OF INCORPORATION OF RADIOACTIVE AMINO ACIDS INTO CYTOPLASMIC PARTICLES FROM PEA ROOTS

Investigator: A. G. Howard
Support: E. I. du Pont de Nemours and Company
Frasch Foundation
It has been shown that each of the purine and pyrimidine bases, as well as the nucleosides of ribonucleic acid (RNA), enhances the rate of enzymatic incorporation of amino acids into particles from pea roots. These particles are normally prepared by centrifugation of whole pea root cytoplasm at forty thousand times gravity for thirty minutes. The particles sedimented at thirty thousand times gravity incorporate glutamate, but the rate of incorporation is not increased by the addition of uracil or uridine. A mixture of the four nucleosides of RNA does, however, enhance the rate of amino acid incorporation. Preparations which are enriched in those particles which sediment between thirty thousand and forty thousand times gravity do not respond more markedly to the presence of uridine than do standard preparations. Previous work on the kinetics of glutamate incorporation into the pea root particles has shown that there is a two-hour lag before the rate reaches its maximum. This lag is almost entirely eliminated if the particles are incubated in a solution of sucrose and buffer for three hours before addition of the substrate.

The initial rate of glutamate incorporation increases with the time of pre-incubation in absence of substrate and is maximal with a pre-incubation period of four hours. With this period of pre-incubation, the subsequent initial rate of glutamate incorporation is approximately forty times greater than that of the non-pre-incubated system. Additions of nucleic acid and nucleosides to the incubation mixture increase the rate of amino acid incorporation with the pre-incubated system.

Pre-incubation of the system in the absence of substrate increases subsequent rate of incorporation of glutamate, alanine, leucine, histidine, aspartate, and glycine.

It is possible that the particles prepared contain free amino acids which are incorporated into protein in preference to externally supplied amino acids. Pre-incubation may exhaust these internal supplies so that the externally supplied glutamic acid may be used more exclusively.

79. FRACTIONATION OF RIBONUCLEIC ACID

Investigator: George C. Webster
Support: E. I. du Pont de Nemours and Company

In connection with other studies on the relation of ribonucleic acid to protein synthesis, it became of interest to attempt to fractionate ribonucleic preparations. A preparation of yeast ribonucleic acid proved to be quite heterogeneous and could be separated into various polynucleotides in any of three ways: (1) by chromatography on a column of one percent cross-linked Dowex-50 by elution with changing concentrations of dilute HCl; (2) by chromatography on a column of one percent cross-linked Dowex-1 by elution with formate-formic acid systems; (3) by salting out the polynucleotide fractions with increasing concentrations of ammonium sulfate. The last method is extremely convenient and effective. That different polynucleotide fractions are obtained is shown by their greatly different effectiveness in promoting amino acid incorporation by a particulate preparation from pea roots.
80. ON THE MECHANISM OF SYNTHESIS OF AMIDE AND PEPTIDE BONDS

Investigators: George C. Webster, Luther W. Eggman, J. E. Varner
Support: E. I. du Pont de Nemours and Company
Charles F. Kettering Foundation Fellowship
U. S. Public Health Service

Work has been continued on the mechanisms involved in the enzymatic synthesis of simple amides and peptides. Information concerning these mechanisms is of great potential importance to an understanding of the manner in which peptide bonds are formed during protein synthesis. One of the principal present tasks has been to prepare peptide and amide bond synthesizing enzymes in unequivocally pure form. The enzyme which catalyzes glutamine synthesis has now been purified 5,300-fold from green peas. This preparation and the 2,000-fold purified preparation used by other investigators have been studied by ultracentrifugation. The 2,000-fold preparation is quite heterogeneous while the 5,300-fold purified preparation consists of either two or three components.

The enzyme which catalyzes the synthesis of the dipeptide glutamylcysteine has now been purified some 3,000-fold. The mechanism of synthesis of this dipeptide now appears to resemble closely the synthesis of glutamine. From these studies, and others on less highly purified preparations which catalyze the synthesis of asparagine and glutathione, a general picture is emerging concerning the mechanism of synthesis of amide and peptide bonds. Preliminary studies have also indicated that techniques employed in these studies can be applied to the study of the synthesis of the peptide bonds in protein.

Publications:


81. THE BIOSYNTHESIS OF ASPARAGINE

Investigators: George C. Webster, J. E. Varner
Support: E. I. du Pont de Nemours and Company
Charles F. Kettering Foundation Fellowship

Asparagine is an important metabolite in living cells, but attempts of various investigators to discover how it is synthesized have all failed. It has now been possible to demonstrate the biosynthesis of asparagine-C¹⁴ from aspartate-C¹⁴ and ammonia in intact lupine seedlings, in cell-free extracts of these seedlings, and in wheat germ. Adenosinetriphosphate (ATP) and magnesium ions are required for maximal rates of synthesis. The enzyme which catalyzes asparagine synthesis has been partially purified from both lupine and wheat germ. The reaction is analogous to that involved in glutamine synthesis, namely:

Aspartate + NH₃ + ATP → Asparagine + ADP + P₃

The reaction is reversible, since the enzyme can catalyze the incorporation of orthophosphate-P³² into ATP in the presence of asparagine and adenosinediphosphate. The enzyme also catalyzes the formation of aspartyl hydroxamate from
asparagine by a transfer reaction.

Publications:


82. THE BIOGENESIS OF CAFFEINE

Investigator: Walter S. McNutt, Jr.
Support: U. S. Army, Office of the Quartermaster General

The possibility that caffeine may arise in plants through methylation of a pre-existing purine ring system has been investigated by use of 8-labeled adenine and uniformly labeled guanine. The labeled compounds have been administered to leaf sections of the coffee plant by vacuum infiltration and were permitted to remain in contact with the leaf in the dark for periods of three days. Under these conditions the caffeine becomes labeled to an extent of three hundred counts per minute per $10^{-6}$ mole of caffeine. Work is in progress to determine the distribution of labeling within the caffeine molecule.

83. PARTIAL PURIFICATION OF GLUTAMIC DECARBOXYLASE

Investigator: John M. Clark, Jr.
Support: E. I. du Pont de Nemours and Company
General Funds

Although glutamic decarboxylase is present in large quantities in brain tissue and in certain plants, this enzyme has not been purified appreciably. It has now been purified some thirtyfold from squash fruits by fractionation with ammonium sulfate and protamine and by adsorption and elution from calcium phosphate gel. The purified enzyme requires pyridoxal phosphate as a cofactor. It is stable in the frozen state only if frozen rapidly at -80° C.

84. METABOLISM OF UREA IN PLANTS

Investigators: George C. Webster, J. E. Varner, A. N. Gansa
Support: E. I. du Pont de Nemours and Company
Charles F. Kettering Foundation Fellowship

The increasing use of urea as a source of nitrogen for the nutrition of plants has raised a question as to how the urea is metabolized by the plant. It was found that the carbon of urea-C$^{14}$, when fed to a plant, is distributed among amino acids with the same pattern as is the carbon from an equivalent mixture of C$^{14}$O$_{2}$ and NH$_{3}$. Although there are certain differences in the kinetics of labeling, all the results are consistent with the view that the metabolism of urea occurs by hydrolysis to CO$_{2}$ and

*California Institute of Technology Undergraduate.
NH₃, followed by incorporation of these substances into the normal metabolic cycles of the plant.

85. STUDIES ON THE MECHANISM OF BIURET ACTION IN LEAVES

Investigators: George C. Webster; R. A. Berner, * A. N. Gansa *
Support: E. I. du Pont de Nemours and Company
General Funds

Although urea is now an important and widely used nitrogen source for plants, its application to certain plants (notably citrus and pineapple) results in leaf injury. Investigation has shown that this injury (a progressive, irreversible yellowing from the tip of the leaf) is not attributable to urea itself, but is caused instead by low concentrations of biuret which are present in many samples of commercial urea. In an attempt to find the cause of the toxic action of biuret, we have studied its effect on various phases of plant nitrogen metabolism. Xanthium pennsylvanicum has proved to be a convenient and sensitive plant for assay of biuret action. Biuret toxicity is particularly marked when the substance is supplied to the plant together with urea. Biuret has, however, no great effect on the uptake or the hydrolysis of urea. Neither does biuret influence amino acid oxidation, transamination, or arginine hydrolysis. In plants supplied with urea-C¹⁴, biuret causes an increase in the radioactivity of the free amino acid pool of the leaves. It inhibits, however, the incorporation of radioactive amino acids into protein, and strongly reduces the protein level of leaves. These results indicate that biuret may act by inhibiting the protein-forming system of leaves.

86. RUBBER FORMATION

Investigators: Walter S. McNutt, Jr., James Bonner
Support: U. S. Army, Office of the Quartermaster General
General Funds

The administration of C¹⁴-labeled acetate or of β-methyl crotonate to seedlings of the rubber-producing plant guayule results in a relatively small incorporation of C¹⁴ into the rubber. Most of the radioactivity of the benzene extract of the plant resides in the fraction which remains after removal of rubber by precipitation with ethanol. This fraction has been examined for possible precursors of rubber. About sixty percent of the activity is accounted for by higher fatty acids which average about twenty carbon atoms per carboxyl group. The infra-red adsorption spectrum of the mixture is characteristic of long chain fatty acids. Upon ozonization and ozonolysis C¹⁴-labeled acetone is formed, indicating that the preparation contains a terpene-like structure.

Reversed-phase chromatography shows the mixture to contain no fewer than four radioactive components.

*California Institute of Technology Undergraduates.
87. METABOLISM OF β-METHYLCROTONATE IN A PLANT ENZYME SYSTEM

Investigator: R. B. Park
Support: U. S. Army, Office of the Quartermaster General
General Funds

β-Methylcrotonate has been found to increase terpene formation in an intact plant system. The present experiments were designed to determine the initial fate of β-methylcrotonate in cell-free enzyme systems. The rubber-producing plants guayule and kok-saghyz were used as the source of enzyme. $^{14}$C-labeled β-methylcrotonate, coenzyme A, adenosinetriphosphate, activation enzyme prepared from spinach, and crude homogenate of guayule or kok-saghyz formed the reaction mixture. After incubation for five hours, the reaction mixtures were extracted with benzene. The benzene extract was then taken up in chloroform and washed with base to remove unreacted β-methylcrotonate. Activity remained in the chloroform extract and this activity was increased fourfold in the presence of activation enzyme. The chloroform extract was then placed on a reverse phase column such as is used for separation of higher fatty acids. Activity appeared in substances of a larger molecular weight than β-methylcrotonate. The quantitative determination of the amounts of these substances and their identification are the subject of present investigations.

88. PURIFICATION OF MYXOMYOSIN

Investigators: Paul O. P. Ts'io, Luther W. Eggman, Jerome Vinograd*
Support: General Funds
Arthur McCallum Summer Scholarship
U. S. Public Health Service

A soluble protein complex that exhibits reversible changes in physical properties in response to physiological concentrations of adenosinetriphosphate (ATP) may be extracted from the plasmodia of the myxomycete Physarum polycephalum by 1.4 M KCl. Under the proper conditions, addition of ATP to this crude extract causes a marked and rapid decrease in viscosity. The magnitude of the decrease, which is reversible, is a measure of the "ATP-response" or "ATP-sensitivity" of the system. A routine assay based on ATP-response has been developed and used for following the active principle during fractionation.

The ATP-sensitive material is precipitated by ammonium sulfate between thirty-two and forty percent saturation. The precipitates redissolve readily in low ionic strength solutions or buffers, and all the activity is retained during dialysis against similar solutions. This makes it possible to perform physical analyses of solutions of known ATP-sensitivity.

The salt-precipitated preparations have been further purified by differential centrifugation in a preparative Spinco centrifuge. Material sedimented at 40,000 r.p.m. redissolves in 0.2 M neutral buffer solutions to give viscous solutions of high ATP-response. These preparations, which represent about a twentyfold purification of the active component, can be further fractionated by repeated dif-

*Division of Chemistry, California Institute of Technology

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ferential centrifugation. Two cycles of alternate high- and low-speed centrifugation result in maximum enrichment of activity.

Twice sedimented and redissolved preparations, referred to as DC-2, have been used for chemical and physical characterization studies (see Reports Nos. 89 and 90). The ATP-sensitive material in these preparations has been designated as myxomyosin.

89. CHEMICAL CHARACTERIZATION OF MYXOMYOSIN

Investigators: Paul O. P. Ts'o, Luther W. Eggman, Jerome Vinograd*
Support: General Funds
Arthur McCallum Summer Scholarship
U. S. Public Health Service

Myxomysin is an ATP-sensitive protein which forms viscous, yellow colored solutions.

Twice centrifuged preparations (see Report No. 88) contain a yellow pigment material, of as yet unknown identity, which has been removed with NH₃-aqueous acetone mixtures and partially purified by adsorption and elution from Filtrol.

About one percent by weight of trichloroacetic acid precipitable material in myxomyosin preparations is phosphorus (TCA-P). The presence of pentose nucleic acid, which was suggested by a strong absorption at 260 mp and by the presence of TCA-P, was confirmed by paper chromatographic identification of adenine, guanine, cytidine and uridine in perchloric acid hydrolysates. Based on TCA-P content and extinction coefficients, about ten percent nucleic acid is normally present in current preparations, although the quantity is somewhat variable between different preparations.

Incubation with pancreatic ribonuclease causes a large increase in the viscosity of myxomyosin. This increase is reversed by ATP, suggesting that the nucleic acid moiety is not necessarily a part of the ATP-responding mechanism of the myxomyosin complex.

90. PHYSICAL CHEMICAL CHARACTERIZATION OF MYXOMYOSIN

Investigators: Paul O. P. Ts'o, Luther W. Eggman, Jerome Vinograd*
Support: General Funds
Arthur McCallum Summer Scholarship
U. S. Public Health Service

The best available preparations of myxomyosin, as judged by ultracentrifugal and electrophoretic homogeneity and magnitude of the ATP-induced change in viscosity, have been examined in detail to identify and characterize the material involved in the ATP-response.

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Electrophoretically, DC-2 preparations contain one major and two minor components, A, b and c, respectively. Both minor components have a higher mobility than that of the major component. Component c, which accounts for three to five percent of the total area in typical electrophoresis patterns, migrates very rapidly and is probably composed of nucleic acid or polynucleotide material. Component b constitutes about fifteen percent of the total area. It migrates only slightly faster than the main component. In the presence of excess ATP, the area of b is increased about threefold at the expense of A. Thus, components A and b appear to be two equilibrium forms of the same material, differing only in their charge per unit mass.

Ultracentrifugally, two components are present in DC-2 preparations dissolved in 0.2 M KCl-maleate buffer. The major component constitutes about seventy percent of the total area, exhibits little boundary spreading, and has a sedimentation constant of about 30S at zero concentration. The second component has a sedimentation constant of about 3 to 5S and is polydisperse.

The 30S component has been identified as the ATP-sensitive material myxomyosin. In the presence of excess ATP, the sedimentation rate of the 30S component is decreased about ten percent and the degree of boundary sharpening is increased. No new components appear, nor does there appear to be a shift in the equilibrium ratio of the 30S to the 3 to 5S components.

The intrinsic viscosity in 0.2 M KCl-maleate buffer is 300 cc./gm. If the myxomyosin molecule may be approximated by a prolate ellipsoid of revolution and if it contains twenty-five percent water of hydration, then an axial ratio of eighty to one may be deduced from the intrinsic viscosity data alone.

The molecular weight calculated from a measured partial specific volume of 0.72, the sedimentation constant, and a frictional coefficient derived from viscosity data, is 6.0 x 10^6. Electron microscopy studies indicate that myxomyosin is a rod-shaped molecule about 50 Å in diameter and 3,500 to 6,000 Å in length, with most of the particles about 4,000 Å long. The molecular weight computed for a cylinder 50 x 4,000 Å is 6.1 x 10^6, which is in excellent agreement with the value derived from viscosity and sedimentation data.

91. ANALYTICAL CENTRIFUGATION

Investigators: Luther W. Eggman, Jerome Vinograd*
Support: Rockefeller Foundation
U.S. Public Health Service

In February of this year, arrangements were made whereby a Spinco Model E Ultracentrifuge was acquired jointly by the Divisions of Biology and Chemistry.

The instrument has been calibrated and a thermister-operated temperature indicating and control device installed. So far the ultracentrifuge has been used routinely for following fractionation procedures, correlating biological activity with particular protein components, and for establishing homogeneity of biologically interesting materials.

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Following is a brief summary of the more important projects which have been investigated. Work on all of them is still in progress.

1. Purification and characterization of myxomyosin. (With Paul O. P. Ts'o.) Routine use of centrifugation in perfecting a fractionation procedure and for determination of the molecular weight of myxomyosin (see reports Nos. 88 and 90).

2. Isolation of a heavy component from human antisera (with D. H. Campbell and the Immunoochemistry group). Centrifugation used to follow fractionation by a differential centrifugation procedure, and to correlate a specific anti-RH antibody activity with a high-molecular weight globulin component in human anti-Rh sera.

3. Proteins of green leaves (with Dr. S. G. Wildman's group, Department of Botany, University of California at Los Angeles). The joint program for isolation and characterization of the normal proteins of green leaves, and particularly the normal nucleoprotein component which was first discovered and partially characterized in these laboratories several years ago, has continued.

4. Homogeneity of glutamyl synthetase enzyme preparations (with George Webster and J. E. Varner). These preparations contain two distinct enzymatic activities, the ratio of which remains constant throughout the fractionation procedure. It has been assumed by some workers that only one protein species is present in 2,000-fold purified material. These 2,000-fold purified preparations have been found, however, to be polydisperse. Even 6,000-fold purified preparations possess at least three distinct components. Redissolved sediments (which are not homogeneous) obtained from this centrifugation and which are about 11,000-fold purified still possess the two enzymatic activities in a 1:1 ratio. (See report No. 80).

5. Characterization of H₂O₂ treated proteins (with Geronimo Terres, Jr.). Analytical centrifugation has been used in determining the homogeneity and molecular weights of H₂O₂ treated ovalbumin, bovine γ-globulin and bovine serum albumin in connection with the immunological properties of these materials.

92. RELATION OF AUXIN TO CELL WALL METABOLISM

Investigators: Lawrence Ordin, James Bonner
Support: National Science Foundation Fellowship
Frasch Foundation
General Funds

The experimental evidence available indicates that the primary effect of auxin on plant tissue is a relaxation of cell wall pressure. This investigation concerns the relation of auxin to cell wall metabolism. The experimental method consisted in determination of the rate of incorporation of C¹⁴-labeled substrates into cell wall components and the rate of turnover of these components. It was found that uniformly labeled glucose is incorporated into the cell wall of Avena coleoptile fractions both in the presence and absence of auxin. Differences due to hormone treatment are slight, if any. Most of the activity is found in the protoseptin and polyuronide hemicellulose fractions. Experiments with previously labeled tissue shows that synthesis rather than turnover is responsible for the incorporation.
It appears unlikely that control of amount of cell wall synthesis is the primary function of auxin. Investigations of cell wall metabolism as related to intermolecular bonding are in progress.

93. THE OSMOTIC RELATIONS OF AUXIN-INDUCED WATER UPTAKE

Investigators: Lawrence Ordin, Thomas H. Applewhite, *James Bonner
Support: National Science Foundation Fellowship
Frasch Foundation
General Funds

A primary effect of the plant hormone auxin is the induction of water uptake by plant tissue.

It was found that Avena coleoptile tissue remains in apparent diffusion pressure equilibrium with the external solution both in the presence and absence of auxin and over a wide range of external solute concentrations. Tissue placed in hypertonic solutions deplasmolyzes and grows if a permeable solute such as sucrose is present externally. Under these conditions internal osmotic pressure increases. In the absence of a permeable solute such as sucrose no deplasmolysis occurs and internal osmotic pressure remains constant.

It is concluded that auxin does not control cell growth by causing an active water uptake. Neither does auxin influence the osmotic concentration of the tissue in any way. The present results imply that auxin acts on the plant cell to reduce wall pressure.

94. PERMEABILITY OF AVENA COLEOPTILE TISSUE TO WATER AS STUDIED WITH DEUTERIUM HYDROXIDE

Investigators: Lawrence Ordin, James Bonner
Support: National Science Foundation Fellowship
Frasch Foundation
General Funds

Since a net uptake of water is the primary expression of the effect of auxin on plant cells, it is of interest to know the absolute rate of water movement in the tissue under various conditions and in the presence and absence of auxin. The use of deuterium hydroxide (DHO) permits investigation of water permeability with uninjured tissue whose geometry remains constant during measurement.

It has been found that external osmotic concentration, auxin, growth-inhibiting concentrations of KCN, and 2,4-dinitrophenol (DNP) exert little if any effect on rate of inward diffusion of DHO (0.5% D2O in H2O). The time required for the internal DHO concentration to attain fifty percent of the external DHO concentration is eight to ten minutes. Rate of outward diffusion of DHO equals rate of inward diffusion in the presence of auxin.

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Dead tissue is only slightly more permeable to DHO than living tissue. This indicates that the cell wall rather than the plasma membrane is the primary barrier to water movement.

It is concluded that the Avena coleoptile is highly permeable to water, and that water movement in this tissue is a pure diffusion phenomenon.

The isotopic water analyses were made by use of spectrometer and carried out by Consolidated Engineering Corporation, Pasadena.

95. EXPERIMENTAL SEPARATION OF AUXIN ACTION FROM CELL ELONGATION

Investigator: Robert E. Cleland
Support: General Funds

Auxins cause plant cells to expand by uptake of water. An attempt has been made to completely separate the action of auxin on the cell from the act of cellular expansion. For this purpose experiments were used in which oat coleoptile sections were first given auxin under conditions in which water uptake was suppressed (0.3 N mannitol), and subsequently allowed to expand in water, the action of auxin being blocked by an inhibitor. Any residual effect of the auxin pretreatment manifests itself as an increased expansion of the auxin-treated sections over similar but non-auxin-treated ones.

It has been shown that the action of auxin can be separated from cell expansion and that the residual auxin effect involves two phases. Under aerobic conditions, auxin initiates a loosening of the cell wall. This loosened condition can persist over short periods of time under anaerobic conditions but is destroyed if all metabolism is blocked by potassium cyanide or dinitrophenol. The residual effect of auxin is partially reversed by addition of antiauxin during the anaerobic expansion period, suggesting that auxin must be present in some form during this second phase.

96. AUXIN INHIBITION OF ROOT GROWTH

Investigator: R. B. Park
Support: General Funds

The water which is transported into a growing root is channeled into two processes, exudation and growth. By considering the total volume of water utilized in these two processes in a given length of time, it is possible to determine whether the presence of auxin in the external medium influences the transport of water into a growing root. It was shown in the case of pea roots that the sum of the volume of water exuded plus the volume used in growth is independent of auxin indoleacetic acid concentration in the range 0-10^{-5} M. However, distribution of water between the two processes is auxin mediated; low concentrations favoring growth and inhibiting exudation. Higher concentrations of auxin favor exudation and thus inhibit growth. These results indicate that water uptake in roots is primarily an osmotic phenomenon, and that no additional transport of water into the root occurs in the presence of auxin.
97. AUXIN-ANTIAUXIN INTERACTION AT HIGH AUXIN CONCENTRATIONS

Investigators: Robert J. Foster, D. Harold McRae, James Bonner
Support: Frasch Foundation
        National Science Foundation
        General Funds

The growth rate of Avena coleoptile sections increases with increasing concentration of added auxin. It attains a maximum and decreases with further increase in auxin concentration. At auxin concentrations below the optimum, growth of Avena coleoptile sections is competitively inhibited by added antiauxin. The auxin-induced growth of Avena coleoptile sections may therefore be inhibited either by high auxin concentrations or by added auxin antagonists. This work concerns the nature of the interaction of these two kinds of inhibitions. It is shown that the high auxin concentration induced growth inhibition is not antagonized by antiauxin. On the contrary, the interaction of antiauxin (4-chlorophenoxyisobutyric acid) and auxin (indoleacetic acid) in Avena coleoptile section growth is quantitatively that expected for interaction between a monofunctional inhibitor and a bifunctional substrate.

98. UPTAKE OF AUXIN BY PLANT TISSUE

Investigators: Mary Perrine Johnson, James Bonner
Support: Frasch Foundation
        National Science Foundation
        General Funds

The uptake of the auxin 2,4-D by Avena coleoptile sections has been followed with the aid of C\textsuperscript{14}-labeled 2,4-D. The auxin is not degraded during the two- to six-hour experimental period. It is accumulated by the tissue and the kinetics of the accumulation resemble those of ion uptake in general. A rapid exchange type of binding, consummated in twenty to thirty minutes, is followed by a continuing active accumulation which is constant with time for several hours. Neither rate nor amount of uptake nor binding of 2,4-D bears any discernible relation to tissue growth rate.

99. EFFECT OF TEMPERATURE ON AUXIN RESPONSE

Investigators: R. J. Foster, Mary Lou Slichter
Support: Frasch Foundation
        National Science Foundation

The effect of temperature on the auxin response of Avena has been investigated at 5° C., 15° C., and 35° C. and compared with the data available at 25° C. At 15° C. the growth is linear with time up to twenty hours as it is at 25° C. At both 5° C. and 35° C. growth is not linear with time for more than nine hours. This and the decreased response at 5° C. and 35° C. make quantitative treatment of data at these temperatures difficult. However, at all temperature a maximum response was found near 10\textsuperscript{-5} M applied auxin.

At 15° C. the auxin response fits the equation used to express it at 25° C.
growth = \frac{V_{ex}}{K_S/S + 1 + S/K_{SS}}

where $V_{ex}$ is the extrapolated velocity.

The effect of temperature on the constants of this equation and on the optimal auxin concentration has been obtained for indoleacetic acid and 2,4-D. Since the auxin response is still well represented by the above equation, the effect of temperature on the inhibition constants for various kinds of antiauxins may now be determined.

100. STRUCTURE AND ACTIVITY IN THE AUXINS

Investigators: R. J. Foster, Mary Lou Slichter
Support: National Science Foundation
Frasch Foundation

In the substituted phenoxyacetic acid series the relation between chemical structure and physiological activity has been shown to satisfy the two-point attachment concept in a quantitative as well as a qualitative manner. That is, the effect of structural changes on the strength of a lone attachment point as determined by the inhibition constants ($K_I$) of selected antiauxins has been reflected in the Michaelis constants ($K_M$) of the corresponding auxins. Work is in progress to test the same correlation in the indoleacetic acid series.

For the success of this approach, antiauxins must be found which have only one point of attachment. Skatole, the decarboxylation product of indoleacetic acid (IAA), was found to be an antiauxin stronger than 2,4-dichloroanisole, the decarboxylation product of 2,4-D. ($K_I$ of skatole 1 x $10^{-5}$, $K_I$ of dichloroanisole (DCA), 2 x $10^{-5}$.)

To find members of the other class of antiauxin, the possible analogs of the ortho positions of phenoxyacetic acids have to be determined for the indoleacetic acids.

4-Chloroindoleacetic acid was actually found to be a stronger auxin than IAA on two accounts; it had a lower $K_S$ and a slightly higher $V_{ex}$ (extrapolated velocity). Blocking of just the 2 position, as in 2-methylindoleacetic acid, led to an auxin with only slightly lowered $V_{ex}$ but with a $K_S$ 100-fold larger than IAA itself. Additional substitution of halogen in the 5 position had practically no further effect on the auxin response. With 2,4-D and p-chlorophenoxyacetic acid as auxins, one type of IAA derivatives proved to be strong antiauxins. These were IAA's with both the 2 and 7 positions substituted. Most intensively studied of these compounds has been 2-methyl, 5,7-dibromoindoleacetic acid. With the auxins of the phenoxyacetic acid series, this compound had a $K_I$ of 2 x $10^{-6}$ M, which is in the same range as the diortho-substituted phenoxyacetic acids.

However, with IAA and 2-methylindoleacetic acid as auxins a completely different behavior resulted. Concentrations of 2-methyl, 5,7-dibromoindoleacetic acid, which were by themselves inactive in the auxin response and which strongly inhibited phenoxyacetic acid auxins, actually enhanced the response of IAA and 2-methylindoleacetic acid. This enhancement was evidenced by an apparent lowering of $K_S$ of the indole compounds. Thus, less IAA was needed to achieve a given elongation in the presence of this enhancer. At higher concentration of the enhancer, pretreatment for four hours in 2-methyl, 5,7-dibromoindoleacetic acid alone, followed by fourteen hours in IAA alone, resulted in more elongation than a treat-
The dual activity of compounds of this sort makes a quantitative correlation of auxin and antiauxin strengths difficult in the indoleacetic acid series but they do show a new facet to the auxin problem.

101. STUDIES ON THE PEROXIDASES OF ETIOLATED PEA SEEDLINGS AND THEIR INDUCTION BY INDOLEACETIC ACID

Investigators: Irwin B. Perlis, Arthur W. Galston
Support: American Cancer Society
General Funds

It has previously been shown that indoleacetic acid (IAA) oxidase and peroxidase activities of pea seedling roots are affected by exogenous IAA in a manner suggesting induced enzyme formation. In the present investigation, the peroxidases of the various parts of the pea seedling have been fractionated in an attempt to obtain more specific information about the nature of the induced enzyme.

There appear to be at least five electrophoretically separable peroxidase components in pea seedlings, with varying substrate specificities. Judged on the basis of ammonium sulfate (AS) fractionation data and chromatographic migration rates, these individual peroxidases are considered to be of varying as well as of low molecular weight. One fraction, chromatographically distinct from the other peroxidases, accounts for sixty-five to eighty percent of the total peroxidase activity of the terminal buds, eight to ten percent of the elongating stem below the bud, and twenty to twenty-five percent of the root whether young or mature. Although IAA fed to tissue enhances the total peroxidase activity of root, bud and stem tissue, it appears to act differently on this same fraction, depending on the organ from which it is derived. In the bud, IAA has no effect on this peroxidase fraction, while in the stem tissue a low IAA concentration induces a twofold increase in this material.

This chromatographically homogeneous peroxidase is remarkably stable. One preparation with fifty times the activity of crude brei per unit protein showed 0.05 percent hematin iron, 0.5 percent total iron, and a persistent yellow-green fluorescence under ultraviolet. These facts suggest a metallo-flavoprotein similar to several recently described. Peroxidase activity is inhibited by cyanide, azide and p-chloromercuribenzoate, implying the essentiality of both heavy metal and sulfhydryl groupings.

102. ON THE NATURE OF THE ENZYMATICALLY-CATALYZED OXIDATION PRODUCTS OF INDOLEACETIC ACID

Investigators: David T. Manning, A. W. Galston
Support: General Funds

In the presence of pea seedling indoleacetic acid (IAA) oxidase, IAA undergoes oxidative conversion into at least two and probably three products. Application of

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chromatographic techniques and color reagents indicated that some of the more obvious hypothetical products cannot account for the observed behavior of the authentic oxidation products. The compounds ruled out as major end products were 3-indolealdehyde, o-formamidoacetophenone (OFA), o-aminoacetophenone (OAA) and 4-hydroxyquinoline. It is also found that neither OFA nor 3-indolealdehyde participates as intermediates in the formation of the final oxidation products.

Color reactions with Ehrlich's reagent and with ferric chloride further indicate that the two most prominent oxidation products contain aromatic amino groups and phenolic hydroxyl groups. At the same time, the presence of a third compound containing an indole ring is suggested by a positive Hopkins-Cole reaction of a chloroform extract of the reaction mixture.

While the behavior of OFA with the various color reagents employed is not identical with the behavior of the actual oxidation products, there are close enough similarities to warrant the assumption that some of these products are close to OFA in structure. It is suggested that the neutral oxidation products of 

103. INDOLEACETIC ACID-OXIDASE AND PEROXIDASE ACTIVITY AND PEROXIDE GENESIS IN THE ROOT OF LENTIL

Investigators: P. E. Pilet, A. W. Galston
Support: Swiss Foundation Fellowship
General Funds

Indoleacetic acid (IAA) oxidase and peroxidase activity and peroxide genesis were studied in the root of lentil (Lens culinaris). It was found, as in the root of Pisum, that IAA oxidase activity, as measured by the amount of IAA destroyed per unit fresh weight or per unit protein, is low in the region of the meristem, and increases sharply in both directions (toward the older cells). Exactly the same gradient is shown by in vivo rates of peroxide genesis. Peroxidase activity, on the other hand, shows a reverse trend. 2,4-Dichlorophenol (DCP) increases both peroxide genesis and IAA oxidase activity in a parallel fashion. It is concluded that a) overall IAA destruction is limited by and controlled by the rate of peroxide genesis; b) as cells age, the production of peroxide increases, and hence then IAA oxidase activity increases; and c) the fact that DCP enhances both peroxide genesis and IAA oxidase activity indicates that some DCP-like factor in the tissue may regulate auxin destruction and auxin-controlled growth processes.

104. MEASUREMENT AND HISTOCHEMICAL LOCALIZATION OF INDOLEACETIC ACID INDUCED PEROXIDASE ACTIVITY IN THE ROOT TIP OF THE BROAD BEAN

Investigator: William A. Jensen
Support: U. S. Public Health Service Fellowship

When certain plant tissues are presented with indoleacetic acid (IAA), their peroxidase activity is greatly increased (Siegel and Galston, Biology 1954). The present work represents a histochemical localization and measurement of the IAA-
induced peroxidase activity.

The first three millimeters of the root of *Vicia faba* (broad bean) were used as they had previously been analyzed morphologically (Jensen, Exptl. Cell Res., in press). Roots were treated with IAA (10⁻⁶ to 10⁻⁸ M) for two hours and peroxidase activity measured and site of activity observed.

It was found that peroxidase activity is normally associated with the root cap, proepidermis, provascular, protophloem and protoxylem tissues. After IAA treatment the distribution was the same but activity in the provascular, protophloem, and protoxylem was much increased. The increased peroxidase activity as a result of IAA administration can therefore be accounted for largely by enhanced peroxidase in the provascular tissues.

If eugenol and H₂O₂ are presented to the sections those cells showing peroxidase activity will form an intermediate in lignin biosynthesis. The following biochemical sequence of events would appear to explain the frequently observed auxin-induced lignification of tissues. In the presence of increased IAA the cell forms a system (IAA oxidase) which destroys IAA; part of this system is a peroxidase that is also active in the formation of lignin. Hence, if a peroxide and eugenol type compound are present, lignin may be formed.

105. THE CYTOCHEMICAL LOCALIZATION OF ACID PHOSPHATASE IN ROOT TIP CELLS

Investigator: William A. Jensen
Support: U. S. Public Health Service Fellowship

The major difficulty in the cytochemical intracellular localization of enzymes is the destruction of either the enzyme or the site by the chemicals used in the initial fixation. The use of frozen-dried tissue and localization procedures that are completed before the tissue is exposed to organic solvents circumvents these difficulties. Until recently frozen-dried plant material was not available. Using the newly devised method for obtaining frozen-dried material (Jensen, Exptl. Cell Res., 7, 572-574) the intracellular distribution of acid phosphatase has been studied in the roots of onion, broad bean, and pea.

The sites of activity are spherical (0.6 µ) or rod-shaped (0.6 x 1.2-2.0 µ) cytoplasmic particles. On the basis of experiments with ribonuclease and alcohol it is concluded that the particles contain small amounts of ribonucleic acid and large amounts of lipids and are therefore probably mitochondria. This is in opposition to workers who used alcohol fixatives. They claim that the sites of activity are the nuclei, chromosomes, cytoplasm and cell walls. Alcohol fixation does not destroy the enzyme but the alcohol does dissolve the mitochondria, thus liberating the enzyme and allowing it to be absorbed on any surface remaining.

106. THE NUCLEIC ACID CONTENT IN CELLS IN THE ROOT TIP

Investigator: William A. Jensen
Support: U. S. Public Health Service Fellowship
The first few millimeters of the root contain the early stages of cell development. These stages are arranged spatially within the root so that the region of maximum cell division is at one level while the following stages of cell development occur at levels above in a predictable pattern. It is therefore possible to obtain data relative to cell development by comparing the various levels on a morphological, physiological, and biochemical basis.

For the present work the first three millimeters of the root tip of Vicia faba (broad bean) were analyzed first for the number and dimensions of the various cells they contain. The amount of ribonucleic acid (RNA) and desoxyribonucleic acid (DNA) was measured in two hundred micron sections.

The results indicate that DNA is constant per cell for the length of root studied except in the region of the general meristem where it is about one-half. The RNA, also low in the region of the general meristem, rises sharply until the cells begin elongation when it remains constant per cell. The rise in RNA occurs while the cells are increasing in diameter and undergoing primary differentiation. Protein per cell follows the same pattern as the RNA except that it decreases as the cells elongate.

107. THE INACTIVATION OF ENZYMES BY COENZYME A AND RELATED COMPOUNDS

Investigators: Helen Sharpensteen, A. W. Galston
Support: National Science Foundation General Funds

The peroxidase of pea roots, which is a compound of the indoleacetic acid oxidase complex, has been shown to be inactivated by coenzyme A (CoA) in concentrations as low as $1 \times 10^{-5}$ M. Other sulfhydryl compounds were tested for inactivation properties but none was found to be as effective as CoA. Of the compounds tested (cysteine, glutathione, and dimercaptopropanol) only glutathione showed inactivating properties, and these were at concentrations 100 to 1,000-fold higher than CoA.

The fact that enzyme prepared by grinding tissue in the presence of p-chloromercuribenzoic acid is stabilized against the spontaneous decrease in activity as well as against the CoA-mediated inactivation is taken to indicate that the group responsible for inactivation is the sulfhydryl group.

Crystalline beef liver catalase was found to be inactivated by CoA, glutathione, and cysteine. Again, CoA was found to be much more effective than cysteine or glutathione.

Spectrophotometric data indicate that there is a combination of CoA with catalase, the Soret band being somewhat broader and lower in the presence of CoA. The same pattern was noted for glutathione.

On the basis of this evidence it is proposed that the spontaneous decrease in peroxidase activity of pea breis is due to inactivation by endogenous sulfhydryl compounds.
The problem of the nature of the receptor pigment in phototropism was once considered solved by the coincidence between the action spectrum for phototropism and the absorption spectrum of \( \beta \)-carotene. Galston and Baker have shown, however, that the same argument would also indicate riboflavin as the receptor pigment and, moreover, that the precision of the determination of the action spectrum does not allow a choice between absorption spectra so similar as those of carotene and riboflavin.

A new approach to the problem has been developed by measuring the phototropic sensitivity of plants which are either genetically free of carotene or nutritionally controlled so as to have a very low carotene content. In a comparison of the reactions of coleoptiles of albino (carotene-free) and green seedlings of barley, it was found that a) given a massive dose of light, there is a larger proportion of reactive seedlings among the albino than among the green plants, and b) for both groups the threshold stimulus is the same, about 120 ergs/cm.\(^2\) of blue light.

In another group of experiments the fungus Phycomyces Blakesleeanus was cultivated in media with different concentrations of diphenylamine, a substance known to inhibit carotene synthesis. The growth rate of the mycelium was found not to be significantly affected by this compound and the sporangiophores of plants grown in such media while greatly reduced in carotene content do not lose the capacity of bending towards the light.

These results are believed to rule out carotene as the receptor pigment for phototropism, both in grass coleoptiles and in fungal hyphae.

**109. INTERACTION OF LIGHT AND AUXIN IN FLOWERING**

It is known that the application of auxin to the leaf of a short-day plant such as Xanthium inhibits the appearance in the leaf of the flowering stimulus or flowering hormone which would otherwise appear as a result of dark reactions which go on during the inductive night. The present work concerns the mechanism of auxin inhibition of the dark processes of photoperiodic induction. It has been found that applied auxin given at the beginning of an inductive dark period does not change the critical night length, that is, the moment of appearance of flowering stimulus in the leaf, but affects the rate of production of flowering stimulus by the leaf. The effectiveness of auxin in inhibiting induction increases as it is applied between zero and three hours after the beginning of an inductive dark period. Between three and eight hours auxin is maximumly effective in bringing about subsequent inhibition of flowering. As auxin is given later than eight and one-half hours in an inductive dark period its effectiveness decreases but it still inhibits effectively even if given at the end of the inductive dark period and when the plants are returned to long day.
Inhibition of induction by a light flash given in the middle of the dark period and inhibition of induction by added auxin are additive in their effects. The hypothesis which appears to be best supported by this work is that auxin brings about the destruction of the flowering hormone. If flowering hormone is already present in the leaf a net destruction ensues. If auxin is applied during the synthesis of flowering hormone by the leaf during an inductive dark period then the rate of net synthesis of flowering hormone is decreased.

Publication:


110. EFFECTS OF METABOLITE ANTAGONISTS ON FLOWER INITIATION IN THE COCKLEBUR

Investigator: L. G. Labouriau
Support: General Funds
National Research Council of Brazil
Rio de Janeiro Botanical Gardens

Metabolite antagonists were administered to cocklebur plants during the inductive dark period, and the effects of these substances on subsequent rate of floral development followed. Administration of the materials was made by solution through a stem flap below the leaf.

The amino acid analogs ethionine, thienylalanine, o-fluoro-phenylalanine and canavanine were tried. Of these only thienylalanine was effective (nineteen percent inhibition) at $10^{-3}$ M.

The purine analogs benzimidazole, isoguanine, and mercaptopurine appear to be practically without effect. Small inhibitory effects of 2,6-diamino purine, 8-azaguanine, and aminopterin were observed.

The pyrimidine analogs, 5-bromo-uracil, 2,6-diamino-pyrimidine and isocytosine have only small effects; 2-thiouracil has a small inhibitory effect but 2-thiocytosine has a considerable inhibitory effect at $10^{-3}$ M. This effect decreases linearly with concentration and, between $10^{-5}$ M and $10^{-4}$ M of the substance is significantly stimulatory. Both the inhibitory and the stimulatory effects are enhanced by uracil at $10^{-3}$ M. There thus appears to be no antagonism between these two substances.

Nucleosides at $10^{-3}$ M did not affect flower initiation, with the exception of guanosine. This produces a small stimulatory effect. A mixture of nucleotides at the same concentration does not affect flower initiation.

111. THE FLOWERING OF PINE TREES

Investigator: James Bonner
Support: General Funds
Programs of forest tree breeding as well as field management of economic forest species would be facilitated by knowledge of how to hasten and control flower production of such forms.

Knowledge of the factors that control the flowering of other plant species is now available. The present study of forest tree flowering will draw on the information already available from other plants. Determination of optimal temperature and day length conditions for the growth of pine trees has been started as a first step in a study of the factors. Single plant collections of seeds have been obtained and germinated. These plants are now growing under a wide range of temperature conditions in the Earhart Plant Research Laboratory. Seeds of two Loblolly pine trees, six Jeffrey pine trees, and five Digger pine trees, all known to have produced seed at an earlier than normal age, were supplied by the Southeast Forest Experiment Station and the California Forest and Range Experiment Station of the U.S. Forest Service.

112. PHYSIOLOGICAL STUDIES OF EROSION CONTROL PLANTS

Investigators: Henry Hellmers, J. M. Kelleher
Support: U.S. Forest Service
General Funds

Following the completion of soil nutrition studies in the Orlando Greenhouse and the first series of climatic adaptability tests for exotic species in Earhart Plant Research Laboratory field tests were started. These tests were designed to apply the laboratory findings in an attempt to increase vegetation density on critically eroding slopes in the San Gabriel Mountains. The exotic species planted were those that had grown well under the laboratory temperature conditions that corresponded to those of the local mountains. Of the twenty-two species tested in the Earhart Laboratory only four were considered suitable for use in the field tests. These were Baccharis pilularis ssp. typica, Colycotome villosa, Lotus bertholotti, and Spartium junceum. All field planting sites were fertilized with nitrogen. The soil nutrition studies have shown that the local mountain soils are deficient in nitrogen to the extent that the deficiency is a limiting factor for the growth of plants, even in exceedingly dry years.

Publications:


113. VERNALIZATION BY SEED EXTRACTS

Investigator: H. R. Highkin
Support: National Science Foundation
Frasch Foundation
The flowering of certain plants requires or is hastened by prior cold treatment of the germinating seed. Such cold treatment which results in effects upon subsequent flowering behavior of the plant is known as vernalization. It has now been found that suitable extracts of cold-treated seeds possess the power of inducing or hastening flowering in non-cold-treated plants. The active extract is made by allowing seeds to germinate in aerated water under aseptic conditions. The test seeds are soaked briefly in and allowed to imbibe the extract or diffusate, and then planted and allowed to develop in the greenhouse. Diffusates of pea, spring rye, and winter rye all induce earlier flowering in treated pea seeds (late varieties) and spring rye, both of which are hastened in their flowering by cold treatment. Such diffusates also induce flowering in non-cold-treated winter rye, a form which otherwise requires cold treatment in order to flower.

Diffusate of winter rye seed contains the flowering activity only if prepared from cold-treated seed. Diffusates of pea and of spring rye, on the other hand, contain flowering activity whether the seeds have been cold treated or not. Evidently cold treatment causes the production in winter rye of a substance which causes flowering and this substance can be extracted by the technique used. Seeds which have no absolute requirement for cold treatment appear to contain the flowering substance even in the unvernalized state.

114. THE ABILITY OF VARIOUS NUCLEOSIDES TO INDUCE VERNALIZATION IN PLANTS

Investigators: Walter S. McNutt, Jr., H. R. Highkin
Support: U. S. Army, Office of Quartermaster General
National Science Foundation
General Funds

It has been known that seeds of certain plants when soaked in water release into the medium substances capable of vernalizing other seeds. The active principle of such extracts has been purified and identified. The activity remains in the supernatant upon precipitation of extraneous material with ethanol and acetone. It is adsorbed on charcoal from which it may be eluted with NH₄OH or pyridine solutions. The purified material was subjected to large-scale paper chromatography. Activity was found associated with areas of the chromatogram corresponding in position to guanosine and adenosine. Uridine, cytosine, adenosine, and guanosine are each active at concentrations of 2 x 10⁻⁴ molar. Guanosine is however the most active. The principle of such extracts which possesses the vernalization activity appears therefore to be a nucleoside or mixture of nucleosides.
Much of the time during the last year was spent in preparing a book on "The Control of Plant Growth," in which a description is given of the Earhart Plant Research Laboratory, its operation, and the results obtained during its first six years of operation. To this end a large number of drawings and photographs were prepared, in which especially Mrs. Rachel Morgan Behnke, Mrs. Helene Fox Metzenberg and Mr. James Cunningham have cooperated. This book is being published by the Chronica Botanica Company.

The flowering behavior of strawberries is now being studied for several varieties with an extreme climatic range. Most of the varieties are short-day plants at high temperatures which will also form inflorescences in sixteen-hour photoperiods at low temperatures (below $15^\circ$ C.). A variety coming from Bolivia differs in that it is a day-neutral and flowers predominantly on a twelve-hour photoperiod. It is able to form runners at a lower temperature than any other strawberry plant. This variety will be used in further studies of the relation between flowering and runner formation.

The development of the aromatic taste substances of the strawberry fruit have been investigated under different temperature and light conditions. It turns out that only when strawberry plants are subjected to light at low temperatures do these aromatic substances develop. A few hours at $10^\circ$ C. is sufficient, but only at high light intensities. At one thousand foot-candles and with two hours of light at $10^\circ$ C., in addition to eight hours' light in a greenhouse, maximum flavor is produced; where-
as at five hundred foot-candles more than four hours at 10° C. is required. The plants have to be subjected for several weeks to these conditions before they respond with better flavor.

117. CARBON FRACTIONATION BY HIGHER PLANTS

Investigators:  S. Epstein, F. W. Went
Support:  General Funds

Since it had been found by the geochemistry group at Caltech that in tree trunks there is a difference in the C\textsuperscript{13} to C\textsuperscript{12} ratio for wood laid down at different times of the year, it was decided that the effect of external conditions on the C\textsuperscript{13} to C\textsuperscript{12} ratio in different plants would be investigated. To this end tomato plants were cultivated in the Earhart Plant Research Laboratory under strictly comparable conditions, and the new growth produced over a two- to three-week period was analyzed by the geochemistry group. It was found that although there is some effect of temperature, the C\textsuperscript{13} to C\textsuperscript{12} ratio is somewhat increased by drought conditions. Different species of plants produce organic materials of different C\textsuperscript{13} content.

118. FLOWERING RESPONSES OF ORCHIDS

Investigators:  R. Casamajor, F. W. Went
Support:  General Funds

After several years of work it has now been established that Cymbidium lowianum can be induced to develop inflorescences at any time of the year if they are subjected to 1) long days, 2) day temperatures of 20° C. and 3) night temperatures of approximately 10° C. to 14° C. These requirements are so strict that a 17° C. or 23° C. day temperature is practically ineffective. Not only the initiation of inflorescences but the opening of flowers occurs best at these lower temperatures and longer photoperiods. This temperature response explains the excellent results obtained with Cymbidium culture in the Santa Barbara area.

For Cattleya trianae flowering could be induced at almost any temperature after the plant had been subjected to short-day treatment. One month of such treatment was insufficient but after two or three months of short-day treatment all new pseudo-bulbs developed flowers. In this way it is possible to cause flowering at an exactly predetermined day provided the plants have been kept on long-day treatment previous to the short-day exposure.

The flowering of Cypripedium is much less controlled by change in the environment and these plants will flower over a wide range of temperatures and photoperiods; best results were obtained in an eight-hour day and with day temperatures of 17° C. and night temperatures of 14° C.

Phalaenopsis is somewhat stimulated in flowering by short days, but inflorescences may be developed in long days as well. Best night temperature is 20° C.

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119. FLOWERING OF PEACHES

Investigator: F. W. Went
Support: General Funds

Dr. Bruce Rogers found that flowering of peaches required two successive cold periods with an intervening warm period. The length and optimal temperature for cold treatment is now under investigation. It has been found that one month at 4° C. to 10° C. is insufficient to cause the opening of flower buds, but that a two-month period at 4° C. is effective. Two months at 10° C. does not cause the opening of flowers.

120. PEA GROWTH

Investigators: F. W. Went, Rachel Morgan Behnke
Support: General Funds

In connection with the use of peas by Thornthwaite as a climatic index, the number of nodes formed per day is directly proportional to phototemperature, nyctotherm, temperature, photoperiod and light intensity. Thus these peas can be used as an integrating light and temperature meter. However, their growth in length has a very different temperature dependence in that between 14° C. and 26° C. there is no effect of night temperature, and above that temperature growth is inhibited. There is a negative correlation between temperature and seed production. This was investigated particularly with the Alaska variety, but others like Vinco and Unica behave the same way. In the latter varieties all interactions between day length, temperature and light intensity were investigated. Since the response of these peas differs so much from the response of tomatoes, potato and many other plants it does not seem permissible to use the development of the pea plant as an index for the growing of a number of others.

121. PETROLEUM FORMATION

Investigator: F. W. Went
Support: General Funds

In the course of observations on smog occurrence in other parts of the world, it was found that a) most large cities in the world have an acute smog problem which can be assessed by the damage done to vegetation, and b) a condition which is indistinguishable from smog except in its severity occurs everywhere in the world where there is active vegetation. This so-called natural smog or heat haze is believed to be due to condensation of volatile plant constituents, probably hydrocarbons since these have sufficient vapor pressure to evaporate into the atmosphere. It is postulated that these volatilized and condensed hydrocarbons then return to the earth in the form of small particles of the size of clay particles which settle in certain areas with a slow sedimentation rate.
122. QUANTITATIVE INHERITANCE IN PEAS

Investigators: H. R. Highkin, Georgia Wagner
Support: National Science Foundation
Frasch Foundation

It has already been shown that pea varieties differ markedly in heat resistance. It has been found that in crosses of heat resistant plants times heat susceptible plants, the temperature under which the pollen parents were grown has a marked effect on the reaction of the F1's when grown under test conditions for heat resistance (26° C., sixteen hours of artificial light). This effect is manifest not only in terms of heat resistance but in marked differences in growth rate, leaf size, serration of leaves and color of leaves. Crosses have been made between resistant plants times resistant plants, susceptible plants times susceptible plants, using as pollen parents plants grown at different temperatures. It is hoped to determine whether this pollen effect is a general one or unique to the cross R x S and the reciprocal crosses.

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

Investigators: H. R. Highkin, E. G. Anderson
Support: National Science Foundation
Atomic Energy Commission
General Funds

The expression of the gene for the Maize character necrotic, which appears only after the formation of the third leaf, has been studied under a spectrum of environmental conditions. The F2 seed was supplied by Prof. E. G. Anderson, who has found that under natural conditions normal and necrotic plants segregate in a ratio of three normal to one necrotic. He has also found that when necrotic is crossed to a normal containing genes for pale or albino seedlings, none of the plants show necrosis or at most very little necrosis.

Experiments in the Earhart Laboratory have shown that under conditions of high temperature and high light intensity, i.e. 26° C. for sixteen hours at one thousand foot-candles of light, the population shows a one to three segregation of normal to necrotic plants as opposed to the expected three to one ratio. At five hundred foot-candles the ratio is one normal to one necrotic, while at two hundred and fifty foot-candles the ratio was the expected three to one.

If the plants are germinated at 26° C. in complete darkness, kept for a period at least long enough for appearance of the third leaf and then put in light of one thousand foot-candles the necrotic character fails to appear.

124. EFFECTS OF AUXIN ON VERNALIZATION

Investigators: H. R. Highkin, Georgia Wagner
Support: National Science Foundation
Frasch Foundation
This work is part of a general study of the principles of vernalization—the hastening of flowering which results from cold treatment of seeds or seedlings of some plants.

Two varieties of peas (Zelka and Unica) have been used. These are quantitatively vernalizable, i.e. cold treatment hastens flowering although the plants ultimately flower even without a cold treatment. It has been found that seeds soaked in solutions containing low concentrations of auxin (0.01 parts per million naphthaleneacetic acid) form flowers earlier than controls soaked in water. Auxin treatment thus appears to substitute for cold treatment. Increasing the concentration results in a decrease in effect until at ten parts per million there is an inhibition in flower formation as compared to the control. The effect of auxin concentration is, however, complicated by the fact that the effects on flowering can be realized only under conditions of low light intensities. Increasing the light intensity results in a loss of both the enhancing and inhibitory effect of the auxin on flowering.

125. THE INHERITANCE OF VERNALIZATION IN PEAS

Investigator: H. R. Highkin
Support: National Science Foundation
Frasch Foundation

The inheritance of vernalization has been reported in spring and winter rye as being due to a single gene difference. Cooper has found, however, that analysis of the data does not support this view either for the spring and winter rye, or for any other plants in which the character has been studied.

Since all late flowering varieties of peas are more or less affected by a cold treatment, indicating a quantitative response, crosses must be made between early varieties, which are not affected by cold, and the later vernalizable varieties. These studies are further complicated by the fact that there is evidence that the early plants flower at the earliest possible node (ninth) since practically all the vegetative nodes are already formed in the mature seed, so that the early varieties may very well carry the gene for vernalization although it would never be expressed.

Crosses between an early variety (L5; ninth node) and a late one (Zelka; eighteenth to thirty-sixth node) indicate that the inheritance of vernalization is indeed more complex than a single factor difference.

One of the segregants was found to both late and non-vernalizable, indicating that the early variety may not carry the factor for vernalizability. Crosses have been made between this plant and Zelka to determine the mode of inheritance of vernalization with the complication of the early and late character removed.

126. ROOT GROWTH AND AUXIN TREATMENTS ON SEEDS KEPT AT LOW TEMPERATURE

Investigators: P. E. Pilet, H. R. Highkin
Support: Swiss Foundation Fellowship
National Science Foundation
Frasch Foundation
General Funds
Beta-indolyl-acetic acid (IAA) was applied to seeds of Pisum sativum (var. Zelka) during imbibition. The seeds were then germinated at 20° C. or 4° C. in continuous darkness or continuous light. The effect of hormonization, i.e. IAA applied to the seeds, on root growth was studied. Without IAA (control), root growth is greater at 20° C. than at 4° C., and greater in continuous darkness than in continuous light at both temperatures used. In continuous darkness, at 20° C., the high concentrations of IAA (1 x 10^-4, 10^-5 and 1 x 10^-6 M) produce a strong inhibition of root growth, which decreases as the IAA concentrations decrease. At a concentration of 10^-7 M, stimulation of root growth occurs. However, after four days, the action of hormonization decreases and is completely nullified after fourteen days. Concentration of 10^-4 M IAA produces a different effect on root growth in the light or in the dark: great inhibition in the dark which decreases with time and a lesser inhibition in the light during the early period of development. In the light however there is a stimulation with time.

Approximately similar results were obtained for seeds kept at 4° C. in continuous darkness or light.

This study was carried out in conjunction with experiments on the effect of auxin on vernalized seeds to determine whether or not the rate of early development of the root could be correlated with the effect of auxin and cold treatment on flowering.

127. CONTROL OF GROWTH OF THE LENTIL BY TEMPERATURE AND LIGHT

Investigator: P. E. Pilet
Support: Swiss Foundation Fellowship
General Funds

The percentage of seeds of the lentil (Lens culinaris) germinating at different temperatures in continuous darkness, in continuous light, and in alternating sixteen-hour dark and eight-hour light periods was determined. The optimal temperature for germination is 20° C. in the dark and 24.5° C. in the light. The length of roots was studied under the same conditions. Results indicate that initial growth rate is small; there is a subsequent acceleration and finally a marked decrease. Light is strongly inhibitory to growth. In both dark and light, root growth is inhibited at 27° C. The optimal temperature for growth in darkness is 23.5° C. and in light, 24.5° C. In alternating sixteen-hour dark and eight-hour light periods, after fifteen days, the optimal temperature is 17° C. (dark) and 18° C. (light). The number of lateral roots formed increases with temperature and the optimal temperature for lateral root length shifts with time. In alternating sixteen-hour dark and eight-hour light periods, the maximum number of lateral roots is obtained at a dark temperature of 27° C. combined with a light temperature of 24.5° C. The maximum length of lateral roots is obtained at a dark temperature of 27° C. and a light temperature of 21.5° C.

The effect of indoleacetic acid (IAA) on root growth at different temperatures in continuous darkness was studied. At 1 x 10^-6 M, the growth of old roots (initially 30 mm. long) was slightly stimulated at low temperatures, while with an increase of temperature an inhibition developed. The growth of young roots (3 mm. initially) was stimulated over the temperature range tested with the greatest stimulation occurring at the lowest temperatures. At the highest concentration tested (1 x 10^-4 M), growth of old roots was inhibited and the degree of inhibition increased.
markedly as the temperature increased. Results are discussed in relation to the preceding papers of the author. The effect of IAA on lateral root formation at different temperatures increased with the IAA concentration.

The optimal temperature for stem growth is the same in continuous darkness or continuous light (26° C.). In alternating dark and light periods, the optimal temperature regime, after fifteen days' growth, was 17° C. in the dark and 23° C. in the light.

Many experiments were performed to establish the relationships between thermo- and photoperiods applied to the root or stem and root growth. Optimal temperature for root growth was determined for a variety of photoperiods and observations showed that optimal temperature decreases with time.

Additional experiments were performed to determine the relations between growth of main roots and lateral roots, using the "fog box." Camera studies were also made to analyze the growth rate of roots kept in varying photo- and thermo-periods.

128. PHYSIOLOGICAL GENETICS OF THE CONTROL OF FLOWERING PEAS

Investigator: H. N. Barber
Support: Rockefeller Foundation
University of Tasmania
General Funds

Work in the Earhart laboratory has shown:
1) That genetically early varieties of peas are non-photoperiodic and non-vernalizable.
2) The late varieties are both vernalizable and quantitative long-day plants. The two processes interact competitively; prior vernalization reducing the size of the photoperiodic response. This shows a new pattern of behavior, contrasting with older work on other plants in which prior vernalization usually enhances photoperiodic responses. Both the vernalization and photoperiodic responses are reversible, vernalization by high temperatures and long-day response by a return to short-day conditions.
3) Photoperiodic and vernalization responses are dominant in F1 between late and early varieties. Growth under constant environments has enabled discontinuous segregation in F2 to be obtained for the first time. Such segregations are obtained by growing progenies under short-day conditions. They have enabled the genetic differences between late and early varieties to be analyzed into two genetic components:

(a) A major gene controlling the development of photoperiodic and vernalization reactions. Under short-day conditions (twelve hours) and without prior vernalization possession of this gene, Sn, causes flowering ten to twelve nodes later than in plants without this gene (ssn). Under continuous light the difference is only about five nodes.
(b) A polygenic component which apparently does not cause a photoperiodic and vernalization reaction, but works by way of some other physiological process. Under short-day conditions the polygenes cause a change of ± 8 nodes in Sn plants, whilst in sn plants they cause a change of ± 3 nodes.
It has not been possible by genetic means to separate the photoperiodic reaction from the vernalization reaction. The same dominant gene controls the development of both reactions, prior vernalization of an F1 or F2 simply causing change in mean flowering node of late segregants. There is no suggestion that prior vernalization can split up the F2 distribution into different classes as would be expected if the vernalization and photoperiodic reactions were under the control of two dominant genes situated at two loci without tight linkage.

4) The experiments have failed to confirm Rasmussen's earlier suggestion that segregation at the Le locus (tall/dwarf) has any effect on flowering behavior. Segregation at the R locus is similarly without effect.

5) Further grafting and cutting work has shown:
   (a) That a vernalized rootstock causes less delay in the flowering of a genetically early scion than an unvernalized stock. This appears to be the first time the vernalization stimulus has been transmitted other than from one apex (vernalized) to another apex (unvernalized).
   (b) That the photoperiodic effect is similarly graft transmissible.
   (c) That cuttings from early varieties taken in the first internode are not altered in flowering behavior whilst cuttings from late varieties flower at a lower node if removed before eight days after germination. Apical cuttings in late varieties (i.e. with a variable number of lower nodes removed) are not changed in flowering behavior if removed after eight days.

129. GROWTH AND DEVELOPMENT OF BROAD BEANS

Investigator: Lloyd Evans
Support: Commonwealth Fund Fellowship

The developmental requirements of broad beans (Vicia faba) have been relatively neglected by previous investigators. It has been found that photoperiod and day and night temperatures affect the rate of growth of broad beans much as they affect the growth of peas.

Some early flowering spring bean varieties show initiation of flowers at the seventh to eighth node regardless of temperature and photoperiodic conditions, but in the later flowering spring and winter bean varieties the node of first flowering is greatly influenced by these conditions. The temperature at which the seeds are set in the pod, the temperature of germination, and the temperature of growth all interact in a quantitative manner to give flower initiation over a range from the sixth to the sixty-fifth node. Temperatures lower than 17° C. may be regarded as vernalizing temperatures for Vicia faba (being progressively more effective in hastening flower initiation the lower they are) and are effective at any time during the life cycle of the plant; temperatures above 17° C. are progressively devernalizing. Most broad bean varieties are quantitative long-day plants, and under fixed temperature conditions will flower at a progressively earlier node the longer the photoperiod, flowering soonest in continuous light. But although flower initiation is possible in short-day conditions floral expansion is not, requiring daylengths greater than twelve hours.

Since the node of first flower initiation responds quantitatively to both vernalization and photoperiodic treatments it has been used as an index of the efficiency of various substances in hastening flowering. Of the chemicals used to date for seed
inhibition the nucleosides have proved to be the most active, lowering the node of first flower initiation as much as do water extracts of seeds of early flowering bean varieties, although not as much as full vernalization at 40°C. would. These chemicals are now being used on plants, such as perennial rye grass, with an absolute requirement for cold temperatures during their development.

130. COFFEE RESEARCH

Investigators: M. G. Mes, H. Metzenberg
Support: IBEC Research Institute

Flower buds of coffee develop up to a size of some six millimeters over a period of a few months. With many coffee varieties the buds then appear to go into a dormant state lasting for many weeks depending on the climate. With renewed growth the bud length may increase to about twenty-three millimeters (open flower) within eight to eleven days. This was found to be accompanied by the formation of the microspore tetrads and a rapid increase in water content. Strong tensions were found to exist in the water columns of the trees. An anatomical study revealed the typical symptoms of plants suffering from water stress.

By supplying resting buds with water in various ways, but particularly by submerging the tops of the trees in water for one to two hours, the "dormancy" could be broken at will.

It is therefore maintained that delayed flowering of coffee trees is due to water stress which is normally reduced by external water application to the tops of the trees through rain.

In the absence of rain it should be possible, once the buds have developed to a particular size, to bring coffee trees into flower on a set date by the use of overhead irrigation.

131. FLORAL INITIATION IN NIGHT-FLOWERING JASMINE

Investigator: Roy Sachs
Support: Lucy Mason Clark Fellowship
Arthur McCallum Summer Fellowship

The requirement for floral initiation in the night-flowering jasmine (Cestrum nocturnum) is satisfied if plants receive long days followed by short days. Continuous short or long days or short preceding long days are ineffective. A minimum of five to seven long days followed by two short days is required for the production of flowers. The sequence of reactions necessary for floral initiation in Cestrum is as follows:

\[
\text{long days} \quad \text{short days} \quad \frac{L}{S} \quad (\text{floral stimulus})
\]

(a) "L" is a heat-labile substance produced in leaves as a result of long-day induction and required for the effectiveness of short-day induction. It is not trans-
located from the leaves.

(b) "S" is a heat-labile substance, possibly identical with the floral stimulus causing floral initiation, formed as a result of short-day induction. It is formed only in leaves which have previously received long-day induction and is translocated to the growing points.

The production of "L" in short-day plants is controlled by daylength independent reactions, and the production of "S" is controlled by daylength independent reactions in long-day plants.

For Cestrum the critical day and night lengths for long- and short-day induction overlap in the 11.5 to 12.5 hour photoperiod range; the low efficiency of the two processes in this range precludes the possibility of an intermediate daylength satisfying both requirements. That is, continuous inductions with a twelve-hour photoperiod is completely ineffective in causing floral initiation. There are plants, however, which flower only when the daylength is within an intermediate range; on either side of this range no flowering occurs. It is possible that these intermediate day plants are related to Cestrum in that they may possess two daylength requirements; however the critical photo- and nyctoperiods for the two reactions overlap sufficiently to permit simultaneous satisfaction of both requirements in an intermediate daylength.

132. GERMINATION AND GROWTH OF CACTI

Investigator: Elie Eichenberger
Support: Lucy Mason Clark Fellowship
          Rockefeller Foundation
          General Funds

The seeds of several cactus species (Cereus Dayami, C. horridus, C. peruvianus, C. Huntingtonianus, Trichocereus telegnosus, Lemairocereus montanus) require continuous light and high temperatures (optimum 26°C. to 30°C.) for germination. Although no germination occurs in the dark the later germination in the light is not affected by a dark pretreatment. Germination is faster in the light when the seeds are laid out on charcoal, which suggests the presence of a water-soluble inhibitor. The lack of germination in the dark cannot be attributed to the presence of a water-soluble inhibitor. A longer pretreatment of the wet seeds at 10°C. reduces germination at high temperatures.

The growth of a large population of one-half-year-old C. horridus plants has been followed for about one year. Although the size of the plants was extremely variable the growth at a day temperature of 30°C. and a night temperature of 17°C. with a sixteen-hour photoperiod is exponential, the rate being approximately the same for all the plants. Under this condition doubling in height takes about four months. The difference in height of the plants has therefore to be attributed to differences in development in the first months after germination.

When the photoperiod of plants grown at 30°C. day and 17°C. night temperatures is reduced from sixteen to eight hours the growth in length decreases considerably and the plants begin to grow in diameter, acquiring in this way a more succulent appearance. On the other hand an increase of the night temperature to 26°C. on a sixteen-hour photoperiod results in a loss in turgescence. A decrease of the night
temperature to 10° C. results in a slight decrease of the growth rate compared with 17° C. (on a sixteen-hour photoperiod) but elongation stays exponential.

133. ON DESERT ECOLOGY

Investigator: Elie Eichenberger
Support: Lucy Mason Clark Fellowship
Rockefeller Foundation
General Funds

As the desert annuals can tolerate considerable water stress and escape elimination under extremely adverse conditions, an understanding of the dynamics of the desert annual vegetation rests on investigation of germination. Seed populations which have been composed from seeds collected in the field have been used to supplement the work done with soil samples. The experiments emphasize the importance of temperature, rain leaching, absorptive properties of the soils, not only on the quantitative aspect of the seedling population but also on the relative distribution of species. Through the presence of water soluble inhibitors in the seeds and special temperature requirements germination is favored under conditions which give a maximal chance for survival of the germinated seedlings.

134. ON DESERT ECOLOGY

Investigator: Jorge Morello
Support: John Simon Guggenheim Memorial Foundation Fellowship
General Funds

Seeds collected at different places in the Sahara and in western Argentine were used for a study of their germination and growth under controlled conditions.

Seeds of annuals from the Sahara germinate very well at intermediate temperatures (17° C.) and poorly at high or lower temperatures. On the other hand the shrubs require higher temperatures for germination (between 26° C. and 30° C.). It has been demonstrated that, with rains of eleven millimeters or less, not a single seedling of annuals from the Sahara will appear, but with less than eleven millimeters (between seven and eleven) some shrubs can germinate. The germination of grasses is delayed or completely stopped by the presence of the husks.

When germination has been great (due to a heavy rain), and later conditions are poor due to lack of further rain, each plant produces small leaves and few flowers.

The same type of growth can be obtained with two or three slight rains, each of an amount near critical for germination; and also with an initial heavy rain and then low temperatures.

In all the cases if depauperate plants are growing in good conditions each germination is reflected in a flowering plant some weeks later.
135. WATER RELATIONS IN PLANTS

Investigator: Elie Eichenberger
Support: Rockefeller Foundation

In the continuation of the work on water relations in plants an attempt was made to distinguish between the physical factors which have an immediate effect on transpiration and biological ones which result from the preconditioning of the plants. When tomato, bean or cucumber plants are grown under uniform conditions transpiration under optimal water supply is strictly proportional to the leaf area. Removal of leaves result in a decrease in transpiration proportional to the reduction in leaf area. The age of the plants affects the transpiration per leaf area not at all or only very slightly. When water stress is allowed to develop, growth is first affected and transpiration decreases only later.

For plants grown under one set of conditions transpiration and water uptake are proportional to the vapor pressure deficit of the atmosphere. The use of automatically recording potometers shows that water uptake follows transpiration very closely and that a removal of leaves results in an immediate reduction of water uptake.

A two to three day treatment of cucumber plants which have been grown at middle temperatures (23° C. day and 17° C. night temperature) in high day temperatures combined with a sixteen-hour photoperiod results in a decrease of transpiration under standard conditions (20° C. and sixty percent relative humidity) in the order of forty to sixty percent. The reduction of transpiration is the more marked the lower the night temperature is after the high day temperature. This phenomenon, which may be of great importance in the day by day changes in transpiration of plants under field conditions, has been further investigated. The results suggest that, besides the immediate conditions under which a plant stays, its history may play an essential role for its water use.

A method has been worked out for measurement of transpiration by measuring the humidity of the in- and out-going air in a plant chamber. The measurement agrees well with gravimetric transpiration determinations and water uptake data with potometers and could be adapted for field studies on transpiration.

136. PERIODICITY OF EXUDATION

Investigator: O. M. van Andel
Support: American Association of University Women Fellowship
General Funds

The rate at which decapitated plants exude liquid from the cut stem has been proved to change periodically, showing a maximum before noon and a minimum around midnight. It was investigated whether this periodicity could be influenced by changes in the cultural conditions of the plants (before the decapitation) or in the conditions during the exudation experiments (after decapitation).

The periodical cycle of cockleburs (Xanthium pennsylvanicum) could be reversed by illuminating by night instead of during the day. Plants grown in continuous light showed a normal periodicity. However, when sunflowers (Helianthus annuus) were
kept in continuous light the periodicity disappeared, the rate of exudation being more or less irregular. Otherwise here, too, the cycle could be shifted more or less by appropriate changes in the period of illumination. The rate of exudation was quite regular throughout the whole day and night when plants—both cockleburs and sunflowers—were put at 10°C. or at a lower temperature after decapitation, no matter under which conditions the plants had been grown. On the contrary the difference between minimal and maximal rate of exudation increased greatly at 30°C. (the ordinary experimental temperature being 17°C + 30°C). A periodical change could be induced by transferring the decapitated plants from low to higher temperature, the time of minima and maxima depending on the time at which the temperature had been changed. A change in temperature at midnight—the time of the normal minimum—resulted in a cycle with minima in the morning, maxima at night.

137. TRANSPERSION AND STOMATAL MOVEMENTS IN RELATION TO ILLUMINATION

Investigator: O. M. van Andel
Support: Rockefeller Foundation

The opening and closing of the stomata of tomato leaves under normal day-night conditions and under various illumination treatments were studied. The opening of the stomata was observed on the lower side of a living leaf by means of an Ultrapak microscope. The plants were kept either in a greenhouse or in dark rooms, illuminated artificially for alternating periods of one, four, or twelve hours, or in continuous light, at light intensities of six hundred or twelve hundred foot-candles. The stomata were opening and closing periodically, as has been found before, but their movement was not influenced much by changes in the illumination. The treatment of the plants before the experiment could be of importance for the course of the movements. Besides the changes in the opening of the stomata the rate of the water uptake by the roots was measured, either by weighing or by means of a drop pump connected with a recorder. No close relation between degree of opening and water uptake under the various conditions could be demonstrated, which indicates that in this case the cuticular transpiration must be rather important quantitatively.

138. GROWTH OF TREE SEEDLINGS IN VARIOUS TEMPERATURES AND PHOTOPERIODS

Investigator: Paul J. Kramer*
Support: National Science Foundation
Duke University

Seedlings of several species of eastern forest tree species are being grown with various combinations of day and night temperatures and long and short photoperiods. The results should assist in explaining why certain tree species grow longer than others in the summer. They should also indicate the extent to which high day and night temperatures limit southward distribution of northern species. The species under study include balsam fir and white pine which have a northern range, loblolly pine which has a southern range, and two deciduous species, white oak and northern red oak, which have rather wide ranges and short growing seasons.

*Department of Botany, Duke University
Although the first growing season is not yet complete, the growth of some of the species shows a definite reaction to both temperature and photoperiod. Shoot growth of loblolly pine was reduced by cool days (17° C.) and increased by high day temperature (30° C.) as compared to growth at intermediate temperatures (23° C.). Warm nights (24° C.) reduced growth, but cool nights (11° C.) did not, as compared with intermediate nights (17° C.). Warm nights and cool days both reduced growth of northern red oak seedlings, the best growth being made with intermediate night temperatures and intermediate or hot days. Balsam fir and white pine showed best survival and growth with cool nights. All species made more growth with cool nights. All species made more growth with a sixteen-hour photoperiod and less with an eight-hour photoperiod than with natural illumination.

139. EFFECTS OF TEMPERATURE, LIGHT, AND NUTRIENTS ON THE DEVELOPMENT OF WEEDS

Investigator: Rüdiger Knapp
Support: National Research Council Fellowship, General Funds

In the ecology of weeds growing below and between the crops, temperature, light, and the available nutrients are of great importance. Their influence was studied under controlled conditions.

Some species and pasture plants, which were also studied for comparison, can germinate over a wide range of temperature. In this way it was possible to observe different processes during the germination, which are sensitive in a different manner to the temperature. These are the time needed for the start and the end of germination, which is strongly affected by differences in temperature, for instance. The final germination percentage in certain species is not much affected by a wide range of temperatures (between 7° C. and 30° C.).

In the most cases the optimum temperature of the germination corresponds to the natural distribution of weeds. But there are remarkable exceptions. For instance, the composite Sonchus arvensis, which grows in Europe, in northern regions, and in the high mountains in a cold climate, can germinate only above 25° C. in constant temperature. Such high temperatures seldom occur and only for short times in the above-mentioned cold regions. In an experiment in which the fruits were subjected to alternating high and low temperatures, it was found that, with a pretreatment in a moist atmosphere for three weeks at 14° C., a six-hour exposure to 30° C. was sufficient to obtain a good germination percentage. This finding explains the ability of the species to germinate in cooler climates.

Many sets of experiments were started, in which plants were grown from seedling stage to maturity. The competition between the crops and the weeds is greater as less nutrient is available. In high nutrient concentration the competition effects nearly disappear, even in very small pots and dense intermixing of the root systems. The length of light period strongly influences the beginning of flowering and the shape of the plants in most of the species. It seems that this effect in the species composition of weed associations is of greater importance than hitherto assumed. Constant low temperatures of 4° C. kill most of the weed species studied after some weeks. Only two species, the groundsel (Senecio vulgaris) and the corn cockle (Agrostemma githago), grew slowly in this temperature.
140. ACTION OF LIGHT AND TEMPERATURE ON GROWTH OF PLANT TISSUE CULTURES

In the present investigation callus cultures of sunflower (Helianthus annuus crown-gall), Boston ivy (Parthenocissus tricuspidata), and a carrot (Daucus carota) were used. For the study of the low temperature effects upon anthocyanin formation in vitro, transplants of root tissues of Daucus carota were employed.

The effect of light intensity was investigated after the optimum temperature for growth had been established. The importance of the effect of the physical factors was manifested by the growth response of different tissues after two months' culture under different conditions of light and temperature. Optimal growth was achieved in continuous artificial light and at air temperatures of 23° C. for the grape, 26° C. for sunflower and carrot.

These experiments show that the optimum temperatures for growth of tissue cultures are higher than those usually observed for growth of intact plants. These observations are in agreement with other workers. Optimal growth was obtained at three hundred and fifty foot-candles. In the growth of tissues under artificial light the increase in tissue temperature due to illumination must be considered. In the roots of Daucus carota low temperature treatment (4° C. for thirty-two minutes) induces anthocyanin formation in the proliferating callus as well as in the original cells of the transplants. In addition it was noted that those tissues immersed in cold water produced a callus (smooth, hard, globular and compact), completely different from the usual loose irregular one. It would appear that this particular form of callus is not due to cold temperature, inasmuch as the phenomenon is not observed in those tissues not cooled by water immersion.

Publication:


141. COMPARATIVE PHYSIOLOGICAL STUDIES IN THE DUCKWEED

Fifty strains of duckweed (Lemnaceae) from a diversity of climates, including ten different groups of species, were grown as clones in pure culture in Erlenmeyer flasks under different temperatures and light intensities for the purpose of comparing their growth rates. The temperature optimum proved to be approximately the same for all strains, being higher than 20° C., and lower than 30° C., probably near 26° C. The strains may differ, however, in:

a) The light intensity at which saturation is reached; some strains reach saturation at six hundred foot-candles, whereas others do not up to one thousand foot-candles.
b) Their tolerance to extremes of high temperature, which may range between 31° C. and 38° C.

c) Their behavior at low temperatures: below 14° C. certain strains grow irregularly or form special resting buds, whereas others still grow quite well at 4° C.

d) Their growth rate in darkness when supplied with sugar.

The addition of sugar to the culture solution will greatly increase the growth rate of all Lemnaceae, at least at lower light intensities. The increase in growth due to the presence of sugar in the medium is approximately additive at low light intensities and temperatures not over 26° C., that is, the growth rate of cultures in the presence of light without sugar plus the growth rate of the same culture in darkness with sugar, yeast extract and casein is the same as in the presence of light of the same intensity with sugar present. The addition of yeast extract and casein to cultures grown with sugar in the light has mostly no effect, but is essential for the growth of cultures in the dark.

There appears to be little relation between the climate of the original habitat of a strain and its behavior under the conditions studied. There are, however, certain similarities in the growth response of strains of the same species group. Flowering was observed in a few strains of two species under certain conditions.

142. INVESTIGATION OF THE EFFECTS OF SUPPLEMENTARY SUGAR FEEDING OF PLANTS

Investigator: Alex M. M. Berrie
Support: Sugar Research Foundation

It has been shown that plants can utilize sugar given as a solution sprayed on their leaves providing some light is also given. The whole problem of the energy relations of the plant and how it utilizes light energy is still at an elementary level. A possible line of approach is to supply the plant with some source of energy which can be adequately measured, then replace this in whole or in part by another source, which also is capable of precise measurement. By equating these quantities it should be possible to find energy equivalents, and eventually some idea of how the plant utilizes its normal source of energy-light.

Preliminary work showed that sugar could replace light but the relationship light energy x time was more important when time was the factor which was kept small. In other words short days, less than twelve hours, restricted the growth of plants and utilization of light, even though the intensity of the light was high.

These findings were carried over to horticultural practice in areas where the winter day length is short, as in Scotland, and it was found that under horticultural conditions sugar had a retarding effect on the crop. This was due not to any effect of the sugar on the plant but to the increase in the content of the microorganisms in the soil. The microorganisms increased to such an extent that anaerobic conditions were set up in the pots and death of the plant roots ensued.
143. INJURY OF TOMATO PLANTS BY CONTINUOUS LIGHT AND OTHER UNFAVORABLE PHOTOPERIODS

Investigator: William S. Hillman
Support: National Science Foundation Fellowship
General Funds

It has long been known that tomato plants exposed to 24 hours of light per day develop leaf injury symptoms and eventually die. Similar injury is induced by repeated light-dark schedules such as 6 hours light - 6 hours dark or 24 hours light - 24 hours dark, but not by 12 hours light - 12 hours dark. During the past year the facilities of the Earhart Plant Research Laboratory have been used to examine this phenomenon further.

Leaf-injury symptoms are caused by continuous illumination over the whole range (30° C. - 14° C.) of constant temperatures tried, but differ widely in appearance depending upon the temperature. Sensitivity to injury is highest in plants with 4 to 7 leaves, declining markedly in older plants. Leaves are susceptible to injury only in an early, active stage of growth; leaves close to maturity when the plant is first exposed are not injured.

Daily temperature changes of at least ten degrees (e.g. 8 hours 17° C., 16 hours 30° C.) will completely prevent injury by continuous light even though either temperature used alone permits it. The occurrence or prevention of injury bears no apparent relationship to rates of stem elongation, leaf development, or weight gain immediately preceding it.

While repeated light-dark schedules of 20 - 4 hours or 16 - 4 are harmless, 8 - 4, 11 - 4 or 14 - 4 is injurious; 8 - 16, however, is harmless.

The general conclusion may be drawn that healthy leaf development in tomato requires some environmental fluctuation with a period near 24 hours. When this is not provided by a normal daily alternation of light and darkness, temperature changes may be substituted.

144. STUDIES OF MORPHOGENESIS OF THE SHOOT

Investigator: Ralph O. Erickson
Support: John Simon Guggenheim Memorial Foundation Fellowship
University of Pennsylvania
General Funds

Observations have been made of the rates of growth of the stem and of initiation and growth of leaves in various conditions. Most of the work has been done on Xanthium growing vegetatively, though some attention has been given to other plants. Automatic photographic equipment has been developed to aid in obtaining data.

145. PHYSIOLOGICAL VARIABILITY IN MONKEY FLOWERS

Investigator: R. K. Vickery, Jr.
Support: National Science Foundation Fellowship
General Funds
The rise of new species through the gradual differentiation of a population into ecologically distinct and isolated sub-populations can be conveniently studied in the numerous polymorphic populations of monkey flowers (the genus Mimulus). The type of physiological differentiation of fifteen Mimulus populations is being analyzed by observing the growth of clone members of individual plants under twenty-two different climates in the Earhart Laboratory. The investigations reveal a remarkably wide and varied range of physiological tolerances in most of the races even though they come from such sharply contrasting climatic areas as Kodiak Island, coastal California, the Great Basin, the high Rockies, and the foothills of the Chilean Andes.

Apparently most of the Mimulus populations now contain sufficient physiological tolerances and variability to invade new environments and repeat the evolutionary pattern of differentiation into ecological races by which they themselves presumably arose. (Work is beginning on the role of interracial hybridization in evolution.)
146. RELEARNING TESTS FOR INTEROCULAR TRANSFER FOLLOWING DIVISION OF OPTIC CHIASMA AND CORPUS CALLOSUM

Investigators: R. W. Sperry, John S. Stamm, Nancy M. Miner
Support: Frank P. Hixon Foundation Fund

It was found by Myers that a pattern discrimination learned with one eye covered transfers readily to the untrained eye in cats that have had the optic chiasma divided in the midsagittal plane. However if right and left hemispheres have been further separated by division of the corpus callosum, the performance drops to a chance level as soon as the blinder is shifted to the opposite (trained) eye. The score then remains at or near chance throughout forty to eighty test trials with the untrained eye, suggesting complete amnesia for the habit learned with the first eye. However, the earlier findings leave open the possibility that a minor amount of latent transfer is present but remains undetected under the above conditions. An investigation has been undertaken to find out if any latent transfer might be demonstrated in an improvement in the scores for relearning the same discrimination with the second eye. Data collected to date suggest complete amnesia with no carry-over from one eye to the other.

147. FUNCTION OF THE CORPUS CALLOSUM IN CONTRALATERAL TRANSFER OF TACTILE DISCRIMINATION IN CATS

Investigators: John S. Stamm, R. W. Sperry
Support: Frank P. Hixon Foundation Fund

In a further investigation of the role of the corpus callosum in learning and memory, experiments are being conducted on tactile discrimination. An apparatus has been constructed in which cats can be trained to distinguish between two objects by feeling them with one front paw. Visual and other extraneous cues are excluded and the use of any but the one front paw is prevented by the design of the apparatus. Three pairs of test objects differ with respect to roughness, softness, and shape, respectively. Four unoperated cats and four cats with the corpus callosum sectioned were each trained to perform all three types of discriminations. After a cat had learned with one paw it was retrained on the same problem with its other front paw.

The operated cats apparently have to relearn the problems from scratch. In the unoperated animals consistent transfer effects were found, i.e. the cat learns much more rapidly with the second paw, requiring on the average one-fourth as many
trials to reach the criterion of learning. Cats with sectioned corpus callosum required about the same number of trials to relearn the problems with the second paw as they did for the original training. These findings are in agreement with those obtained on interocular transfer of visual discrimination of cats with sectioned corpus callosum and optic chiasma.

148. FUNCTIONAL CAPACITY OF THE ISOLATED VISUAL CORTEX IN CATS

Investigators:  R. W. Sperry, Ronald E. Myers
Support:  Frank P. Hixon Foundation Fund
Southern California Society for Mental Hygiene

Earlier brain lesion studies have suggested that the visual cortex may mediate visual functions autonomously in the absence of connections with other cortical areas. Perceptual theory, on the contrary, has suggested that the perceptual process is less insulated and involves extensive integration with other parts of the cortex. The question is being tested by removing all parts of the neocortex except the visual area. The extensive removals and functional tests are restricted to one hemisphere by masking one eye after surgical section of the optic chiasma and the corpus callosum in the midline. The two hemispheres then function independently with respect to visual learning and memory. Leaving one hemisphere intact prevents general paralysis of nonvisual functions. Severe impairment of visual perception has been found in two preliminary cases with the visual cortex isolated as described.

149. FUNCTIONAL RECOVERY FOLLOWING ALTERATIONS IN NERVE-MUSCLE CONNECTIONS OF FISHES

Investigators:  R. W. Sperry, Norma Deupree Sperry
Support:  Frank P. Hixon Foundation Fund
National Institute of Neurological Diseases and Blindness

In contradiction to the results in man and other mammals, it has been found (Weiss, Sperry) that surgical disarrangement of nerve-muscle connections fails to produce any functional disorder in the limb coordination of urodele amphibians and in the fin movements of certain marine fishes. As a possible explanation it has been suggested that the synaptic terminals on the regenerated motor cells may undergo some kind of chemotrophic breakdown in the centers followed by a reestablishment of new central hook-ups patterned to suit the new nerve-muscle connections in the periphery. Such a synaptic interchange would be in harmony with various other phenomena that point to specific chemoaffinities as a basis for the orderly ontogenesis of synaptic associations in the vertebrate nervous system. In the hope of finding ultimately some direct morphological evidence in support of this theory, the effects of surgical section and cross-union of motor nerves have been studied in two Caribbean fishes having special morphological adaptations to the requirements of the experiment. Some interesting complications in nerve-muscle physiology have been uncovered but the findings remain indecisive on the main issue.
Evidence that the skin can impose local specificity upon its nerve supply failed to appear in some earlier studies in which the ophthalmic branch of the trigeminal nerve was cross-united with the mandibular branch and came to innervate the skin of the mandible. The efts and newts lowered the head when the skin of the mandible on the operated side was touched. These reversed (ophthalmic) responses persisted even after section and central regeneration of the trigeminal root.

On the assumption that neuronal specification had already reached an irreversible status in these post-larval animals, the study has been extended to larval amphibians. The left ophthalmic branch of cranial nerve V was crossed to the right mandibular branch in frog tadpoles (Rana pipiens, R. catesbeiana, and R. clamitans) at different stages of development. Specification of the ophthalmic nerve by the mandibular skin and formation of appropriate central relations would make it possible upon stimulation of the skin of the right jaw to elicit reflex brushing of the left mandible by the forelimb. This response, indicating integumental specification of the nerve fibers, has been obtained in some animals whereas in others the skin around the left eye has been wiped. The period during which specification of the cranial nerve fibers occurs is still under investigation.

Cutaneous local sign in skin grafts rotated 180° in frog tadpoles was found after metamorphosis to be correlated in most cases with the intrinsic specificity of the grafted skin rather than its location on the animal. Further investigation to determine the exact period during which integumental specification of cutaneous nerve fibers occurs is in progress.

Tadpoles in paddle, foot, and early metamorphic stages were prepared as described above with belly skin on the back and dorsal skin on the abdomen. Tests for specific cutaneous localizing reflexes have been completed on the animals operated on in the paddle stage. They show clear-cut responses. When the belly skin of the graft on the back is touched the frog rubs the belly with the forelimb. When the dorsal skin of the graft is touched the back is brushed by the hindlimb.

It has been suggested that the entire integument undergoes a highly specific local differentiation and induces a parallel specification in the primary sensory neurons via their terminal contacts. This local specification of the cutaneous fibers then
determines the patterning of functional relations in the centers. The question arises whether a differentiation of the skin in two planes is necessary or whether a differentiation in the dorsal-ventral direction combined with segmental innervation could account for the development of cutaneous localizing reflexes in the trunk region of the frog.

In an attempt to detect a differentiation of the skin along an anterior-posterior axis which could be tested for in cutaneous reflexes, two sets of animals were prepared. In the first group a large strip of dorsal skin extending from just behind the eyes to the tail of the tadpole was rotated 180° to reverse its anterior-posterior orientation. In the second group, animals used in another experiment were selected. In these a wide rectangular strip of skin extending from the dorsal midline to the ventral midline on one side was rotated 180°. The ventral and dorsal areas of skin, in addition to being abnormally located were reversed in the anterior-posterior direction.

153. CUTANEOUS LOCALIZING REFLEXES ELICITED FROM CORRESPONDING SKIN AREAS OF FORE- AND HINDLIMBS

Investigator: Nancy Miner
Support: Frank P. Hixon Foundation Fund

Hindlimb buds transplanted to the back region in frog tadpoles became innervated by dorsal cutaneous nerves rather than limb nerves. After metamorphosis stimulation of the transplant elicited responses in the normal ipsilateral hindlimb that were specific for the areas touched. In the more anteriorly placed transplants, however, the forelimb responded with movements that appeared, in general, to be appropriate for the homologous skin areas stimulated. Several questions raised by these results are under investigation. Are the sensory nerves from corresponding skin areas of the fore- and hindlimbs biochemically indistinguishable in the nerve centers? Does a forelimb bud grafted to the back near the tail form central reflex relations appropriate for a hindlimb? How do the central reflex relations formed by a homograft compare with those of an autograft?

154. EFFECT OF TRIGEMINAL NERVE SECTION ON THE SYNAPTIC ENDINGS IN MOTOR NUCLEUS V

Investigators: R. W. Sperry, Harbans L. Arora
Support: Frank P. Hixon Foundation Fund

Further investigation of chemotrophic effects in the formation and maintenance of synaptic connections has been started using teleost fishes. At present fishes seem to offer the most promising possibilities for morphological demonstration of the central nervous changes that follow peripheral reinnervation of foreign muscles to bring about restoration of normal motor coordination. The functional effects of section and regeneration of cranial nerve V are under investigation. Subsequently histological staining procedures will be applied to the brain in an attempt to demonstrate any morphological changes that may be present in the synapses of the motor V nucleus.
Publications:


155. THE HOST-VIRUS RELATIONSHIP IN BACTERIA LYSOGENIC FOR PHAGE P2

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

It is known that prophage occupies a chromosomal site in the (lysogenic) host bacterium, and that it behaves in bacterial crosses like any other bacterial marker. Doubly lysogenic strains, i.e. carrying two (differently marked) P2 prophages, have now been investigated in this respect. In crosses between a doubly lysogenic strain and a non-lysogenic strain singly lysogenic recombinants are obtained. This indicates that the prophages occupy two different sites on the bacterial chromosome. A second, independently isolated doubly lysogenic strain was crossed with the first doubly lysogenic strain. In this cross two of the prophages (one from each parent strain) behaved as alleles (site I). The other two appeared to be at different chromosomal sites (II and III), since triply lysogenic recombinants were obtained. The two sites occupied by the prophages of a doubly lysogenic strain can also be distinguished in a different manner: phage with the markers of the prophage at site I seems to be liberated in larger amounts than phage with the markers of prophages at sites II or III. Since these results are independent of the markers used to distinguish the two prophages, this difference can be considered a position effect. By crossing several independently isolated singly lysogenic strains, and by superinfecting doubly lysogenic strains with a third P2 phage, it was demonstrated that site I is the site most commonly occupied by P2, both in the process of lysogenization and in the process of prophage substitution. This had been suspected on the basis of previous observations on spontaneous and ultraviolet induced prophage recombination in doubly lysogenic strains, a process which is not symmetric in respect to prophages occupying different chromosomal sites.
156. SENSITIVITY OF INTRACELLULAR PHAGE P2 TO ULTRAVIOLET LIGHT

Investigator: L. Elizabeth Bertani  
Support: General Funds

It is known that the resistance to ultraviolet light of a virulent phage infecting a cell varies in the course of the infection, and thus may serve as an index to phage development inside the cell. Similar studies are being carried out with the temperate phage P2 and one of its virulent mutants. The resistance of the infecting virulent mutant remains equal to that of the free phage for the first five minutes after infection, and then increases rapidly to a maximum level which it retains until lysis occurs. For the temperate phage, one must distinguish between infection leading to phage production and infection leading to lysogenization. About seventy percent of the cells infected with P2 lyse, and about thirty percent become lysogenic. One thus must follow the survival after irradiation of both the infected cells that lyse and form plaques (infected centers), and the infected cells that are lysogenized. The latter is possible as the resistance to ultraviolet light of P2 is lower than that of its host cell (either uninfected or lysogenic). The resistance of the temperate infective centers remains equal to that of the free phage for fifteen minutes after infection, and then increases rapidly to the same maximum level as for the virulent mutant. The resistance of the lysogenizing cells remains equal to that of the free phage for five minutes after infection, and then increases rapidly to a value which corresponds to the resistance of the host cell. The sensitivity to ultraviolet light of the phage-cell complex is thus a useful index of the development of phage P2 inside its host cell, and allows a separation, at an early stage, of the lytic from the lysogenic process, either one of which may be the outcome of infection by a given temperate phage particle.

157. MULTIPLICITY REACTIVATION OF PHAGE T2 INACTIVATED BY IONIZING RADIATIONS

Investigators: J. J. Weigle, G. Bertani  
Support: National Foundation for Infantile Paralysis

Phage inactivated by radiations may not reproduce in single infection (one phage particle per host cell), but may do so in multiple infection (two or more "inactive" particles per cell). This is called "multiplicity reactivation" and was first described for ultraviolet inactivated phage. It was found a year ago that fairly efficient multiplicity reactivation could be obtained with gamma ray inactivated phage. This contrasted with results obtained by others with X-ray inactivated phage. In the past few months the effects of three types of ionizing radiations have been compared with respect to multiplicity reactivation. None was found with soft X-rays, some with hard X-rays, and more yet with gamma rays. In all cases, though, the amount of multiplicity reactivation was lower than that obtainable with ultraviolet inactivated phage. Several lines of evidence suggested that perhaps the difference between ultraviolet and ionizing radiations with respect to multiplicity reactivation was only a secondary one: if X-rays produced an additional damage affecting an early step in the infection process, the conditions for occurrence of multiplicity reactivation could not be realized, even though a part of the damage was multiplicity reactivatable. It was decided therefore to irradiate with soft X-rays not free phage, but phage pre-adsorbed (at various multiplicities of infection) onto the host cells, and indeed a large multiplicity effect was obtained, of the same order as that ob-
tained after ultraviolet irradiation. Pre-adsorption thus bypassed the effects of
the early step damage (or made impossible its production), and allowed multi-
plicity reactivation to occur. Additional experiments seem to indicate that the two
damages (the early step damage, and the multiplicity reactivable damage) are two
different expressions of the same event (ionization or ionization cluster) produced
by the radiation.

158. THE NUMBER OF LINKAGE GROUPS IN PHAGES T2 AND T4

Investigators:George Streisinger, V. G. Bruce
Support:National Foundation for Infantile Paralysis Fellowship
Abbott Laboratories

Hershey and Rotman (1948) and Doerman and Hill (1953) have respectively re-
ported three linkage groups in phages T2 and T4. The methods used by them and
by others would not have detected linkage of distant loci.

It can be shown on the basis of the Visconti-Delbrück model of phage mating that
a three-factor cross with very unequal multiplicities and early lysis constitutes a
much more sensitive test of linkage. The very early lysis reduces the number of
rounds of mating, and the unequal multiplicity assures that matings of minority
parent type will be predominantly with the majority parent type. Two factors on
the same linkage group and one factor on another linkage group were used. The
ratios of selected recombinant classes indicate linkage of all factors from the three
linkage groups. The order of linkage can also be determined. These results sim-
plify the genetic structure of phages T2 and T4 as no "orderly" mitotic mechanism
is required to keep three separate linkage groups together.

159. FINE STRUCTURE OF THE h REGION OF PHAGE T2

Investigators:Naomi Franklin, George Streisinger
Support:U. S. Public Health Service Fellowship
National Foundation for Infantile Paralysis Fellowship

A study of genetic fine structure may contribute to an understanding of basic
genetic mechanisms. A genetic investigation was therefore undertaken of mutations
occurring at the h (host range) locus in phage T2L. This locus controls the adsorp-
tive specificity of the phage: wild type T2 adsorbs to and infects Escherichia coli
strain B, but not strain B/2, whereas the mutant T2 h adsorbs to and infects both
strains. Since phage adsorption is believed to be a function of the phage tail, it
is hoped to compare various genetic changes in the h region with possible altera-
tions in the tail protein.

A series of different T2 strains with the adsorptive properties of the wild type
were obtained by spontaneous mutation from T2 h. These different mutant strains
are all alike in showing, as the wild-type T2 h', no measurable adsorption to B/2,
but differ widely in their mutation rates to the h phenotype, and slightly in their
adsorption rates to B. Crosses of the different mutants to each other, as well as
to the wild-type T2 h', yielded h recombinants with low frequencies characteristic
for each pair of strains. On the basis of the frequencies of recombination between
them, a reasonably linear map can be constructed, covering approximately 2.5
crossover units. The distances between the loci of this group are roughly additive, though in general the longer distances are greater than the sum of the shorter. This indicates perhaps a finite length for the mutated areas. The closest pair of loci give a recombination frequency of 0.003 percent. Assuming that the recombination frequency is proportional to the physical length of the genetic element, believed to be made of Watson-Crick desoxyribonucleic acid, a recombination frequency of 0.003 percent represents a distance of at most four nucleotides.

160. THE DISTRIBUTION OF PARENTAL BACTERIOPHAGE DNA AMONG PROGENY PHAGE

Investigators: N. K. Jerne, G. Stent*
Support: National Foundation for Infantile Paralysis

Experiments have been conducted at the Virus Laboratory of the University of California, Berkeley, with the object of finding the quantitative distribution of parental desoxyribonucleic acid (DNA) from bacteriophage T4 among the progeny obtained in single infection in Escherichia coli B.

The following facts were known: a) phage labelled with radioactive phosphorus P$^{32}$ are inactivated exponentially by the P$^{32}$ disintegrations; b) in single infection of cells with P$^{32}$ labelled phage, close to 50 percent of the label is recovered in the phage progeny; c) no detectable inactivation is observed in phage progeny obtained from labelled parent phage. These facts suggested that the P$^{32}$ transferred from parent phage to progeny may be unevenly distributed among the progeny particles, the majority of which (even when obtained by premature lysis) would contain less than 0.2 percent each of the parental label, while a small minority would contain most of the transferred phosphorus.

The experiments now carried out attempted to characterize this suspected small fraction of heavily labelled progeny particles by a method which distinguishes it specifically from the unlabelled fraction. The heavily labelled fraction is killed at a rate proportional to the P$^{32}$ content of each particle of this fraction. As the particles get killed they also become unable to transfer P$^{32}$ in a second generation transfer experiment. By measuring the decrease of transfer with time between the transfer experiments an estimate can be made of the death rate of the heavily labelled fraction and thereby of the P$^{32}$ content of these particles. In contrast to the method employed previously, viz., direct measurement of the death rate of the total first generation progeny, the new method measures the death rate of the labelled fraction only.

A highly radioactive phage stock was made, and first generation progeny phage was prepared. This was used in a series of second generation transfer experiments, at various times after preparation of the first generation phage. From the results obtained one can calculate that each of the more heavily labelled fraction of first generation phage particles contains about two to five percent of the parental phosphorus. In one cycle of phage reproduction, most of the DNA of the parent particle thus reappears in about eight to fifteen progeny particles.

*University of California, Berkeley.
161. INACTIVATION OF BACTERIOPHAGE T4r BY SPECIFIC ANTISERUM

Investigator: Joseph D. Mandell
Support: National Foundation for Infantile Paralysis Fellowship

The inactivation of phage T4r by anti-T4r horse serum diluted in low ionic strength medium was studied, and a theory was worked out to predict the time course of inactivation for any input ratio of phage and antibody.

The discovery by Jerne and Skovsted that the rate of inactivation of phage T4r by specific antiserum is a thousand times faster in a medium of low ionic strength than in 0.1 M salt solution has made possible the study of the kinetics of the reaction at extremely low concentrations of antiserum. The use of low concentrations of antiserum has allowed the investigation of the reaction in conditions of phage excess, at such low concentrations of phage that aggregation of phage does not take place. From a study of the kinetics of inactivation at low concentrations of phage and antibody in a low ionic strength medium estimates were made of the number of sites on a phage particle to which antibody molecules may attach (700), the number of these sites occupation of any one of which results in the inactivation of the phage particle (10-30), the number of specific inactivating antibody molecules in the antiserum used (10^16/ml.), and the efficiency of killing of a phage particle per collision of antibody with the tip of the tail (0.8).

Publication:


162. ACTIVATION OF T4.38 BY UREA

Investigator: Gordon Sato
Support: General Funds
Arthur McCallum Summer Scholarship

Phage T4.38 must first react with any of a number of cofactors before it can adsorb to its bacterial host. It has been found that urea, a well known denaturing agent, can activate (confer adsorbability) upon T4.38.

Urea both activates and kills the phage. The kinetics of both processes are similar to the kinetics of denaturation of protein by urea. Both the activation and killing rates decrease as the temperature is raised from 0° C. to 37° C. and increase as the temperature is raised from 37° C. to 47° C. Both rates are proportional to a high power of the urea concentration (circa 12th power), and both rates increase as the pH of the urea solutions is raised from the isoelectric point of the phage. Using S°35 labeled phage, it has been shown that after exposure to urea, the fraction of the total population which can adsorb to bacteria is equal to the adsorbable fraction of the survivors of the urea treatment.

Experiments designed to characterize the adsorption rate and to measure the stability of the activation produced by urea have led to the tentative conclusion that urea activated phages are not adsorbable to bacteria immediately after diluting of the urea, but that some rate limiting transformation occurs when the urea is
diluted away, which results in adsorbability of the phage. The physical picture suggested by this hypothesis is that urea must desorb from the phage, or that new intramolecular bonds must be formed in the protein of the phage organ of adsorption before urea treated phage can adsorb to its host.

Publication:


163. PHYSICAL CHANGES PRODUCED BY TRYPTOPHANE ON PHAGE T4.38

Investigator: P. Cheng
Support: National Foundation for Infantile Paralysis Fellowship

The adsorption of phage T4.38 to its host bacterium requires activation of phage by a cofactor such as tryptophane. The activation must involve a change in the protein structure of the phage tail, which is the organ for adsorption. Two approaches were used to shed more light on the nature of the change produced by tryptophane:

a) Viscosity. It was found that a minute amount of tryptophane greatly increased the solution viscosity at room temperature of a phage sample, and that the effect was efficiently suppressed by the presence of indole.

b) Thermal inactivation. It was found that the presence of tryptophane caused a considerable drop in phage survival at temperatures between 55°C and 60°C in all salt media used. The drop was accompanied by an increase in the amount of desoxyribonucleic acid (DNA) liberated by the phage. The effect appears to be specific for L-tryptophane. Both glycine and D-tryptophane showed little effect. Furthermore the effect was again inhibited by indole. The same specificity is shown by these substances in relation to the cofactor effect on adsorption.

Together with the inference that cofactor acts on the phage tail, the present findings suggest firstly that the tail is an outlet for DNA, and secondly that the cofactor effect involves the denaturation of the protein of the phage tail. The effect described under (b) could be used as a simple and sensitive method for the detection of L-tryptophane.

164. A NEW THEORY OF ANTIBODY FORMATION BASED ON NATURAL SELECTION

Investigator: N. K. Jerne
Support: National Foundation for Infantile Paralysis

Studies of the interactions between bacteriophage T4 and specific antibody showed that a change in quality of the antibody takes place in the immunized animal during the course of immunization: the combining power of the antibody seems to increase. Phage T4 treated with serum obtained early during immunization is inactivated reversibly, whereas a treatment with serum obtained at a later stage in the immunization course results in irreversible inactivation. This is similar to earlier results obtained with the diphtheria toxin-antitoxin system. It was also found that a
type of anti-T4 antibody which increases rapidly during the first days after the onset of immunization is already present in small concentrations in the sera of normal animals. These observations—the presence of "natural" antibody in untreated animals, and the "improvement" in quality of antibody produced as a response to immunization—led to speculations about the mechanism of antibody formation. The two current theories, the "antigen-template" theory and the "modified enzyme" theory, both assume that the antigen is the direct inducer of antibody formation. The "antigen-template" theory requires the continued presence of the antigen in the antibody-producing cells, whereas the "modified enzyme" theory requires the antigen only to initiate the process which can then continue in the absence of antigen.

The theory now proposed assumes 1) that natural antibodies for all antigens to which an animal can respond are already present in the untreated animal in small concentrations, 2) that the role of the antigen is to carry the natural antibody which attaches to it to the antibody-producing cell, and 3) that the antigen can then be dispensed with, antibody formation being essentially a preferential replication of the antibody that was selected by the antigen. This theory can account for a good number of immunological observations, such as the finding of natural antibodies against a large number of antigens, the secondary response effect, the improvement of antibody quality (by natural selection), the anamnestic response, the continued production of antibody in the absence of antigen, etc. Attempts are now being made to extend this theory so as to find a consistent explanation for observations such as the absence of auto-antibodies, the specific inhibition of antibody production in adults by antigen treatment in utero, and immunological paralysis.

165. THE ARRANGEMENT OF RIBONUCLEIC ACID WITHIN TOBACCO MOSAIC VIRUS

Investigators: J. D. Watson, D. L. Caspar
Support: Merck and Company
Rockefeller Foundation
U. S. Public Health Service Fellowship

Tobacco mosaic virus (TMV) is now known to be built up of small protein sub-units arranged in a helix about an axial core of ribonucleic acid (RNA). From x-ray diffraction measurements the radial electron density distribution in the virus has been determined. The RNA core is found to be hollow, with all the phosphorous atoms at a common radius of about 24 Å and thus in close contact with the protein shell. Several lines of evidence lead to the conclusion that the RNA is related to the protein in a regular way, so that there are an integral number of nucleotides for each protein sub-unit. A division of the crystallographic sub-unit into two equivalent halves is indicated by chemical and x-ray evidence, which suggests that there are an even number of nucleotides associated with each crystallographic sub-unit. This number is most likely six, from the phosphorous content of TMV and the number of sub-units per particle. Since the helical arrangement of the protein sub-units is known from x-ray results, the configurations which the RNA may assume can be delimited.

The distance between successive phosphorous atoms in a fully extended poly-nucleotide chain is about 7.5 Å and so the maximum length of a six nucleotide unit
is 45 Å. This places a severe restriction on the possible ways the nucleotides can be linked between equivalent points. Only the location of the lattice points is determined by the x-ray data, and these points can be joined together in a number of different ways. With one strand passing through each lattice point, the points of the TMV helix containing twelve and one-half sub-units per turn can be connected using one or between nine and fifteen equivalent strands. The one-strand helix can be made with one, two or three polynucleotide chains per lattice points, and the twelve-strand helix with one or two chains per lattice point. For the other possible strand numbers there can be only a single chain per lattice point. All other models are ruled out since they would require more than six nucleotides to bridge the distance between two lattice points.

From biological considerations it seems desirable to have the RNA chains run the entire length of the virus. Otherwise it is difficult to imagine why the virus length is constant, and why infectivity always accompanies the particles of 3000 Å length. The fact that the molecular weight of isolated TMV RNA falls rapidly from that of the intact core to units of molecular weight between \(2 \times 10^5\) to \(3 \times 10^5\) suggests that the core is composed of a number of RNA chains. This idea is strengthened by the observation of R. Hart that RNA filaments in partially degraded TMV particles frequently show fraying into unequal strands. We consider therefore the arrangements with strand numbers of nine or greater as more attractive. All models with a single chain per lattice point must have the chains running in the same direction; otherwise the crystallographic sub-units would not be identical. Crystallographic evidence suggests that the TMV particle is non-polar, and the RNA core can only be non-polar if there are two chains per lattice point, running in opposite directions. Two chains per lattice point are possible with a strand number of twelve, and this gives us a twenty-four-chain core in which the chains are fully extended with the maximal phosphate-phosphate separation of 7.5 Å.

This type of model is contingent upon the presence of six phosphorous atoms per crystallographic sub-unit. With four, five or seven, the core would be most likely polar, but still multi-stranded. In any case, it is clear that the RNA following extraction has a different structure from that which it assumes in the virus. Extracted RNA has helical features suggesting a diameter of about 20 to 25 Å and a pitch no greater than 30 Å, whereas the RNA core of TMV has a diameter at least twice this, and is probably a nine- fifteen- or twenty-four-chain helix. The twenty-four-chain helix with a pitch of 850 Å is favored from considerations of symmetry.

166. X-RAY DIFFRACTION STUDY OF THE STRUCTURE OF SPHERICAL PLANT VIRUSES

Investigators: D. L. Caspar, D. W. Berreman
Support: U. S. Public Health Service Fellowship
U. S. Atomic Energy Commission

Low-angle X-ray diffraction patterns from southern bean mosaic virus (SBM) have been obtained using the recently developed point-focusing monochromator. The positions of the diffraction maxima correspond approximately to those calculated for a uniform density sphere of diameter 282 Å. Small shifts in the positions of the

* Norman Bridge Laboratory of Physics, California Institute of Technology.
maxima relative to those calculated for a uniform density sphere are observed, sug­
gestig that there are measurable radial density fluctuations in the virus involving
large dimensions. By analogy with the structure of tobacco mosaic virus, which
consists of a hollow cylindrical core of ribonucleic acid (RNA) surrounded by a
protein shell, we might expect that the RNA of spherical plant viruses, such as
SBM, forms a hollow spherical core surrounded by protein. It has been established
for turnip yellow mosaic virus that the RNA forms a central core inside a spherical
shell of protein; it is not known if the core is solid or hollow. The observed shifts
in the diffraction maxima of SBM are not inconsistent with a hollow core RNA model,
but accurate intensity measurements will have to be obtained to calculate the radial
density distribution. Work has also been started on the x-ray diffraction from
tomato busby stunt virus.

167. THE LIGHT GROWTH REACTIONS OF PHYCOMYCES

Investigators: M. Delbrück, W. Reichardt
Support: National Science Foundation
General Funds

The average growth rate of the sporangiophores of Phycomyces has a constant
value, presumably determined by the rate of supply of precursor material. The
actual growth rate deviates transiently from the average value when the intensity
of illumination is varied. The illumination thus controls the detailed time course
of growth. Methods have been devised for studying this control system in detail
and a theoretical set of notions has been introduced to interpret the data. On the one
hand the illumination determines the level of adaptation A, according to a simple
differential equation. On the other hand, the organism is able to evaluate a quantity
called the subjective intensity, equal to the ratio of the objective intensity to the
level of adaptation. The time course of the subjective intensity is related to the
growth response in a simple manner.

A paper discussing this work and reviewing the general problems of biological
stimulus-reaction systems, entitled "System Analysis for the Light Growth Responses
in Phycomyces" was presented at the Growth Symposium in Amherst, June, 1955.
The proceedings of this symposium will be published by Princeton University Press.

168. PROPERTIES OF A PLAQUE-TYPE MUTANT OF POLIOMYELITIS VIRUS,
TYPE 1

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

The isolation of the plaque type mutant designated as r has been mentioned
in the previous report. Additional work on this mutant has been carried out, partly
in collaboration with Dr. Herbert Wenner of the University of Kansas Medical
School, who performed the pathogenicity tests in monkeys.

1. Reproducibility of the plaque character. It was found that the r plaque char-
acter was expressed only under certain cultural conditions which consisted usually
in developing the plaques in a suboptimal growth medium. In an optimal medium,
for example, in the presence of serum, the plaques produced by the mutant and the wild-type strain were indistinguishable.

2. Growth characteristics. One-step growth curves of the mutant and of the wild-type virus on suspensions of monkey kidney cells as well as on monolayer cultures were obtained under identical conditions. No significant difference was found, either in the length of the latent period, or in the slope of the rise period. The final virus yield per cell was slightly higher in the case of the wild type.

It was thus impossible to detect the selective mechanism by which the mutant strain was isolated.

3. Neuropathogenicity in the monkey and its correlation with plaque character. The previous finding of a greatly reduced neuropathogenicity of the \( r \) mutant was confirmed: only one out of twelve monkeys inoculated intracerebrally with \( 10^5 - 10^8 \) plaque-forming particles of the \( r \) line developed a paralysis, whereas fourteen monkeys inoculated intracerebrally with \( 10^2 - 10^4 \) particles of the wild type all developed a paralysis. By serial passage in rhesus monkey kidney cultures of the virus stock from which the \( r_1 \) mutant had been obtained, another line was isolated which showed the plaque character of the \( r_1 \) mutant in a still higher degree. This line (\( r_2 \)), when tested in monkeys, was found to be highly pathogenic. This shows that the plaque character and the pathogenicity character are independently determined.

4. The \( r_1 \) line and the wild-type line were found to differ in another character: the sensitivity of inactivation at \( 44^\circ \) C. at a pH of 7.4. The \( r_1 \) line is more sensitive to this condition. The \( r_2 \) line, on the other hand, is approximately as sensitive as the wild type. The relationship between the characters heat sensitivity and pathogenicity has not yet been determined.

169. MUTANTS OF POLIOMYELITIS VIRUS, TYPE 1, RESISTANT TO HEAT INACTIVATION AT \( 44^\circ \) C.

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

Heat resistant mutants were selected for to be used in genetic crosses.

The heat inactivation at \( 44^\circ \) C. of poliomyelitis virus, type 1, in a saline solution, depends strongly on the pH. The virus has its maximum stability at pH 5.0; the stability decreases much more strongly toward the alkaline than toward the acid side. At pH 7.4, the wild type shows after thirty minutes at \( 44^\circ \) C. a survival of approximately \( 10^{-3} \); the \( r_1 \) line, a survival of \( 2 \times 10^{-4} \). This inactivation is almost absent in the presence of a solution of lactalbumin hydrolysate, a medium used in the cultivation of the cells and of the virus.

The virus particles surviving three cycles of inactivation at pH 7.4 and \( 44^\circ \) C. were found to give rise to highly resistant lines. These lines showed after a heat treatment of sixty minutes a survival of twenty to forty percent.
Heat resistant lines were obtained both from the wild type and the r1 line. The pathogenicity character in monkeys was not affected by the change in the heat resistance.

170. STUDY OF THE CHANGES IN UV-SENSITIVITY OF POLIOMYELITIS VIRUS, TYPE 1, AFTER ITS ADSORPTION TO MONKEY KIDNEY CELLS

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

To get some information on the changes occurring in poliomyelitis virus subsequent to its adsorption onto susceptible cells, the sensitivity of the virus to UV irradiation was determined immediately after its adsorption to monkey kidney cells (= time zero), and after one hour of intracellular virus growth. To obtain the UV-sensitivity at time zero, the virus was adsorbed to the cells in the presence of cyanide. To reduce the screening effect of the cells, the virus adsorption and irradiation were done on monolayer cultures. Under these conditions, the screening by the cells was found to be negligible since not more than fifteen percent of the incident light was found to be adsorbed by monolayer cultures. After the irradiation, the infected cells were brought into suspension by exposure to a sodium versenate solution and the number of cells capable of releasing virus was determined by the plaque technique. The values were corrected for the loss of the ability of the cells to support virus growth at the corresponding UV doses. The necessary correcting factor was obtained from an independent determination of the survival curve of the ability of the cells to support virus growth if irradiated before infection.

The results of these experiments showed a very marked increase in the UV-resistance of Brunhilde virus immediately after adsorption followed by a further, although less pronounced, increase after one hour of intracellular virus growth. These results indicate that the virus undergoes deep physico-chemical changes immediately after its adsorption onto the cells. It is hoped that a parallel study of the sensitivity changes to X-rays of the virus will throw some additional light on the nature of the processes causing these changes.

171. KINETICS OF THE RELEASE OF POLIOMYELITIS VIRUS FROM SINGLE CELLS

Investigators: A. Lwoff, R. Dulbecco, M. Vogt, M. Lwoff
Support: Rockefeller Foundation
Abbott Fund
General Funds

These experiments were initiated with the ultimate goal of studying the problem of interrelations of viruses and cells at the level of individual cells. The cells used for these experiments were monkey kidney cells that had been previously infected with Brunhilde virus and repeatedly washed to eliminate non-adsorbed virus particles. With the technique of DeFonbrune, microdrops of nutrient medium containing individual infected monkey cells were placed on a coverslip immersed under paraffin oil. At various time intervals, samples of the microdrop fluid were withdrawn and assayed for the presence of plaque-forming particles. It was found that the infected cells did not release any virus during a period of approximately five and one-half hours which
corresponds to a latent period approximately one hour longer than that of cells in mass cultures. After this time, the individual cells released the bulk of their virus (one hundred to two hundred plaque-forming particles) within a period of one hour. This virus release is, thus, significantly more rapid than that of Western equine encephalomyelitis virus from chick embryo fibroblasts. The rapidity of the release of the poliomyelitis virus may be correlated with the steepness of the initial part of the growth curve of poliomyelitis virus in mass cultures, which is the steepest growth curve so far obtained among the animal viruses studied.

It was, furthermore, possible to correlate certain morphological changes in the individual cells during virus release. The most striking change was the occurrence of a hyaline zone at the periphery of the cells, which was transformed into a vacuolized area during the time of maximal virus release. The virus release appears to be correlated with a lysis of important portions of the cell cytoplasm.

Publications:


172. QUANTITATIVE RELATIONS BETWEEN CELLS AND VIRUS IN THE ROUS SARCOMA

Investigator: Harry Rubin
Support American Cancer Society Fellowship
Boswell Fund

The Rous sarcoma virus has been found to cause both ectodermal and mesodermal tumors in the chick embryo. The type of tumor has been shown to depend only upon the type of cell originally infected and is not due to virus variation. Thus it seems likely that the mechanism of tumor production by the virus is not highly specific and may be due simply to the release of the cell from its normal regulatory processes.

The rate of virus multiplication in cultures of sarcoma cells is much slower than that of viruses which cause cell death. This rate remained unchanged over many sarcoma cell passages in young chicks, thus suggesting an intimate and direct role for the virus in perpetuating the malignant properties of the cells.

The slow but constant rate of virus production suggested an analogy to temperate phage multiplication in cultures of lysogenic bacteria. A series of experiments was performed to determine whether this finding reflected a basic similarity between the two systems at the level of the individual cell. Sarcoma cells in tissue culture were irradiated with a dose of ultraviolet light which resulted in death of all the cells within forty-eight hours, yet permitted them to continue to release virus for about twelve hours. It was found that a high proportion of such cells released virus after they had been placed on the choriallantoic membrane of the developing chick embryo. This result suggested that all the sarcoma cells may release virus during a period of time corresponding to one cell generation. This is in sharp contrast to lysogenic bacteria where only a very small fraction of cells yield virus.
The distinction from lysogenic bacteria received further confirmation from experiments with small numbers of sarcoma cells kept in suspension for short periods of time. A relatively large fraction of these cells yielded virus in small amounts. This contrasts with lysogenic bacteria where the rare "spontaneously lysing" cell yields large bursts of virus.

Experiments with small numbers of cells observed in microdrops for extended periods of time are still in progress. Although incomplete, they are thus far consistent with the interpretation that all the cells release virus in relatively small amounts without dying.

It has been possible to maintain the sarcoma cells in tissue culture under conditions where they fail to multiply but continue to grow in volume. The rate of virus production under these conditions has been found to be proportional to the cell volume and unrelated to cell division.

The time required for release of the mature Rous sarcoma virus from an infected cell has been found to be considerably longer than that required for release of certain necrotizing animal viruses.

173. EFFECT OF VESICULAR STOMATITIS VIRUS ON THE PHOSPHORUS METABOLISM OF CHICK EMBRYO FIBROBLASTS MAINTAINED IN TISSUE CULTURE

Investigator: P. D. Cooper
Support: Gosney Fellowship
Boswell Fund

As a basis for chemical studies, one-step growth curve experiments have been made with tissue culture monolayers of chick embryo fibroblasts infected with vesicular stomatitis virus. By using radioactive phosphorus (P) the paths of phosphate transfer have been compared in virus-treated and control monolayers immediately after infection (latent period) and during virus release, with the object of locating the site in the cell of virus nucleic acid formation. This host cell-virus system appears radically different from bacteriophage systems in that virus infection causes no general rapid rearrangement of P metabolism, although P incorporation slows in most particulate fractions towards the end of virus release. There is no "burst" during virus release, and no gross lysis of nuclei, mitochondria or microsomes. Even in this tissue culture system, where some metabolic processes such as desoxyribonucleic acid duplication are nearly non-functional although virus reproduction is as rapid as in truly growing cells, it is evident that the virus commandeers only a very small proportion of the cell's P metabolism. The results suggest that reproduction of this virus involves ribo- rather than desoxyribonucleic acid, and that it may not originate from any sub-cellular particle.

174. THE GROWTH OF VESICULAR STOMATITIS VIRUS ON CHICK EMBRYO FIBROBLAST CULTURES

Investigator: R. Franklin
Support: American Cancer Society Fellowship
Boswell Fund

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The vesicular stomatitis virus produces plaques on a variety of cells; the number of plaques produced on chick embryo monolayer cultures by a given sample of this virus at various dilutions is proportional to the virus concentration. The number of plaques produced by a given virus sample on different cultures varies according to the Poisson distribution. Thus, a plaque of this virus, like that of other viruses so far tested, is produced by a single virus particle.

The release of vesicular stomatitis virus from chick embryo fibroblast cells was compared with the intracellular appearance of infectious virus, as detectable by disrupting the cells by freeze-thaw cycles.

Suspensions of cells, infected with the virus, were incubated at 37° C. in a nutrient medium. At certain time-intervals, samples were taken and assayed for free and intracellular virus.

The results obtained are very similar to those of Dr. Rubin on the intracellular growth of Western equine encephalitis virus. There is an initial latent period of approximately two hours, after which virus appears in the medium; thereafter, the virus increases exponentially both in the medium and within the cells. This exponential period lasts about five hours. There is always less intracellular than extracellular virus, the highest value being ten virus particles per cell. The release of virus from the cell occurs one to two minutes after it has become infectious.

During the early phases of the latent period infectious virus can be recovered by breaking the cells. This recovery cannot be attributed to elution of adsorbed virus; it is likely that it is due to a slow transformation of the infecting virus into the non-infectious "vegetative" form. This point is being investigated further.

175. THE COMPOSITION OF THE NEWCASTLE DISEASE VIRUS

Investigator: R. Franklin
Support: American Cancer Society Fellowship
Boswell Fund

Newcastle disease virus (NDV) is suitable for chemical work because it can grow on a variety of host cells, including the uniform HeLa cells, is stable, can be easily purified, and can hemagglutinate red cells.

A chicken embryo fibroblast monolayer was grown in the presence of P32 for twenty-four hours, then infected with NDV, and kept in labeled medium until the virus was harvested. The virus was treated with ribonuclease and desoxyribonuclease, then adsorbed onto chick red cells; the cells were washed and the virus was then eluted. The virus was concentrated by centrifugation. In the process of concentration infectivity, hemagglutinating activity and radioactivity remained in constant ratios, showing that the radioactivity was in the virus.

Schmidt-Thannhauser fractionation of such a preparation gave the following distribution of radioactivity:
The DNA value could easily be accounted for by contamination with RNA suggesting that NDV is actually an RNA virus. However, the cells used had little label in their DNA; the amount of viral DNA could be therefore underestimated if it were synthesized from cellular DNA. The experiments are now being repeated using uniformly labeled cells.

176. THE EARLY STAGE OF VIRUS INFECTION

Investigator: R. Franklin
Support: American Cancer Society Fellowship
Boswell Fund.

A $^{32}$P-labeled preparation of Newcastle disease virus (NDV) was used to infect chicken embryo fibroblast monolayers (cold cells). After an initial adsorption period all non-adsorbed virus was washed away and the cells were treated with receptor-destroying enzyme to remove any virus material from the outside of the cells. The cells were then ground in .25 M sucrose and fractionated by differential centrifugation into four fractions—nuclear, mitochondrial, microsomal, and soluble. Each fraction was chemically fractionated by a modified Schmidt-Thannhauser procedure and the distribution of $^{32}$P was determined.

$^{32}$P was found in each cell fraction, thirty percent in nucleus, thirty percent in mitochondria, thirty percent in microsomes, and ten percent in soluble. The chemical distribution of $^{32}$P was nearly the same in each cell fraction and similar to the chemical distribution of $^{32}$P in the free virus. These data suggest that the virus which had penetrated the cell had not undergone any deep change when the cells were broken down. The observed distribution of the viral material may be due to a non-specific adsorption. The observed results may be in agreement with a slow transformation of the infecting virus into the vegetative state.

177. INTERACTIONS AMONG ANIMAL VIRUSES

Investigator: M. Baluda
Support: General Funds
Arthur McCallum Summer Scholarship

Western equine encephalomyelitis virus (WEE) and chicken embryo cells were tested for occurrence of multiplicity reactivation and cross reactivation of virus inactivated by ultraviolet light. Neither phenomenon was found. Test for genetic recombination among plaque size mutants were made; the experiments were so far negative.

Interference between WEE virus and vesicular stomatitis virus (VS) was studied
by means of statistical single cell technique. The experiments suggested that VS excludes WEE but WEE does not exclude VS, at least within the time intervals so far explored. This experiment is, however, complicated by the fact that WEE is adsorbed to the cells at half the rate of VS, under the conditions used.

178. TECHNICAL DEVELOPMENTS IN THE STUDY OF THE INTERACTIONS BETWEEN ANIMAL VIRUSES AND THEIR HOST CELLS

Investigators: P. Cooper, M. Vogt, R. Dulbecco
Support: Gosney Fellowship
National Foundation for Infantile Paralysis
Boswell Fund
General Funds

The following technical developments have been obtained in the work with animal viruses. They all center round the plaque technique:

1. Plaques can be produced by using cell suspensions instead of monolayer cultures. This technique is identical with that used in bacteriophage work. It has the advantage of a higher plating efficiency if compared with that of monolayer cultures. For free virus it varies from 1.4-fold for vesicular stomatitis virus to about twofold for poliomyelitis virus; for assays of cells infected with vesicular stomatitis virus the plating efficiency is two- to threefold that of monolayer cultures.

2. By using a tris-(hydroxy methyl)-amino methane (Tris) buffer it is not necessary to incubate the cell cultures in a CO₂-air atmosphere. Cells can be grown and plaques developed in this buffer. It is preferable to grow certain cell types, such as chick embryo fibroblasts and monkey kidney cells, in a bicarbonate-CO₂ buffer. The use of the (Tris) buffer is particularly advantageous for the work with individual cells in microdrops. The virus yields per cell in Tris buffer are the same as in a bicarbonate-CO₂ buffer.

3. Spot-testing techniques have been found satisfactory for the scoring of viral characters, such as heat resistance. Replica plating can also be used in conjunction with cell suspensions (see under 1).

4. Several simplifications include the use of versenate instead of trypsin to prepare cell suspensions, the growth of HeLa cells in media containing very little or no human serum, the use of secondary as well as primary monkey kidney cultures for virus growth.

Publications:

VISITING LECTURERS

Eighteen visitors gave lectures or seminars in the Division of Biology during the year. Several of the visits were made possible by grants from the Eversole Lecture Fund. The lecturers and their subjects were:

Dr. K. G. Grell, Max Planck Institute, Tübingen, Germany. "Life Cycles of Foraminifera."

Dr. C. P. Stewart, Professor of Clinical Chemistry, University of Edinburgh, Scotland. "Place of Ascorbic Acid in Tyrosine Metabolism."

Dr. Hewson Swift, Department of Zoology, University of Chicago. "Some Cytological Studies on Nucleic Acids."

Dr. Albert Siegel, University of California. "The Biology of Tobacco Mosaic Virus."

Prof. Frits Zernike, University of Groningen, Netherlands. "Facts and Fallacies about the Formation of Microscope Images, Including the Phase Contrast Image."

Dr. Irving Klotz, Northwestern University. "The Oxygen-Carrying Proteins: A Comparison of the Oxygenation Reaction in Hemocyanin and Hemerythrin with that in Hemoglobin."

Mr. Charles Horvath, Department of Zoology, University of Southern California. "The Biology of the North Pole."

Prof. Ralph Erickson, University of Pennsylvania. "The Use of Developmental Indices."

Dr. M. D. Thomas, Stanford Research Institute, Stanford. "Air Pollution."

Prof. Donald B. Lindsley, University of California at Los Angeles. "Brain Organization and the Role of the Brain Rhythms in Behavior."

Dr. W. S. Stewart, Professor of Horticulture, University of California, Riverside. "The Chemicalization of Agriculture."


Dr. M. C. Niu, Stanford University. "Recent Approaches to the Problem of Embryonic Induction."

Dr. Seymour Cohen, University of Pennsylvania. "Unbalanced Growth and Death; A Study in Thymine Metabolism."


Prof. Henry Gastaut, University of Marseille, France. "Psychomotor Epilepsy."

Dr. Bernard Horecker, The National Institutes of Health, Bethesda, Maryland. "The Pentose Phosphate Pathway in Carbohydrate Metabolism."

Prof. Erwin Chargaff, College of Physicians and Surgeons, Columbia University. "Universal Regularities in the Composition of Ribonucleic Acids and Their Bearing on Template Function."

GRADUATES

Twelve men were awarded B.S., M.S. or Ph.D. degrees in Biology in June, 1955. The names, degrees conferred, the titles of doctoral theses, and the plans of the men for future work are as follows:
Charles J. Brokaw, B.S. To the University of Cambridge, England.
John W. Brookbank, Ph.D. Thesis: "The Effect of Specific Antisera on the Cleavage of the Sea Urchin Egg." He has received an appointment as Assistant Professor of Zoology at the University of Florida.
Ernest A. Dernburg, B.S. To the Graduate School of the University of California at Los Angeles.
Ernest Dzendolet, B.S. To the Graduate School at Brown University.
Edwin J. Furshpan, Ph.D. Thesis: "On Certain Sensory and Motor Systems of Decapod Crustaceans." He has been awarded a National Science Foundation Postdoctoral Fellowship for research at the University College, London.
Joseph D. Mandell, Ph.D. Thesis: "Inactivation of Bacteriophage T4r by Specific Antiserum." Has been awarded a fellowship by the Carnegie Institution of Washington and will spend a year at the Carnegie Institution in Cold Spring Harbor.
John W. McKee, Ph.D. Thesis: "Indole Inhibition of Cofactor Activated Bacteriophage." He is now working on aircraft nuclear propulsion at Douglas Aircraft.
Bruce J. Rogers, Ph.D. Thesis: "Studies on the Amino Acid Metabolism of Higher Plants." He is now on the staff of Purdue University in the Department of Botany and Plant Physiology.
Roy M. Sachs, Ph.D. Thesis: "Floral Initiation in Cestrum nocturnum." Has been awarded a National Science Foundation Postdoctoral Fellowship for research at the University of Parma in Italy.
Toyoo Tomita, M.S. He will return to Japan.
David R. Viglierchio, Ph.D. Thesis: "Investigations of Some Plant Wound Hormones." He is now doing research at the University of California, at Davis, in the Department of Plant Nematology.
Donald A. Vogel, B.S. He is a development chemist at the Aerojet-General Corporation in Azusa.

GOSNEY FELLOWSHIPS

Dr. Peter D. Cooper was awarded a Gosney Research Fellowship to carry out a year's work in virology at the Institute. He has been on leave from the Wright-Fleming Institute, St. Mary's Hospital, London.

Dr. Paul R. Whitfeld was awarded a Gosney Research Fellowship to work in biochemistry at the Institute. He is a research officer with the Commonwealth Scientific and Industrial Research Organization in Australia.

NUTRITION FOUNDATION-McCALLUM FOUNDATION FELLOWSHIPS

Mr. David R. Viglierchio, a graduate student in bio-organic chemistry, held a Nutrition-McCallum Fellowship during the first term in 1954.

Mr. Edwin J. Furshpan, a graduate student in animal physiology, held a Nutrition-McCallum Fellowship during the second term in 1954.

ARTHUR McCALLUM SUMMER SCHOLARSHIPS

Support for graduate students who continue their thesis research during the summer months is provided by the Arthur McCallum Scholarships. In 1954, ten graduate students were awarded these scholarships.
LUCY MASON CLARK FELLOWSHIP IN PLANT PHYSIOLOGY

Mr. Roy M. Sachs held this fellowship beginning in September. His work was on the night-blooming jasmine and was carried out in the Earhart Plant Research Laboratory.

DU PONT FELLOWSHIP

The E. I. du Pont de Nemours and Company Postgraduate Fellowship in Biology was awarded to Mr. Robert L. Lester, a graduate student in biochemistry.

OTHER FELLOWSHIP APPOINTMENTS

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

During the year 1954-1955, six men held predoctoral fellowships awarded by an agency operating outside the Institute, usually on a national basis. These were distributed as follows:

<table>
<thead>
<tr>
<th>Foundation / Organization</th>
<th>Number of Fellowships</th>
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<tr>
<td>National Foundation for Infantile Paralysis</td>
<td>3</td>
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<tr>
<td>National Science Foundation</td>
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Thirty-two persons held nationally awarded postdoctoral fellowships during this period. These were distributed as follows:

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<td>American Association of University Women</td>
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<tr>
<td>American Cancer Society</td>
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<td>Commonwealth Fund</td>
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<tr>
<td>John Simon Guggenheim Memorial Foundation</td>
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<tr>
<td>Charles F. Kettering Foundation</td>
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<td>Merck Senior</td>
<td>1</td>
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<tr>
<td>National Academy of Sciences - National Research Council</td>
<td>1</td>
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<tr>
<td>National Foundation for Infantile Paralysis</td>
<td>3</td>
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<td>National Science Foundation</td>
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<td>Rockefeller Foundation</td>
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<td>Smith-Mundt</td>
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<td>Swiss Foundation</td>
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<tr>
<td>U. S. Public Health Service</td>
<td>9</td>
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The financial support that made the work 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. Government agencies; and from contributions to general funds provided by Industrial Associates and Institute Associates. During the year special endowment funds were set up in support of the Institute's program of research and instruction in chemical biology. Such funds will be matched out of the $1,500,000 contingent grant made to the Institute by the Rockefeller Foundation. The funds used during the year came from the following sources:

### Institute Endowment Funds

<table>
<thead>
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<th>Fund</th>
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<tr>
<td>William G. and Louise E. Kerckhoff</td>
<td>General biology support</td>
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<tr>
<td>A. C. Balch</td>
<td>General biology support</td>
</tr>
<tr>
<td>Mrs. Henry M. Robinson</td>
<td>Biological and chemical research, particularly as related to cancer</td>
</tr>
<tr>
<td>Charles B. Corser</td>
<td>General biology support</td>
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<tr>
<td>John H. Eagle</td>
<td>Biological and chemical research, particularly as related to disease</td>
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<tr>
<td>Frank P. Hixon</td>
<td>Neurophysiology, physiological psychology, and related research</td>
</tr>
<tr>
<td>E. S. Gosney</td>
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<tr>
<td>W. F. Prisk</td>
<td>Cancer research</td>
</tr>
<tr>
<td>Gloria Gartz</td>
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<tr>
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<td>Studies on plant cell ageing, protein synthesis, nucleic acids, and tissue differentiation</td>
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<tr>
<td>American Cyanamid Company</td>
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<td>James G. Boswell Foundation</td>
<td>Virus research</td>
</tr>
<tr>
<td>Carnation Company</td>
<td>Milk protein research</td>
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<tr>
<td>Carnegie Institution of Washington</td>
<td>Comparative physiological studies</td>
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<tr>
<td>Norman W. Church</td>
<td>General support</td>
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<tr>
<td>E. I. du Pont de Nemours &amp; Company</td>
<td>Predoctoral fellowship; nitrogen metabolism research</td>
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<tr>
<td>Earhart Foundation</td>
<td>Earhart Laboratory support</td>
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