The deoxyribonucleic acid double helix at the patio entrance to the new Gordon A. Alles Laboratory for Molecular Biology
A Report for the Year 1959-1960
on the Research and other Activities
of the
Division of Biology
at the
California Institute of Technology
Pasadena, California
RESEARCH REPORTS

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

Alles Laboratory

Approaching the Biology Laboratories centrally from Wilson Avenue, one faces the forecourt or patio of the new Gordon Alles Laboratory for Molecular Biology. Worked into the design of an iron-work fence are skeletonized formulas of molecules of biological significance — amino acids, hormones, etc. To the right of the patio entrance is a five-foot double helix in anodized aluminum. It is symbolic of DNA, molecular basis of heredity in creatures as simple as the smallest known virus and as large and complex as man. Beyond the patio with its attractive tables, benches, arbor shades and planting boxes is the entrance to the Alles Laboratory itself. A part of the Institute’s Development Program, this modern research laboratory became a reality through a generous gift by Doctor Gordon A. Alles — alumnus of the Institute (A. B., M. S., and Ph. D.), widely known pharmacological and organic chemist, and Research Associate at the Institute — plus a matching construction grant by the Health Research Facilities program of the United States Public Health Service. Alles Laboratory completes the Biology group in essentially the form planned more than thirty years ago by the architectural firm of Goodhue Associates for the Biology Division then established under the chairmanship of Thomas Hunt Morgan. Connecting Kerckhoff and Church Laboratories to form an H-shaped structure, it houses a central and efficiently organized Division Stockroom, a laboratory for electron microscopy under the direction of Professor Alan Hodge, newly arrived from the Massachusetts Institute of Technology, and other units of Biology such as psychobiology, biochemistry, biophysics, and immunogenetics that were previously inadequately provided for in Kerckhoff or Church.

Campbell Laboratory

The early riser or the night owl driving past the Division’s new Campbell Plant Research Laboratory just before dawn may well wonder why all the bright lights at such an hour. In this temperature-controlled laboratory, with its 2500 square feet of glass-covered growing area, 216 feet of fluorescent lights are turned on by time clock at four in the morning for a four-hour stretch and for a like period at four in the afternoon. They are there to provide for a daily 16-hour photoperiod throughout the year for the experimental plants that grow inside. Length of day is a critical factor in the lives of many plants and like temperature it must be controlled if reproducible experimental results are to be expected. Campbell Laboratory, another unit of the Development Program, was made possible by a sizable grant from the Campbell Soup Company plus a matching construction grant from the Health Research Facilities program of the United States Public Health Service. It supplements and complements the Dolk, Clark and Earhart Plant Research Laboratories, the four units being interconnected for convenience and efficient operation. Like Earhart it operates under a quarantine system designed to exclude insects and diseases. Not only does it add space for experiments, but in addition it provides a large area for growing plants under standardized conditions before and after they are subjected to one or more of the many combinations of environmental factors available in Earhart. In this way it
greatly increases the usefulness of the latter. Campbell Laboratory completes a long standing plan to consolidate all plant research work of the Division in a small area conveniently near the Kerckhoff-Church-Alles unit. With its completion and provision for some adjacent outdoor garden area, the ten-acre experimental garden in Arcadia and the Orlando Street Greenhouse in San Marino are no longer needed.

**Plant Physiology and Biochemistry**

In a small garden plot just north of Earhart Laboratory there grows a luxurious crop of eight-foot-high cockleburs. Pampered with floodlights and the best of culture, they are a far cry from their spiny-seeded brethren that the midwestern farmer laboriously machetes from his fields of corn during the hot humid weeks of August. Why raise cockleburs? These are a special breed and for many years they have been used at the Institute for investigations of the physiology of flowering. Fourteen years ago ancestral seeds were collected by Professor Bonner on Midway Airport, Chicago. After inbreeding for four years to insure uniformity, they are now grown as a special Caltech strain. The following paragraphs briefly review the general problem of induction of flowering in the cocklebur and in other plants, as a background into which samples of current work in this area can be fitted. Investigations on flowering have been and are likely to continue to be of major interest to Institute plant biologists.

The cocklebur is a good example of a so-called short-day plant. It begins to form flowers only if the nights are at least 8 1/2 hours long. This is why the seed plot is equipped with floodlights. The nights are kept shorter than the critical 8 1/2 hours to keep the plants in a vegetative state. In this way they grow very large -- and eventually produce a high yield of burs. They would never flower if the nights were always kept short. So, after the plants attain a large size, the floodlights are turned off. Within a few days flower primordia begin to form and within weeks a large crop of fine burs results. Since experimental cockleburs are started in the Campbell Laboratory, it is necessary that the nights there be kept shorter than 8 1/2 hours, for otherwise the plants would flower prematurely. This explains the evening and early morning light schedule. An interesting and long known bit of cocklebur lore is the fact that the burs, which a technical botanist calls fruits, contain two seeds each. One normally germinates the spring after it matures whereas the second defers its sprouting for another year. It is a matter of oxygen permeability. The second seed must age an extra year to become sufficiently permeable to this essential gas. It is a fine adaptive mechanism for bypassing a season disastrously unfavorable to the species.

What makes a cocklebur plant produce flowers when the nights become longer than 8 1/2 hours? Despite much work at the Institute and elsewhere, no one yet knows the complete answer. Nonetheless a great deal is known about the process. For example a single long night is sufficient to induce flowering. But if such a long night is interrupted by a flash of light a few minutes long, induction does not occur. This fact, incidentally, is used in a practical way to prevent flowering in sugar cane and thus increase yield of sugar. This is why there are rotating searchlights in the sugar cane fields of Hawaii. Only a single leaf needs to be given a long night. A black envelope slipped over one leaf of a plant at the proper time will do the trick. Since the rest of the plant then flowers, obviously something must move from the darkened leaf to the rest of the plant. Now if a part of a plant induced to flower by several long nights is grafted to an un-induced plant, the recipient of the graft will flower in short-night environment. The process of transfer by grafting can be repeated serially apparently indefinitely. What is the something that moves from the treated leaf? It has been assumed to be a flowering hormone -- a florigen. But it has not been identified chemically.
The fact that it can be multiplied more or less indefinitely, once induced, suggests that it may be virus-like. It has so far been transferred from plant to plant only through graft unions — that is, through direct contact between living cells. The induction of flowering can be inhibited by various chemical substances. For example, it has been found during the year that treatment with 5-fluorouracil, an analog of one of the pyrimidines of ribonucleic acid, prevents flowering in a cocklebur plant otherwise under conditions that would lead to flowering. This suggests that nucleic acid synthesis may be directly involved.

There are other interesting mechanisms by which flowering is induced. The peach tree requires for flowering two cold treatments, separated by several months of high temperature. Morea, an African iris frequently grown in Pasadena, flowers about a week after a day of low light intensity. Hence all Moreas in Pasadena bloom on the same day, regardless of their treatment in other respects. Tropical orchids require a sharp temperature drop for the opening of their flowers. The final enlargement and opening of the flower of the coffee tree likewise depends on a temperature drop. This often accompanies rain or results from it. This fact discovered in the Earhart Laboratory several years ago by Professor Went and coworkers may provide a basis for control of flowering of plantation coffee trees via overhead sprinklers.

The European black henbane plant Hyoscyamus, a member of the potato family, is normally a biennial plant. The first year it grows as a rosette. The second year it sends up a flower stalk, a process technically known as bolting. An annual variety of Hyoscyamus differs from the biennial by a single relevant gene. Hence a gene change of the appropriate kind can make cold treatment unnecessary. Professor Lang, recently arrived from U. C. L. A. and now in charge of the Clark-Earhart-Campbell Laboratory complex, has discovered that treatment with the plant hormones known as gibberellins will induce bolting and flowering in the biennial henbane. Any one of the three factors — hormones, the gene for annual habit, or the low temperature treatment — serves to speed up cell divisions in the growing part of the plant and to modify their orientations. In the rosette stage the cell divisions are few in number and are oriented pretty much at random with respect to the root-shoot axis. Bolting is a result of a large increase in rate of cell division and a shift in the distribution of the planes of division. It is found that after gibberellin treatment divisions are increased in frequency and the axes of the division spindles are largely parallel with that of the root-shoot axis. Thus the cells are multiplied predominantly in a vertical direction, rather than in a random way, and many times more rapidly. This is an example of work now being continued in the Earhart-Campbell laboratories.

Virus-induced Tumors

In last year's report it was pointed out that Rous sarcoma virus produces morphological changes in chick embryo cells in tissue culture. Presumably this change is associated with malignancy, for such cells transplanted to a chicken produce tumor. However the tumor may result indirectly, for the transplanted cells are known to release virus particles and these by themselves are capable of inducing the tumor. It has recently been shown that the so-called polyoma virus of the mouse will produce morphological changes in hamster embryo cells in tissue culture and that these cells, which do not release free virus, grow into tumors when implanted in a hamster. In this case the observed morphological change seems clearly associated with the ability of the cell to grow as a tumor. This is one of a series of investigations now in progress in the Institute's animal virus laboratories.
Investigations on flowering and tumor-inducing viruses are a small part of the total research activity of the Division. The abstracts that follow summarize work done during the year 1959-60 in these and other areas.

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Alfred H. Sturtevant, Ph.D., D.Sc.
Antonie van Harreveld, Ph.D., M.D.
Cornelis A. G. Wiersma, Ph.D.

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Biology
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Biophysics
Hixon Professor of Psychobiology

Visiting Professors

Boris Ephrussi, D. Sc.
Willis E. Pequegnat, Ph.D.

Biology
Zoology

Research Associates

Gordon A. Alles, Ph.D.
Henry O. Eversole, M.D.
Albert E. Longley, Ph.D.
William S. Stewart, Ph.D.
Jean J. Weigle, Ph.D.

Biology
Plant Physiology
Biology
Biology
Biophysics

* On leave

† U.S. Department of Agriculture
Senior Research Fellows

Jacob W. Dubnoff, Ph.D.
Henry Hellmers, Ph.D.
Geoffrey L. Keighley, Ph.D.
Matthew S. Meselson, Ph.D.
Marguerite Vogt, M.D.

1 U.S. Forest Service

Assistant Professor

Robert S. Edgar, Ph.D.

Visiting Assistant Professor

Charles J. Brokaw, Ph.D.

Instructor

Charles M. Steinberg, B.A.

Gosney Fellows

Harriet Ephrussi-Taylor, Ph.D.
Inge Rasmussen, Ph.D.

Research Fellows

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David Norman, Ph.D.
Masahiro Ogaki, D.Sc.
Eiji Ohnishi, Ph.D.
Daphne J. Osborne, Ph.D.
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<td>R. L. C. Filgrim</td>
<td>3 Ph.D. International Cooperation Administration Fellow</td>
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<td>Clifford J. Pollard</td>
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<td>Ernst Reinhard</td>
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<td>Joon Hee Rho</td>
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<td>Tripl cane K. Sundagaranjan</td>
<td>Ph.D. U.S. Public Health Service Fellow</td>
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<td>Alfred S. Sussman</td>
<td>Ph.D. U.S. Public Health Service Fellow</td>
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<td>Shosuke Takemura</td>
<td>Ph.D. U.S. Public Health Service Fellow</td>
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<td>Howard M. Temin</td>
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<td>Yasushi Watanabe</td>
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<td>Roger Weil</td>
<td>22 M.D. U.S. Public Health Service Fellow</td>
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<td>J. A. D. Zeevaart</td>
<td>24 Ph.D. U.S. Public Health Service Fellow</td>
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**Graduate Fellows and Assistants**

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<tr>
<td>Dennis Barrett, A.B.</td>
<td>(on leave) National Science Foundation Research Training Grant</td>
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<tr>
<td>Harris Bernstein, B.S.</td>
<td>John R. Menninger, 8 A.B.</td>
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<td>Juan P. Boszzi, B.S.</td>
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<td>E. R. Moser, M.S.L.S.</td>
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**Research Aides**

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<td>Yvette Alais</td>
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RESEARCH REPORTS

ANIMAL PHYSIOLOGY

STAFF:
Professors: A. van Harreveld, C. A. G. Wiersma
Graduate Student: Martin Mendelson
Student Assistant: Jan R. Conrad
Research Assistant: Ruth E. Estey
Research Aides: Yvette Alais, Elizabeth A. Constantian, George I. Hickey, Jr., Nancy G. Kennedy, Mary J. Martin

1. ELECTROPHYSIOLOGY OF SPINAL CORDS WHICH HAVE RECOVERED FROM ASPHYXIATION

Investigators: A. van Harreveld, D. Spinelli
Support: U.S. Navy, Office of Naval Research
General Funds

Preparations in which the spinal cord had been asphyxiated for periods between 30 and 90 minutes have been examined electrophysiologically. Where possible the following experimental arrangements were used:

1) Stimulation of the motor nerve to one of the gastrocnemius heads with single and double stimuli of various time intervals. Action potentials were led off from the appropriate ventral roots.

2) Inhibiting effect of stimulation of the sural nerve on the reflex action potentials produced by stimulation of the gastrocnemius nerve.

3) Stimulation of a dorsal root and leading off from the ventral root at the same spinal level and from the dorsal root of an adjacent segment (dorsal root reflex). Double stimuli of various time intervals were used.

4) Stimulation of the dorsal roots with frequencies of 50 per second and higher.

5) Recording of antidromic (Renshaw) inhibition.

6) Recording of dorsal root potentials. A series of control chronic spinal cats have been subjected to the same course of investigation.

Preparations in which the spinal cord was asphyxiated for periods of 30 to 50 minutes showed reflex action potentials after two weeks. These potentials were different from those in the control preparations in the following respects:

1) The (ventral root) reflex action potentials were smaller than in the controls.

2) There was less, sometimes no, multisynaptic activity. In the latter case the reflex action potential consisted of a single mono-synaptic spike.

3) There was less facilitation. In preparations which had been asphyxiated for the longest periods of time there was no facilitation whatsoever. With a time
interval of 1 to 2 msec between conditioning and test stimulus the second response was smaller than the first (refractory period). After 3 msec and longer the reflex response equaled the conditioning (maximal) response. This seems to indicate that in such cords there is no subliminal fringe that can be facilitated by a conditioning stimulus, which would be indicative of an increased excitability of the motor neurons.

4) The asphyxiated cords showed less inhibition than in the control preparations. Antidromic (Renshaw) inhibition was decreased and in some of the most severely damaged cords it was completely abolished. This decrease of inhibition is also manifest in a decrease of the depression of reflex activity caused by a stimulus of a dorsal root which in the normal cord usually lasts several seconds (post-activation depression). In the most severely damaged cords this depression was entirely absent.

5) The absence of Renshaw and post-activation depression makes it possible to drive the motoneurons at high frequency (50 per second and 100 per second) without a marked decrease of the reflex action potentials.

The dorsal root reflex is subject to a similar post-activation depression as the ventral root reflex. This depression was also decreased in the asphyxiated preparations and was completely absent in the most severely damaged ones. It was found that the dorsal root reflex was still present in preparations in which all neuronal elements had been killed by long (90 minutes) asphyxiation.

2. A HISTOCHEMICAL METHOD FOR THE DETERMINATION OF THE LOCATION OF CHLORIDE AND SOME OTHER ELECTROLYTES IN TISSUE

Investigators: A. van Harreveld, R. L. Potter
Support: National Institutes of Health, Public Health Service
General Funds

A histochemical method for chloride (and some other anions) based on substitution fixation of tissue in alcoholic silver nitrate solutions at -25°C has been used previously. The precipitated silver compound can be made visible in histological sections of the treated tissue by exposure to light. This method was subjected to a thorough investigation.

It could be shown that only water-(alcohol)-soluble compounds are detectable with this method, since previous alcohol fixation and leaching of the tissue with distilled water yielded unstained preparations with the silver substitution method. It therefore seemed possible to investigate the reacting matter in extracts of tissue (cerebral cortex). The extracts were chromatographed and showed after spraying with silver nitrate and exposure to light four spots, three of which could be identified as chloride, phosphate, or carbonate. The fourth spot is probably an organic anion; it has not been identified. The ratio of these various compounds in producing the brown material in the histological preparations was determined. About 2/3 of the color is caused by the chloride, 1/6 by the phosphate, 1/20 by the carbonate and about 1/10 by the organic anion. By treating either the tissue or the histological preparations with nitric acid all the silver precipitates but the chloride can be dissolved, making it possible to study the location of the chlorides only.
3. ELECTROLYTE MOVEMENTS IN CEREBELLAR CORTEX AFTER CIRCULATORY ARREST

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

Previously an asphyxial transport of electrolytes (mostly chloride) and water from an extraneuronal space into the apical dendrites of nerve cells in the cerebral cortex and in the Ammon's horn was observed. This transport is accompanied by a marked increase of the impedance of these structures. This investigation was extended to include the cerebellum. Circulatory arrest also produces an impedance increase of the cerebellar cortex. Histological preparations of this tissue treated with the histochemical method mentioned above showed an electrolyte transport into the main dendrites of the Purkinje cells in some preparations; in other preparations the transport had taken place primarily into fibers which ran straight through the molecular cortex, from close to the granular layer to the cortical surface. The appearance of these fibers suggested they were the fibers of Bergmann, a cellular element generally considered to be of glial nature. This identity of the fibers was supported by the observation that the ratio of these fibers with respect to the Purkinje cells is the same in these preparations as in preparations stained especially for Bergmann fibers. A 30% swelling of these fibers was found, attesting to the water transport which takes place in addition to the electrolyte movement. The interest of this observation is that the cell membrane changes which have been considered previously to be responsible for the electrolyte transport do not seem to be restricted to neuronal elements, but can also occur in glial fibers.

4. EFFECTS OF CEREBRAL ASPHYXIATION ON CONDITIONED REFLEXES

Investigator: A. van Harreveld
Support: National Science Foundation
General Funds

A few minutes after cortical asphyxiation there occurs a rather sudden swelling of the apical dendrites. This volume increase is quite considerable and is accompanied by a massive transport of electrolytes into the same structures. Such major changes in the cortex, which develop before actual cell destruction due to lack of oxygen sets in, could conceivably affect the memory traces developed during the acquisition of a conditioned reflex.

Rabbits, which show the asphyxial changes in a marked degree, were conditioned in a Skinner-type box in which the animals by pressing a lever were rewarded with food. After a number of reinforcements (ranging from 600 to 20) the brain was asphyxiated by raising the pressure of the cerebrospinal liquor above the blood pressure. Periods of asphyxiation of 12 minutes or longer resulted in histologically observable damage to the brain. Especially a decrease in the number of Purkinje cells in the cerebellum was observed. Periods of asphyxiation of 8 and 10 minutes were used on the trained animals. These times in general produced little or no cell death, but were ample to cause the transport of water and electrolytes mentioned above. About 20 preparations have been studied up till now. The effect on the conditioned reflex has been small. In most of the animals the reflex was present a few days to one week after the asphyxiation. In a few animals the reflex remained absent for a month after the asphyxiation. These animals were then again taught the reflex, which it was possible to do in a
very short time. It would seem from this that the ion and water transport causing large volume changes of the apical dendrites and to a smaller extent of the pyramidal cells has little effect on the memory trace.

5. METRAZOL AND CELL PERMEABILITY

Investigators: R. L. Potter, A. van Harreveld
Support: National Institutes of Health, Public Health Service

The convulsant drug metrazol has been shown to influence the physiological effect of L-glutamic acid on the cerebral cortex. Experiments with uniformly labeled C-14-L-glutamic acid and rat brain slices indicated that metrazol may slow down some metabolic reactions which utilize this amino acid. Yet ammonia production by cortical slices or whole brain homogenates was unaffected by 3.5 x 10^-3M metrazol.

The possibility that the drug might affect the permeability of cells in brain slices was tested for influx of glutamate and its efflux. About 100 mg brain slices accumulate almost all of 10 µM of L-glutamate from buffered-glucose-medium in two hours at 38°C. The presence of 3.5 x 10^-3M metrazol did not alter this inward movement of the amino acid.

However in the same medium lacking glucose the endogenous amino acids move out of the slices and in 1 to 2 hours reach a measurable level in the medium. However if metrazol is added the amino acid level rises as high in 1 to 2 minutes and remains essentially at this level for an hour.

Attempts are now being made to quantitate these results and establish which amino acids participate in this outflow. Glutamate appears to be the prominent amino acid.

6. POST-SYNAPTIC EXCITABILITY AFTER ANTIDROMIC EXCITATION IN THE MONO-SYNAPTIC REFLEX

Investigator: D. Spinelli
Support: U.S. Navy, Office of Naval Research

Post-synaptic excitability in a reflex arc was tested by the method of antidromic invasion of the cell-bodies, as proposed by Renshaw. It was found that post-excitation depression of the mono-synaptic reflex is due to at least two factors. Following antidromic stimulus there is a marked depression of post-synaptic excitability, as expressed by the decreased number of cell-bodies invaded by the antidromic testing pulse; this depression does not last more than 150 msec. After this period the excitability of the somas appear to be normal. If the reflex arc is tested orthodromically the depression is still about 50% at 300 msec. This result localizes the late part of the post-excitation depression in the afferent fibers. The results obtained are in agreement with those presented in the literature and obtained with more indirect methods.
7. EFFECTS OF THE RETICULAR FORMATION ON THE RECOVERY CYCLE OF A SPINAL REFLEX

Investigator: D. Spinelli
Support: U.S. Navy, Office of Naval Research

Stimulation of the bulbar reticular formation with 200 per second rectangular pulses of .1 msec duration and about seven volts' intensity modifies markedly the recovery cycle of the monosynaptic reflex activity. The early facilitation of the second response (between 1.5 and 5 msec) is strongly depressed without changes in the amplitude of the first one. The late part of the recovery cycle is also markedly affected. The reduction of the second response typical for that part of the cycle is less pronounced and the duration of the cycle is very much reduced. There can be a complete disappearance of depression at 200 msec during reticular stimulation, whereas without stimulation the amplitude of the second response is usually about 30 to 40% of the first. A similar reduction of the depression of the response of the dorsal root reflex to a second stimulus has been observed. These findings suggest that higher centers may control excitatory interneuronal chains and that the late part of the recovery cycle of the monosynaptic reflex should not be ascribed to depletion of transmitter substance but to activity of inhibitory interneurons.

8. THE FUNCTIONAL SIGNIFICANCE OF THORACIC RECEPTORS IN DECAPODS

Investigators: C. A. G. Wiersma, R. L. C. Pilgrim
Support: National Science Foundation
International Cooperation Administration
General Funds

In the thoracic cavity of lobsters, Alexandrowicz (1952) has reported the presence of five sense cells, whose dendrites intermingle with normal muscle fibers. This is in contrast to the dendrites of the stretch receptors in the abdomen and the posterior thorax in which they connect with specialized receptor muscles. The function of the five N-cells was problematic. In the crayfish (Procambarus clarkii) and in the rocklobster (Panulirus interruptus) it was found that each cell's axon ran in a different main thoracic nerve, and that an unbroken series of receptor cells is present from the first thoracic up to the fifth abdominal ganglion. The N-cells react to stretch of the muscle on which they are located, quite similarly as the slowly adapting stretch receptors of the abdomen, though with lower frequencies of nerve impulses. They are now considered to be homologous with the slowly adapting stretch receptors of the abdomen. In the crayfish the two anterior cells are placed on a small slip of the muscle which attaches the carapace to the rest of the body and discharge when a pull on this muscle is present. This might come about by filling of the thoracic cavity, or by an increase in blood pressure. Of the more posterior cells, two are located on a muscle, the contractor epimeralis, which has an opposite effect, and the signals of these cells are increased under opposite circumstances. The fifth cell signals stretch of a muscle when the abdomen is moved sideways on the thorax. In the rocklobster the three anterior cells are located on a slip of the attractor epimeralis, the last two on the abductor of the abdomen, and the contractor epimeralis muscle is absent. Sideways movement of the tail in both species is also signalled by the first of the slowly adapting stretch receptors with a specific receptor muscle, whereas the last of the thoracic receptors indicates bending of the first abdominal segment of the tail on the thorax. In contrast to all other stretch receptors, those signalling side motions are reciprocal in their activity on the two sides. All
receptors are about equally sensitive to the exciting influence of acetylcholine and the inhibiting one of γ-aminobutyric acid, though all anterior cells appear to be without inhibitory innervation. Since in the true lobster the five anterior cells are again differently arranged, it is concluded that these cells have originated from slowly adapting receptors which lost their muscular part and are now free to attach themselves to any other muscle whose state of stretch may be of functional significance.

9. THE ANATOMICAL PHYSIOLOGY OF THE CRAYFISH THORACIC NEURO-MUSCULAR APPARATUS

Support: International Cooperation Administration
National Science Foundation
General Funds

During the preceding experiments it was observed that the descriptions of the thoracical musculature and of the segmental innervation in the crayfish as given by Schmidt and by Keim, which are considered standard, were seriously at fault in certain aspects. This has led to considerable confusion in the nomenclature of muscles, nerves and sense organs. Hence a close investigation of different aspects of anatomy, checked by physiological experimentation, was needed. Certain muscles described as lateral extensor muscles of the thoraco-abdomen were found not to extend the tail but to move it sideways. For these the name M. abductor abdominalis has now been coined. The insertion of this musculature is not, as previously thought, on the first abdominal segment, but on a remarkable set of three chitinous pieces, in shape not unlike the auricular ossicles. To this structure the name secula has been given. It was found that the second roots of the thoracic ganglia, through which the axons of the stretch receptors enter, show a course which makes it easy to mistake the root of a given ganglion for a root of the next posterior ganglion. This has led to a wrong nomenclature for the segmental thoracic nerves. In addition it has become necessary to rename all stretch receptors in such a way that from now on those from one species can be homologized with those of another. Whereas in the crayfish as in the rocklobster, all main second roots of all thoracic and abdominal ganglia up to the fifth abdominal ganglion carry the axons of a slow adapting receptor, in the rocklobster an additional fast receptor appears only from the sixth on; in the crayfish, not before the seventh nerve. In crabs no stretch receptors were found at all, though the epimeral attractor muscle is certainly as extensive as in the previous species, and correspondingly anterior slow adapting cells were expected.

10. FURTHER DATA ON FACTOR I IN MAMMALIAN BRAIN

Investigators: G. Hickey, C. A. G. Wiersma
Support: National Science Foundation

It was shown last year that Factor I of mammalian brain contains in addition to γ-aminobutyric acid and to γ-hydroxybutyric acid a component which has no acylatable amino group. During the past year several attempts have been made to elucidate the composition of this component. These trials have so far not met with success, mainly because the strong activity of fresh preparations, as tested on the crayfish stretch receptor, disappeared the more the substance was purified.
It has been shown by Wiersma that the legs of certain decapod crustacea (Pachygrapsus) contain organs specifically sensitive to the velocity of movement of the joints. He has further shown that each component cell of the organ begins to show activity at some characteristic velocity and then reaches a maximum output at some higher characteristic velocity, the range usually being quite narrow. In each organ a number of elements, each with a different sensitivity, is present. These units all possess a characteristic maximal frequency and each unit responds specifically for movement in one direction.

It is of interest to know the intracellular events which give rise to these characteristics and to this end an investigation is in progress in which ultrafine micropipette electrodes are inserted into the cells of the receptor organ and the leg is passively moved at various velocities. The electrical changes inside the cells are recorded. Preliminary data indicate that the movement stimulates the cells by causing a positive change in their intracellular potential which is proportional to the velocity of the movement and which reaches a level where the cell fires spikes. A negative change of potential appears to take place for the direction of movement to which the cell is insensitive and thus does not arouse nerve impulses. No satisfactory data yet exist for an explanation of the quick saturation of impulse frequency reached during fast movements or for the constancy of the maximal frequency.
12. BIOLOGICAL SYNTHESIS OF PROTEIN

Investigator: H. Borsook
Support: National Institutes of Health, Public Health Service
U. S. Atomic Energy Commission

The biological material used has been rabbit reticulocytes. These are obtained from the blood of rabbits made severely anemic with phenylhydrazine. Usually after six daily subcutaneous injections of phenylhydrazine practically all the circulating red cells are reticulocytes. C-14-labeled amino acids are used to follow the synthesis of protein.

In order to maintain the maximum rate of protein synthesis the saline solution in which the cells are suspended needs to contain a mixture of amino acids, an energy source (glucose), iron and an iron-chelating agent.

Added iron has a synergistic action with the added amino acids and glucose. The longer the incubation the greater is the need for added iron. In order to maintain the maximum effect of the added iron it is necessary to add a chelating agent to the reaction medium, otherwise the added iron becomes unavailable. Chelating agents we have found useful for this purpose are fructose-amino acids which are present in liver, citrate or one of the proteins in plasma.

The influence of the protein in plasma is seen best as a factor which contributes to the maintenance of the initial rate, without which it falls off progressively, so that in four hours the rate without the added plasma is only 50% of what it is in the first hour.

The effective protein in plasma, previous experiments indicate, may be transferrin. But this has not yet been proved rigorously. The purification of the effective protein is in progress; the best preparations obtained so far exert their maximum effect at a concentration of 40 µg per ml.

The main difficulty in the purification of the effective protein is associated with separation of transferrin from haptoglobin. In testing the interaction of reticulocytes and plasma from a number of different species it was found that homologous plasma was always the most effective, and that the effectiveness of any one heterologous plasma was variable on reticulocytes of a number of different species. It would appear that in the transfer of iron from transferrin to the reticulocyte cell wall there is a more or less specific fit of the transferrin on the cell wall, i.e. it is analogous to an immunological phenomenon. And the iron
receptor in the cell wall has an affinity for iron as great or greater than has transferrin, which is the protein in plasma with much the greatest affinity for iron.

When reticulocytes are lysed their rate of incorporation of labeled amino acids into protein is 0.1% (or less) of that in intact cells. Furthermore, whereas the process continues in intact cells for at least four hours without much, if any, slowing down, in lysed cells or preparations from these even the very slow rate (compared with that of intact cells) stops within one half hour's incubation. Nevertheless by using sufficiently heavily labeled amino acids it was possible to observe the synthesis of hemoglobin (and other proteins) in a system derived from lysed cells containing microsomes, pH 5 enzymes, S-RNA and supernatant solution enzymes. It is possible thus to combine cell fractions from different species, e.g., rabbit microsomes with sheep pH 5 enzymes, etc.

Two findings have emerged from such experiments. One is that the hemoglobin species into which the C-14-amino acid was incorporated predominantly was that of the microsome and not that of the other cell components used. This finding suggests that the template for hemoglobin synthesis in reticulocytes is in the microsomes. If this suggestion is confirmed the next question is how the hemoglobin passes from the microsome template out into the solution. A second finding made with this cell-free system appears to bear on this question. There appears to be in the supernatant solution (after removal of microsomes, pH 5 enzymes and S-RNA) a factor or factors which are involved in the counts appearing in the hemoglobin free in the solution. One of the difficulties in working with the cell-free system is that of getting the labeled amino acid which has been incorporated into the microsome protein to pass from the latter into the solution as soluble protein. As stated above our findings suggest that such a process may be dependent on factors in the supernatant solution.

Much experimentation has been devoted to studies on lysed reticulocytes with the aim of greatly increasing the rate of synthesis of protein and of nucleic acids. In this connection the reversal of lysis is being studied in both erythrocytes and reticulocytes. Last year a report appeared in which an observation of 35 years ago was revived: that when the solution in which erythrocytes have been lysed with water is again made isotonic the cells again are able to retain their hemoglobin and potassium ions, they regain the ability to behave as osmometers. This observation was confirmed. The lysis of reticulocytes can also be reversed, but they differ from erythrocytes in two important respects found so far: 1) the reversal of the lysis of erythrocytes can be effected with saline solutions whereas only in plasma is there any significant degree of reversal of reticulocytes; 2) whereas 100% reversal of erythrocytes appears to have been obtained, about 66% is the highest degree of reversal of reticulocytes obtained so far. A small amount of plasma gives a higher reversal of lysis also of erythrocytes.

In the process of the maturation of reticulocytes most of their RNA disappears. An outcome of studies of the maturation process was the finding of a ribonuclease in reticulocytes. Such an enzyme is present in the microsomes and attached to the water-insoluble proteins. In intact microsomes the enzyme appears to be inactive. When the microsome protein was digested with chymotrypsin the ribonuclease became active. When the microsomal RNA was attacked by pancreatic ribonuclease the microsomal protein was more rapidly digested by trypsin. These observations suggest that the attachment of protein to RNA in ribosomes protects both from digestive enzymes.

Other workers in this Institute have taken up studies of hemoglobin synthesis in reticulocytes in vitro. They were encouraged and helped by the working out of conditions for a high rate of amino acid incorporation in vitro into the hemoglobin
of rabbit reticulocytes. Similar conditions were found to be operative on reticulocytes of other species: guinea pig, human, rat, sheep. An example of one such study is that of Dr. Vinograd's (Crellin Laboratory, California Institute of Technology) in which he was able to make and demonstrate hybrids of normal and S-hemoglobin by dissociation and recombination of their constituent peptides.

13. ERYTHROPOIETIC FACTOR

Investigators: Geoffrey Keighley, Peter H. Lowy, Henry Borsook
Support: American Cancer Society
General Funds

Some severely anemic animals have in their blood a hormone which when injected into a normal animal causes an increase in red cell volume. The hormone may be found in anemic human blood and sometimes is excreted in the urine. Highly enriched materials have been isolated from several sources, all of which are mucoproteins. Similar mucoproteins without activity, however, can be isolated from normal animals. It has now been shown that the enzymatic removal of sialic acid destroys the activity. Sialic acid, a characteristic constituent of mucoproteins, appears to be an integral part of the active molecule.

In collaboration with Dr. Elizabeth Russell work has continued with genetically anemic mice, which unlike normal mice do not respond to the erythropoietic hormone. Red-cell-forming tissue from normal mice, transplanted to anemic mice, does respond to the hormone.

The standard sample of the hormone which was distributed by this laboratory last year has been assayed in six other laboratories, by ten different methods, with satisfactory agreement. A report has been prepared for publication.

In collaboration with Dr. Denman Hammond at the Children's Hospital of Los Angeles a survey has been made of the amount of the hormone in the serum and urine of a sample of patients with congenital hypoplastic anemia.

Publications:


A recent review of this subject:


14. ERYTHROPOIETIC-STIMULATING FACTOR FROM URINE OF ANEMIC RABBITS

Investigator: G. S. Hodgson
Support: International Cooperation Administration
American Cancer Society

An erythropoietic-stimulating factor (ESF) has been obtained from urine of rabbits, made anemic by phenylhydrazine treatment, using ethanol fractionation and ion-exchange chromatography on a DEAE column. ESF from rabbit urine is a
mucoprotein similar in properties to ESF obtained from rabbit plasma, as concerns its behavior on DEAE, its thermostability and the fact that it is inactivated by neuraminidase and trypsin.

15. STUDIES ON THE RETICULOCYTE RIBOSOME

Investigator: T. A. Sundararajan
Support: National Institutes of Health, Public Health Service

A good deal of evidence has accumulated implicating microsomal particles in protein synthesis. The present studies were aimed at obtaining more information on the nature of these particles with particular reference to the mode of attachment and formation of the ribonucleoprotein complex (ribosome). Reticulocytes were used as starting material in view of the relative ease of isolating the ribosome from them, and also for the reason that these cells make a single type of protein, namely, hemoglobin.

The isolation and characterization of RNA from microsomes has been reported by several workers. The isolation of the structural protein in native state presents some difficulty since the use of detergents or ribonuclease for disruption of the ribosome results in the formation of protein with poor solubility. By using acetic acid to disrupt the ribosome it was possible in the present studies to isolate the protein in the native state in a 35% yield. The protein contained only traces of nucleic acid material (less than 2%) as judged by its UV absorption spectrum and behaved as a basic protein in its solubility characteristics and electrophoretic mobility. Electrophoresis on starch gel at pH 4.6 showed the protein to be comprised of at least two major components and several minor ones. The protein formed an insoluble complex with nucleic acids, a ribonuclease digest of RNA and ATP; it was not formed with nucleoside monophosphates. This suggests an involvement of secondary phosphate groups of the nucleic acid in the complex formation with the protein. The ribonucleoprotein complex was readily degraded by trypsin and by ribonuclease at rates comparable to that observed with the separated RNA and protein components of the ribosome.

The enzymatic properties, if any, of the microsomal protein are being investigated. The possibility of reconstituting the ribonucleoprotein complex, following disruption of the ribosomes, is being explored.

16. FACTORS DETERMINING THE SPECIFICITY OF HEMOGLOBIN SYNTHESIZED IN A CELL-FREE SYSTEM

Investigators: Hildegard Lamfrom, Miriam Wright
Support: National Institutes of Health, Public Health Service

In the cell-free system from reticulocytes, hemoglobin is synthesized which is characteristic of the cell species. The major objective of this study was to determine how the specificity in hemoglobin synthesis was attained. Cellular components -- microsomes, pH 5 fraction and supernatant -- were prepared from the reticulocytes of rabbit and sheep. Various combinations of these components were incubated under appropriate conditions. C-14-leucine and C-14-valine were used to label the newly formed hemoglobins, which were first separated from the microsomal particles and then isolated by chromatography. In all experiments the highest incorporation of C-14-amino acids occurred in the hemoglobin corresponding to the microsomal fraction. This lends support to the view that the chromatographic characteristics of the final protein, and presumably the amino acid sequence, are determined in the microsomal particle. However,
in every mixed system a significant amount of both types of hemoglobin was synthesized. The possibility exists, therefore, that there may also be some specificity determined by the supernatant.

Publication:


17. THE RELEASE OF PROTEIN FROM THE MICROSOMAL INTO THE SOLUBLE PHASE: AN ADDITIONAL EXPERIMENTALLY DISTINGUISHABLE STEP IN PROTEIN SYNTHESIS

Investigators: Hildegard Lamfrom, Miriam Wright
Support: National Institutes of Health, Public Health Service

Of all the cell-free systems thus far studied, only in the reticulocyte and pea systems does a large fraction of the incorporated radioactive amino acids appear in soluble protein. In other systems the synthetic process seems to stop with the assembly of protein or large peptides on the microsomes. Experiments in this laboratory indicate the existence of a factor in the supernatant fraction of reticulocytes which seems to be concerned with the release of soluble protein material, i.e., hemoglobin, from microsomes. Similar fractions from other sources, e.g. guinea pig liver, do not seem to have this activity. The factor from all reticulocytes tested (rabbit, sheep, duck, and human A and S) functions interchangeably in all the systems listed. However the factor in the reticulocyte supernatant did not mediate the release of radioactive proteins from microsomes in the cell-free systems of lymphocytes of immunized rabbits, suggesting that the factor is specific for hemoglobin.

18. DOES SOLUBLE RNA ACCOMPANY THE AMINO ACID INTO THE MICROSOME?

Investigators: Hildegard Lamfrom, Miriam Wright
Support: National Institutes of Health, Public Health Service, Gosney Fund

In the currently accepted scheme of protein synthesis one of the steps is thought to involve the coupling of a specific amino acid to a specific soluble RNA. This event is believed to be followed by the transfer of the amino acid to the microsome. It is not yet clearly established whether or not the soluble RNA, or a portion thereof, accompanies the amino acid into the microsome.

In an attempt to test for this possibility the following experiment was carried out. Soluble RNA was labeled with C-14-adenosine and/or C-14-uridine by incubating whole rabbit reticulocytes under appropriate conditions. The labeled soluble RNA was isolated and subsequently incubated in a cell-free system together with unlabeled microsomes, supernatant fraction, ATP and GTP. The microsomes were reisolated after incubation and analyzed for radioactivity. In control experiments the incorporation of C-14-amino acids was followed both into microsomes and into soluble protein.

Preliminary results indicate that soluble RNA does become associated with microsomes during amino acid incorporation. It appears that no stable linkage is formed between soluble RNA and the microsome and that perhaps only one soluble RNA is associated with one microsomal particle at any one time during
a period when the microsomes incorporate amino acids at a high level. The possibility seems ruled out that the polymerization of amino acids into a polypeptide is accompanied by the polymerization of soluble RNA on the microsome on anything like an equimolar basis.

Alfred Gierer, who was in residence at the Institute as a Gosney Fellow during 1958-1959, collaborated in this work.

19. PEPTIDES AND OTHER AMINO ACID DERIVATIVES IN DROSOPHILA LETHAL MUTANTS

Investigator: H. K. Mitchell
Support: National Science Foundation
General Funds

Prior to the initiation of the research carried out in Zürich it was observed that the fly Drosophila contains a large number of peptides and other little known amino acid derivatives. With the objective of ascertaining how these substances are concerned with protein synthesis, detailed analyses were made of these materials in three lethal mutants of the organism. Two of the mutants 1 tr and 1 me were known to have abnormalities in protein synthesis and were found also to have abnormal patterns of peptides and other amino acid derivatives. A third mutant, 1 21, did not differ significantly from the wild-type fly. Time did not permit examination of other available mutants since each of the above required analyses of several hundred fractions. However, the correlation between abnormal peptide patterns and protein synthesis deficiencies is encouraging and excellent material for further work has been provided.

Identification of one of the non-peptide amino acid derivatives prominent in Drosophila (tyrosine-O-phosphate) was nearly completed and enzymological work on its biological importance was initiated.

All phases of the work of the past year, which was carried out at the University of Zürich, will now be continued at the California Institute of Technology.

20. DEVELOPMENT OF URICASE IN THE ONTOGENY OF THE EUROPEAN GRASS FROG

Investigator: Dennis Barrett
Support: National Science Foundation

Phase specificity of the appearance of enzymes is an interesting phenomenon of itself, and may facilitate a study of the control of enzyme synthesis in general. In this project an enzyme was sought which would be present in later stages of embryogeny, but absent in the earliest stages. Then an interval would be available when various agents could be tested to see whether they could speed or slow the enzyme's appearance in a system of known genetic competence.

After a search, uricase in the European grass frog was found suitable. A crude spectrophotometric assay technique was developed, and the normal enzyme levels throughout ontogeny were charted. Whereas uricase is undetectable until stage 18 (Kopsch) -- with heavy feeding at 18°C, about the 17th day after fertilization, just after disappearance of the swimming tadpoles' external gills -- at this stage it does appear and then rapidly increases, at least 200 fold before metamorphosis. Protein determinations for specific activity calculations are not yet complete.
The enzyme has been traced to the liver and pancreas. Dissection and use of these organs only has facilitated more sensitive determinations, but failed to reveal any activity before stage 18. While it cannot be said that the enzyme is absent in early stages, the upper limit which would go undetected by the method is quite low. And it can certainly be said that a phase specificity attaches to the large-scale proliferation of the enzyme.

Attempts to influence the course of uricase development with inhibitors of the enzyme, using injected or soaked eggs, have not yet been definitely successful. Work will continue in this direction, as well as in characterization and purification of the enzyme and clarification of the somewhat anomalous results indicating that it is mitochondria-bound.

This work was done at the University of Zürich.

21. MORMONIELLA BIOCHEMISTRY

Investigator: Dennis Barrett
Support: National Science Foundation

The biochemistry of hymenoptera is as yet unexplored. A modest beginning consists in studies of the fluorescent compounds in the small parasitic wasp Mormoniella, advantageous for chemical genetic studies because many morphological mutants are already available.

In addition to other pteridines and several compounds as yet unidentified, an inventory included 2-amino, 4-hydroxy-pteridine and isoxanthopterine. Since 2,4 in other systems is the only known precursor of isoxanthopterine, the wasps were tested for the presence of the enzyme which accomplishes this transformation, xanthine dehydrogenase. In a chromatographic-fluorimetric modification of Glassman and Mitchell's method, wasp extracts did indeed show a weak xanthine dehydrogenase activity, to the extent roughly of 15 µ moles per hour per gram of protein nitrogen.

Qualitatively, adenosine deaminase activity was demonstrated, but uricase activity was lacking. Excretion patterns are now under investigation.

This work was done at the University of Zürich in collaboration with George B. Saul II, Zoology Department, Dartmouth College.

Publication:

22. BIOCHEMICAL STUDIES ON POKY

Investigators: Boyd Hardesty, H. K. Mitchell
Support: National Institutes of Health, Public Health Service General Funds

A previous report (Biology 1959) has described the presence of an electrophoretic heterogeneity in the cytochrome c isolated from poky, a mutant of Neurospora having a maternally inherited phenotype. Further work has indicated that the primary component of cytochrome c isolated from fast poky, mi 3 or
wild-type Neurospora is electrophoretically very similar to or identical with the major component of poky cytochrome c.

Young poky accumulates up to 16 times the amount of cytochrome c found in wild-type Neurospora. The distribution of this cytochrome in the subcellular fractions of three-day-old poky has been found to be quite different from that of wild type, in which the cytochrome c is found primarily in the mitochondrial fraction. Only a small portion of the cytochrome c of homogenized poky was precipitated by high-speed centrifugation. The amount of cytochrome c present in mitochondria isolated from poky is approximately equal to that found in mitochondria from wild type. In such cases most of the cytochrome c remains in the supernatant or soluble fraction, comprising about 25% of the total protein of that fraction.

Poky has also been found to contain relatively large amounts of free (not bound by covalent bonds) fatty acid. Less than 4% of the total fatty acid of wild type is present as the free acid compared with about 47% for three-day-old poky. Further, the specific composition of the free fatty acid of poky only poorly reflects the composition of the bound fatty acids, a finding suggesting that the free fatty acids did not arise directly from the bound fatty acids by degradative processes.

23. FATTY ACID ACTIVATION OF CYTOCHROME C TO DESTRUCTIVE OXIDATION BY HYDROGEN PEROXIDE

Investigators: Boyd Hardesty, H. K. Mitchell
Support: National Institutes of Health, Public Health Service
General Funds

Herzenberg (California Institute of Technology, Ph.D. thesis, 1956) isolated an acidic ether soluble material from poky, a maternally inherited mutant of Neurospora, which was capable of inducing a very rapid decline in the Soret band absorption of cytochrome c in the presence of low concentrations of hydrogen peroxides. This material is apparently identical with the free fatty acid found in relatively large amounts in poky. The reaction has been studied in detail using horse heart cytochrome c with the common fatty acids and some related compounds. Palmitic acid was found to have the highest activity on a molar basis, although lauric acid and laurel sulfate gave the highest maximum rates of destruction.

The hydrogen peroxide oxidation is a complex reaction apparently involving at least the oxidation of the porphyrin. Free fatty acids apparently affect cytochrome c by modifying the tertiary structure of native cytochrome c so that the porphyrin is more directly exposed to attack by hydrogen peroxide.

Fatty acids have also been shown to impair the reduction of the iron of cytochrome c by ascorbic acid and to have a hyperchromic effect on the Soret band absorption of the molecule. Although most work has been done using horse heart cytochrome c, similar effects have been demonstrated with cytochrome c isolated from Neurospora.

24. γ-PEPTIDES OF GLUTAMIC ACID AND SOME OF THEIR PROPERTIES

Investigator: Hans-Joachim Burkhardt
Support: National Science Foundation

In the course of a study on the biosynthesis of peptides and proteins in this laboratory it became of interest to investigate some special properties of
synthetic γ-glutamyl peptides. For this reason the γ-glutamyl di-, tri-, and tetrapeptides were synthesized by coupling the appropriate N-carbobenzoxy benzyl esters containing a free γ-carboxyl group with glutamic acid dibenzyl ester using the mixed anhydride method developed by Boissonnas (R. A. Boissonnas, Helv. Chim. Acta 34, 874, 1951). The resulting carbobenzoxy benzyl esters were reduced to the free peptides with palladium and hydrogen.

The optical purity of the free peptides was confirmed and studies on the stability of the γ-peptide bond, the chromatographic behavior, the optical rotation and the reaction of the peptides with ninhydrin before and after hydrolysis were performed.

25. THE ISOLATION OF A VERY ACTIVE DERIVATIVE OF VITAMIN B₁₂

Investigator: J. W. Dubnoff
Support: National Institutes of Health, Public Health Service

Several new derivatives of vitamin B₁₂ have been isolated in recent years and a coenzyme function has been ascribed to at least two of these. The mechanism of action has not yet been defined nor are these factors active at biological concentrations of vitamin B₁₂.

Recently a new form of the vitamin was isolated in this laboratory. It is effective at concentrations as low as 10⁻¹² molar in activating certain preparations of tyrosine oxidase.

Other enzyme systems are being studied to determine whether this factor is a non-specific activator of inactivated enzymes as appears to be the case from earlier work on the effect of vitamin B₁₂ on enzyme systems in E. coli.

26. STUDIES ON OZONE CRACKING AND ON THE CONCENTRATIONS OF OXIDES OF NITROGENS IN TOBACCO SMOKE

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

Work has been done on ozone cracking in the Los Angeles area and on the occurrence of high concentrations of oxides of nitrogen in tobacco smoke. The latter finding is of importance in the study of the effects of smoking on respiratory diseases.

Publications:


27. THE PROTEIN OF BACTERIOPHAGE QX174

Investigator: Edward A. Carusi
Support: Jane Coffin Childs Memorial Fund for Medical Research

An investigation of the physical and chemical properties of the protein isolated from QX174 has been undertaken. The purpose of this work is to determine the number of protein subunits which make up the external protein shell of the phage, and the spatial arrangement of these subunits in the virus. Additionally, it is hoped that information regarding the sequential arrangement of the amino acids within the subunit of the wild type and mutant types of QX174 can be correlated with specific alterations in the nucleic acid of the virus.

It has been found that dissociation of QX174 at pH 11.5 yields a low molecular weight protein subunit which can be freed entirely from contaminating nucleic acid. These preparations exhibit a single boundary in the ultracentrifuge with a sedimentation coefficient of about 4S. The subunits apparently are not denatured during preparation since they are able to cross-react serologically with antiserum against whole virus.

The ultraviolet absorption spectrum of the subunit is distinct from that of the virus, exhibiting a maximum at 275 millimicrons and a minimum at 250 millimicrons in aqueous solution, pH 8.5. A spectral shift occurs at pH 11 and above with a maximum and minimum at 290 and 270 millimicrons, respectively.

Aqueous solutions of subunit are stable for long periods between pH 8.5 and 11.5. At lower pH values the protein aggregates in an apparently disordered fashion and precipitates. Attempts to reaggregate the subunits around QX DNA have been unsuccessful.

Preliminary amino acid analysis revealed a high content of acidic amino acid residues. From the least abundant amino acid (cystine) content a tentative minimum molecular weight of about 25,000 could be deduced.
28. ANTIGENIC PROPERTIES OF BACTERIOPHAGE $\Phi X174$

Investigator: Ursula Rolfe
Support: National Institutes of Health, Public Health Service

An investigation of the antigenic properties of bacteriophage $\Phi X174$ and related particles, including the non-infectious 70S component, the heated virus, and the 4S subunit has been undertaken. All immune sera studied contained phage-inactivating antibodies. Kinetic studies indicate that inactivation is a one-hit process, and suggest that it is partially reversible.

The number of zones of precipitation obtained between the various antisera and antigens by the agar double diffusion technique is tabulated below. The results indicate that the virus possesses three antigenic sites responsible for the formation of precipitating antibodies. One of these sites is not accessible in the intact phage, but is exposed in the 70S component, and in the heated virus. The presence of antibodies specific for this hidden site in anti-$\Phi X$ serum suggests that breakdown of the virus occurs prior to antibody formation.

Of the remaining two sites only one is present in 4S material. The other is apparently destroyed when the protein coat is dissociated into its subunits. This latter site does not stimulate production of inactivating antibodies since all inactivating activity may be removed from anti-$\Phi X$ serum by adsorption with 4S material.

Zones of Precipitation by Double Diffusion

<table>
<thead>
<tr>
<th>SERA</th>
<th>Anti-$\Phi X$</th>
<th>Anti-70S</th>
<th>Anti-heated $\Phi X$</th>
<th>Anti-4S</th>
<th>Control Serum (obtained before immunization)</th>
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<tr>
<td>$\Phi X$</td>
<td>2</td>
<td>2</td>
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<td>1</td>
<td>0</td>
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<tr>
<td>70S</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>1</td>
<td>0</td>
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<tr>
<td>heated $\Phi X$</td>
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<td>3</td>
<td>3</td>
<td>1</td>
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<tr>
<td>4S subunit</td>
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</table>

29. RESEARCH UPON BACTERIOPHAGE $\Phi X174$

Investigators: Robert L. Sinsheimer, Barbra J. Staib, Faith N. Poole, Lesley Menninger
Support: National Institutes of Health, Public Health Service General Funds

Phage $\Phi X174$, labeled with respect to density by replacement of 96% of the N atoms with N-15, and made radioactive by incorporation of P-32 (approximately 0.1 P-32 atom/particle) has been prepared by growth of the virus on host cells grown in appropriate media. Methods have been developed for the isolation of this doubly-labeled virus in pure form. By comparison of the plaque-titer with the P-content of such preparations, it can be estimated that 35 to 40% of the particles are capable of forming plaques.
Over 80% of the radioactivity of such preparations specifically adsorbs to susceptible bacteria within 2 minutes at 37°. (This adsorption is blocked by addition of anti-phage serum.) However within 6 to 8 minutes at 37°C, 60 to 70% of the adsorbed radioactivity is back in the medium. It is in the form of large particles, judged from filtration and sedimentation experiments to be comparable in size to the whole virus. A fraction of the radioactivity is now accessible to the action of deoxyribonuclease. These particles, which appear to represent the non-plaque forming fraction of the preparation, apparently make an unsuccessful attempt to penetrate the bacterium after adsorption, and, upon failure, are released into the medium.

The fate of the radioactivity which actually penetrates the host cells has been studied both by lysis of the cells before the normal time of phage burst and by measurement of the transfer of activity to progeny phage. Density gradient analysis of the DNA from infected cells, after deproteinization with phenol, reveals that the radioactivity from the parental phage is mostly still in large molecules and may be found in two species, banding at different densities. One species bands at the density to be expected for N-15 DNA. The other species bands at a lighter density, even lighter than the density of N-14 DNA. The nature of the latter band is under investigation. With increasing time after infection, more of the radioactivity appears on the latter band.

By purification of the progeny phage (with addition of carrier) it can be shown that less than 2% of the radioactivity of parental phage that actually enters the cell appears in the progeny phage.

30. INFECTION OF PROTOPLASTS FROM Escherichia coli BY SUBVIRAL PARTICLES FROM BACTERIOPHAGE QX174

Investigator: George Guthrie
Support: National Institutes of Health, Public Health Service
General Funds

Investigations have been made to develop a system wherein purified deoxyribonucleic acid (DNA) from bacteriophage QX174 could be used to initiate an "infection" in protoplasts with the subsequent production of mature QX174 virus.

Infection of protoplasts by subviral particles of T2 virus has been reported by Spizizen (1957) and Fraser, et al. (1957). These reports indicated that protoplasts, which are bacteria whose cell walls have been removed by enzymatic digestion, were capable of performing all the biochemical reactions necessary to make mature phage. However, the T2 infective particles described in both of these reports contained not only DNA but also part of the viral protein from T2.

Bacteriophage QX174 is a minute particle containing a single molecule of single-stranded DNA. Protein containing subviral particles can be obtained from this phage, by heat treatment, which are infectious in a manner similar to the subviral particles of T2. In addition QX DNA can be extracted from the viral protein "coat" by two different methods and further purified by banding in a cesium chloride density gradient. QX DNA molecules purified in this manner are capable of infecting protoplasts from the QX174 host bacteria, E. coli C, and also protoplasts from other strains of E. coli which are resistant to infection by whole QX174 virus. Studies of the characteristics of such infections of protoplasts indicate certain differences from ordinary virus infection of whole cells.
A report on this work has been accepted by the Journal of Molecular Biology. The system is now capable of being used for the study of viral multiplication, initiating the infection with pure nucleic acid. In addition, investigations on the state of viral DNA within infected cells during the latent period are being made.

References:


31. SINGLE-BASE DEAMINATION AND INACTIVATION OF BACTERIOPHAGE QX AND ITS ISOLATED DNA-MOLECULE

Investigator: K. W. Mundry
Support: International Cooperation Administration
National Institutes of Health, Public Health Service
General Funds

From previous experiments with the RNA-molecule of tobacco mosaic virus (TMV) it is known that a single deamination occurring at one of its 6,000 bases is mutagenic (inactivation = lethal mutation). The single-stranded DNA-molecule of bacteriophage QX174 resembles in size and structure closely the RNA-molecule of TMV. Therefore, a quantitatively similar response to deamination was expected, but was not found in preliminary trials. This has proven to be due to a dependence of inactivation upon ionic strength which had not been previously investigated with TMV-RNA.

If deamination with nitrous acid is done at different concentrations of KCl, with all other conditions kept constant, the complete phage particle of QX174 shows a higher stability against the reaction with HNO₂ at high salt concentrations than at lower ones. Quantitatively the same behavior was found with the isolated, infectious DNA-molecule of this phage.

It is known from experiments with double-stranded DNA that amino groups involved in hydrogen bonding are rather efficiently protected against the reaction with HNO₂. Hence, intramolecular hydrogen bonds might be the cause for the increasing stability of both particles with increasing salt concentrations.

The identical behavior of the complete phage and its isolated DNA molecule indicates that in the phage particle under the conditions of these experiments the DNA is the only material showing detectable reaction with HNO₂ and that there is no inactivation by prevention of phage attachment to its host. Further this gives some evidence that the structure of the DNA inside phage QX174 allows intramolecular hydrogen bonding, depending upon the environment, to the same extent as the isolated DNA in solution. Small differences possibly remaining between the sensitivities of QX DNA and TMV-RNA might be due to small differences in molecule size and/or redundancy. Both affect the probability of a particular deamination being lethal.

References:

K. W. Mundry und A. Gierer, "Die Erzeugung von Mutationen des Tabakmosaikvirus durch chemische Behandlung seiner
32. INVESTIGATION OF NUCLEOTIDE SEQUENCES IN NUCLEIC ACIDS THROUGH THE USE OF CHEMICALLY SYNTHESIZED OLIGONUCLEOTIDES

Investigators: Robert L. Sinsheimer, Donald E. Hoard
Support: National Cancer Institute, Public Health Service
General Funds

The code in which genetic information is transmitted from parent to offspring is believed to reside in the sequence of nucleotide residues in the chains of DNA molecules. Continuing efforts are being directed toward determination of the maximum lengths of certain of the pyrimidine nucleotide sequences. Such information will serve to test current hypotheses concerning the nature of the genetic code. The isolation of radioactive deoxynucleotides for use as starting materials in the chemical synthesis of suitable oligonucleotide markers is in progress.


33. STUDIES ON THE RIBONUCLEIC ACID OF TOBACCO MOSAIC VIRUS

Investigator: Pen Ching Cheo
Support: National Institutes of Health, Public Health Service

The role of metals in the function and structure of many enzymes has been well documented. Their occurrence or possible role in the structure and function of nucleic acids has not been determined.

Further studies of magnesium ion on the ribonucleic acid from tobacco mosaic virus indicated that the effect of magnesium is characteristic of a strong interaction. The hypochromic effect due to the presence of magnesium is more than double that produced by an equivalent increase in ionic concentration of monovalent ion. In measuring the optical density of RNA as a function of temperature, a rapid rise is observed beginning at about 35°C when measured in acetate buffer, and upon addition of NaCl. If magnesium is present in the buffer, a higher temperature (about 55°C) is required to initiate the same rise.

In the presence of copper ion at room temperature, the optical density of the RNA is raised (hyperchromic effect) gradually as the concentration of the copper ion is increased; and at the same time, there is a shift of maximum absorption region from 257 mµ to 259 mµ. At the lower CuCl₂ concentrations (0.002 to 0.004%) an increase of temperature enhances the hyperchromicity of the RNA. Thus a 10% increase in optical density above that at room temperature is observed at 40°C in 0.002% CuCl₂ and at 30°C in 0.004% CuCl₂. Further increase in temperature results in no further increase in optical density in either case. When the CuCl₂ concentration in the buffer is 0.008%, the hyperchromicity of the RNA reaches its saturation point almost instantly at room temperature. Increase in temperature does not result in further increase in optical density.

The sedimentation coefficient of the RNA is increased upon addition of copper ion. At a concentration of 0.004% of the CuCl₂, the S value of the RNA is increased 26%. However, there is no increase in molecular weight as indicated by light scattering measurements at that concentration. A 47% increase in S value
is observed when the concentration of CuCl₂ is 0.008%.

The biological activity of TMV-RNA containing copper ion is reduced when the concentration of the CuCl₂ is above 0.002%. The infectivity lost at high concentrations of CuCl₂ can be recovered by adding versene to the Cu-RNA, followed by precipitation with ethyl alcohol.

Further investigations on the optical rotatory properties of Cu-RNA are in progress.

34. MUTATION IN TMV-RNA AND ITS RELATION TO THE STRUCTURE OF THE VIRUS COAT PROTEIN

Investigator: K. W. Mundry
Support: International Cooperation Administration
           National Institutes of Health, Public Health Service
           General Funds

Among the mutants of wild-type tobacco mosaic virus (TMV), chemically induced in vitro by deamination with nitrous acid, there is a fairly high percentage of strains which are difficult to transfer successively. Experiments have demonstrated that infectious material can be isolated out of homogenates from single infective centers (lesions) (ground in pH 7.5 buffer at room temperature) obtained from non-deaminated control material with 99 to 100% success. However, only 70% of lesions formed by infections obtained from "5-hit"-deaminated RNA yield infective material which is transferable (30 to 40 misses among 100 transfers tried).

At present, some independent and testable hypotheses exist to explain this finding:

1) Since lesion size is a genetically controlled character of TMV strains, and often reduced by mutation, the 30 to 40% misses might be the little lesions because of reduced virus multiplication, or spread, and thus contain too little virus to observe infectivity. This explanation was dismissed when it was observed that even large and very well growing lesions among those obtained from deaminated RNA often fail to contain easily transferable infectious material.

The remaining hypotheses are:

2) Mutation may affect the amino acid composition of the TMV coat protein and thus may induce changes of the secondary structure of the coat protein polypeptide unit which are sufficiently serious to weaken the structure of the final virus coat, normally protecting the enveloped RNA.

3) Mutation may destroy the information necessary for, and thus block, the production of coat protein. In this case, RNA will be reproduced, but not coated by protein. Hence transmission could be achieved only when RNA is extracted and, during isolation, is protected against ribonuclease. Experiments to isolated infectious RNA out of single infective centers are under way but have not yielded convincing results as yet.

4) The RNA-molecule may possess a distinct part which contains a highly specific structure necessary to start the proper combination of coat protein and RNA in order to form a stable nucleoprotein (this has been suggested by D. L. D. Caspar). If this structure is changed by mutation, even unchanged coat protein would be unable to envelop the RNA properly. In this case also, free RNA would be the infectious material in the lesion.
Such experiments are of some general interest relative to the problem of how RNA-information is related to protein structure and protein formation. Mutants fitting hypothesis 3) might present a valuable tool in studies relative to the formation of specific proteins in RNA replication.

The procedure of inducing mutants with HNO₂ in vitro permits to some degree an estimation of the number of nucleotides in a nucleic acid molecule concerned with a specific information. For one particular group of mutants this number is about 200. Since "misses" are roughly as frequent as this particular type of mutation, a similar number of nucleotides seems to control the "easy transmissibility" of the wild-type TMV-nucleoprotein.

References:


35. A SCREENING PROGRAM FOR PHOTO-MUTANTS IN PHYCOMYCES

Investigators: Diana Burkhardt, Lois Edgar, D. Varju, M. Delbrück
Support: National Science Foundation
General Funds

To assist in studies of the photoreaction in Phycomyces, it was decided to institute a search for mutants with altered characteristics in their responses to light. It is proposed to test about 3,000 isolates from X-rayed zygotes. The isolates are tested for their reaction to unilateral illumination with light of three different wavelengths, as follows:

1) Blue light of an intensity near the threshold for the parental strains. The parental strains grow towards this light.

2) Light of wavelength 312 mµ, and higher intensity than under 1), above. The parental strains are indifferent at this wavelength, growing neither towards nor away from this light.

3) Light of wavelength 254 mµ, of an intensity near the threshold for the parental strains. The parental strains grow away from this light.

The peculiar inversion of the phototropism from positive to negative on passing the neutral point near 312 mµ is due to a screening pigment, gallic acid, in the sporangiophores. At longer wavelengths the sporangiophores are highly transparent, permitting the focusing effect of the cylindrical stalk to confer an advantage on the distal side, stimulating it more strongly than the proximal side, thus causing the specimens to grow towards the light. For wavelengths below the inversion point, gallic acid screens the light from the distal side. Only the proximal side is then stimulated, and the specimens grow away from the light.
Mutants might be expected to produce altered amounts of visual pigment, or of screening pigment, or they might produce a visual pigment with altered spectral characteristics. Any such changes may be expected to show up as an altered response under at least one of the above named conditions.

Tests are performed on batches of 50 to 100 isolates, cultured individually in small shell vials carried on turntables which transport the vials past a narrow slit admitting light of the appropriate quality. The turntables rotate at the rate of three turns per minute.

It is apparently difficult to obtain genetically pure mutants when the multinucleate vegetative spores are irradiated. This has been the experience of Helga Harm. In the present work, germinating zygotes are X-rayed. These zygotes, after 3 to 6 months' dormancy, germinate and form a sporangiophore and sporangium very similar to that formed by the vegetative mycelium. The spores are multinucleate, like the vegetative spores. It is believed, however, that the nuclei of the spores in the zygote sporangium are derived by nuclear division after spore formation, whereas the nuclei of vegetative spores are believed to have finished dividing before spore formation. Therefore, if a mutant is produced by irradiation during an early stage of the germination of the zygote, one may expect the spores to be homokaryotic.

About 3,000 zygotes, resulting from two crosses, were prepared by Helga Harm in Frankfurt and shipped here. A few hours after germination the zygotes are irradiated with 3,000r; the spores are collected four days later and plated out. Six to nine isolates are made from any one sporangium. From each isolate 10 cultures are made for photoreaction tests and one for tests of growth factor deficiencies. About 100 isolates are processed per week. To date 900 isolates have been tested. Several isolates have shown abnormal reactions and have been set aside for further tests.

36. ACTION SPECTRUM OF PHOTO-GEOTROPIC EQUILIBRIUM

Investigators: Lois Edgar, D. Varju, M. Delbrück
Support: National Science Foundation
General Funds

Over a very wide range of intensities sporangiophores (spphs) illuminated unilaterally with a horizontal blue light beam grow towards the light source with an average deviation of 30° upwards from the horizontal. Dennison (Thesis, 1958) interpreted this deviation as an equilibrium between phototropic and geotropic activities. However, this deviation from the horizontal does not occur when using UV light for illumination. The spphs are then growing horizontally away from the light source. This phenomenon suggests an interpretation of the 30° equilibrium angle in the visible based on the dioptric properties of the cylindrical spph. At this angle the focusing line produced by the cylindrical spph is located on the distal cell wall. For stronger inclination the illumination on the distal side is very diffuse and the phototropic reaction possibly reverses sign. This might explain the 30° angle between the direction of illumination and the axis of the specimen. The fact that the deviation is "up" from the horizontal is certainly due to geotropism. In the case of UV illumination a focusing does not occur since the distal side is perfectly screened. Therefore the negative tropic reaction produces a growth horizontally which is not appreciably distorted by a weak geotropic effect.

At very low intensities a real competition between geotropism and phototropism comes into evidence: as the intensity is lowered the specimens grow in directions more and more nearly vertical. It seemed of interest to compare the
action spectrum of this photo-geotropic equilibrium with the pure phototropic one reported last year. At these low intensities there might be present more visual pigment and self-screening distortion of the action spectrum. Such distortion would be expected to make the action spectrum more uniform.

Wavelength has been plotted versus the reciprocal quantum flux which produces an angle of 60°, midway between the purely geotropic and the purely phototropic position. This spectrum is fairly uniform within the measured range (370 μm ≤ λ ≤ 490 μm) in contrast to the purely phototropic action spectrum measured at much higher intensities. The average value of the equilibrium quantum flux amounts to 5.5 x 10⁵ quanta/cm²sec.

37. STIMULATION OF NARROW TEST AREAS WITH UV LIGHT BELOW 300 μm

Investigators: D. Varju, M. Delbrück
Support: National Science Foundation
General Funds

The internal screening of light below λ = 300 μm by the Phycomyces sporangiophores (spphs) is so strong that light impinging from one side stimulates the proximal side only. Tropic responses are therefore much stronger than in the visible where both proximal and distal side are stimulated and the tropic response is the result of the slight differential between these effects. The tropic responses in the UV are strong enough even for short stimuli and narrow test areas to give significant responses and should thus permit definite conclusions regarding:

1) localization of the sensitive zone;
2) autonomy versus integration of the parts of the growing zone;
3) association of the receptors and the adaptation system with the cell wall, or with some of the particles in the protoplasmic upstream or downstream, or with some other component such as an hypothetical "inner wall", postulated as a result of previous work.

A suitable optical system has been developed permitting illumination with high intensity, free of components above 300 μm, of narrow areas of adjustable width, uniform in intensity within this width and sharply bounded.

Periodic tropic responses were used to determine the range of tropic sensitivity within the growing zone (GZ). Stimuli of one second duration, 1 mm wide, were given alternately at 5 minute intervals to opposite sides of the spph. It was found that the sensitive zone ranges from 0.6 mm to some point beyond 1.7 mm below the sporangium. Former investigations using visible light and the growth response had suggested a similarly limited sensitive zone.

In contrast, illuminating small areas of the GZ within the range of 0.2 to 0.6 mm below the sporangium continuously, tropic reactions can be observed. However the reaction time increases from the 3.5 minutes for the sensitive zone as defined above to 10 minutes for a stimulus 2 mm from the sporangium. Moreover the kink appears always some 0.5 mm below and keeps moving away from the sporangium. The movement of the kink center away from the sporangium is common to all experiments illuminating small areas of the sensitive zone at a constant distance from the sporangium, no matter how large the distance.
Stimulations of the sporangiophore at constant height relative to the ground produce sharp kinks which stay with the line.

The proper motion of an adaptive configuration has also been investigated. Ten minutes after increasing the adaptation level in the upper or lower half of the GZ short narrow stimuli were applied on one or on the other side of the separating line. It turned out that the edge of an adapting configuration originally 1.0 mm below the sporangium is moving away from the sporangium with a speed approximately 10 µ/min. This is much less than the speed of the tropic response or the growth velocity (~50 µ/min) and is also less than the speed of a marker on the cell wall (15 to 20 µ/min at 1.0 mm below the sporangium).
38. PREVENTION OF PODOPHYLLIN POISONING IN SEA-URCHIN EGGS BY DNA DIGESTS

Investigator: Joseph Butros
Support: International Cooperation Administration
National Institutes of Health, Public Health Service
General Funds

The toxicity of podophyllin has been attributed to action on the nucleus, clumping the chromosomes and leading to cell death at metaphase. Cleavage can be blocked in the various organisms tested, at a concentration of the order of 0.5 mg/liter.

In view of the previous finding in this laboratory that DNA digests had a promoting effect on the cleavage of sea-urchin eggs, a testing was done for possible protective action of these digests against podophyllin poisoning.

Soon after fertilization of Lytechinus (sea urchin) eggs, they were distributed into: 1) podophyllin (0.4 mg/liter) solution; 2) a mixture of the same podophyllin solution and DNA digest (30 mg/liter); 3) sea water. While eggs in sea water divided normally and reached blastula, podophyllin-treated eggs showed abnormal cleavages and extreme vacuolization spreading over the cytoplasm. The centers of such eggs were pycnotic (dark) indicating cytolysis. The podophyllin-DNA-treated eggs did not show any cytolytic and vacuolization areas. Eggs in this mixture had perfect shape and consistency, although at this elevated concentration of DNA they did not divide. In further experiments tests will be made to determine whether or not there may be some particular concentration of DNA digest, or the right fraction, whereby the eggs could be protected from the toxic effect of podophyllin and go on with their development. The DNA degradation products could possibly be supplying what the podophyllin was inactivating or binding in the nuclear and chromosomal cycle.

Publication:


39. INDUCTIVE ACTION OF DNA DIGESTS ON FRAGMENTS OF CHICK BLASTODERMS

Investigator: Joseph Butros
Support: International Cooperation Administration
National Institutes of Health, Public Health Service
General Funds

There is evidence that ribonucleic acid (RNA) and RNA-proteins are capable of inducing differentiation in the ectoderm of amphibian embryos. The
present investigation concerns the possible inducing action of DNA digests using chick embryos as experimental animals.

Twenty-hour blastoderms were excised and cut transversely at a level 0.5 mm posterior to Hensen's node. They were cultivated in vitro on an albumen-agar medium. As has been shown by others, fragments of the blastoderm behind Hensen's node (the organizer) do not develop into embryos or embryonic organs. In the present experiments fragments were usually cultured 24 to 48 hours, then fixed, sectioned and stained for microscopic study. The hydrolysates and digests to be tested were prepared initially from commercial DNA (herring sperm and calf thymus); later the DNA's from adult and embryonic chick tissues have been used.

Control fragments grown on the albumen-agar medium show the three germ-layers with no axial structures such as somites, notochord and neural tube. The cells are mainly primitive and undifferentiated except for the presence of blood cells towards the end of the culture period. Upon incorporating the DNA digests into the basic medium, however, rough axial organization was discernible. Neural tubes and possibly sensory placodes were found. Tubular aggregations of mesoderm with round cavities looked like somites and mesonephric tubules. Lateral mesoderm was split in many places into the two usual layers. The middle germ-layer was enormously thickened with abundance of mitotic figures. There was clear tendency for cell aggregation into acini and other configurations suggesting vague organogenesis. Fibroblasts, mesenchyme cells, blood cells and possibly other types were found. The work is still in progress; subculturing treated fragments on the chorio-allantoic membrane or a plasma-clot may permit further expression of their induced differentiation. The possible specificity of differentiation depending upon the source of DNA will be examined.

40. **ANIMALIZATION OF SEA-URCHIN EGGS WITH DIGESTS OF HOMOLOGOUS DNA**

**Investigator:** Joseph Butros  
**Support:** General Funds

Certain chemical substances, notably lithium chloride, cause endodermal structures of embryos to develop excessively while ectodermal organs are suppressed. This effect is called vegetalization. Other substances, such as sodium thiocyanate, have the reverse type of effect termed animalization. Gustafson and Hörstadius have recently shown that nucleic acid metabolites, among other agents, have an animalizing action similar to thiocyanate, even in the presence of lithium chloride. The possibility that degraded DNA may have similar effects was suggested by the previous finding that it promoted action on sea-urchin cleavage.

Highly polymerized DNA was prepared from the sperm of the sea urchin Lytechinus, digested with DNAs and fractionated on an Ecteola column, or used whole, unfractionated, after inactivating the enzyme at 80°C.

A group of fertilized eggs were placed in 2.5% LiCl; a second group in a mixture of 2.5% LiCl and DNA digest of 5 mg/100 ml final concentration; and a third group were left as controls in sea water. Eggs cleaved in all groups and reached the blastula stage.

The blastulae of all groups were removed and placed in fresh sea water to resume development. Examination of the eggs was made when the sea water group reached the pyramidal pluteus stage. It was found that the eggs treated
with LiCl developed typical wavy, constricted exogastrulae; i.e. were vegetalized. The eggs that were supplied with DNA digests in addition to the LiCl were small and remained rounded as in thiocyanate animalization.

Experiments are planned to determine whether or not it may be possible to find the proper conditions and concentrations such that the DNA hydrolysate will just counteract the effect of LiCl and permit normal development, and to determine if there is specificity of action of different fractions of the digest.

41. ATTEMPT TO CULTURE MOUSE EGGS SHORTLY AFTER FERTILIZATION AND IN EARLY CLEAVAGE STAGES

Investigators: Joan Malone, Louise M. Fessenden, Bithika Raychaudhuri, Albert Tyler
Support: National Institutes of Health, Public Health Service

Several earlier investigators have observed that fertilized eggs of rodents taken before cleavage, or in the 2- to 4-cell stage, very rarely divide when placed in various culture media. However, later stages generally proceed well to blastocysts in simple culture media. This was confirmed in a large number of experiments with mouse eggs (mostly previously reported) employing diverse culture media (various combinations of balanced salt solutions with or without serum albumin, containing an assortment of various amino acids, vitamins and other growth factors as devised by Eagle and by Puck, with and without extracts of chick and mouse embryos, mouse-, rat-, horse-, and human-sera and other materials).

Cultures of an additional 2,661 eggs, in 1- to 4-cell stages, from 189 mice have been studied during the past year. The best results have again been obtained with addition of estradiol (10^-6%) and slices of autologous Fallopian tube to Eagle's medium containing horse- and human-serum. Over all about 15% (as compared with 14% last year) of eggs so cultured made at least one division. Two or three cleavage divisions were made by 4% of these eggs.

On the basis of experiments by J. Butros (Exp. Cell Research, 18:318-332, 1959) showing an improvement of cleavage in underripe sea-urchin eggs by addition of hydrolysates of DNA, these, as well as undergraded preparations of DNA, and various combinations of nucleotides, nucleosides and purine and pyrimidine bases, were tested on the mouse eggs. No improvement in cleavage was obtained.

42. ACTION OF 2-DEOXY-D-GLUCOSE ON SEA-URCHIN EGGS

Investigator: Robert E. Black
Support: National Institutes of Health, Public Health Service

Metabolism of glucose is known to be competitively inhibited by the analog 2-deoxy-D-glucose (2DG) in yeast and in rat tissues. Also 2DG is capable of inhibiting cell division in cultured chick fibroblasts and tumors, and budding in yeasts.

In the present work 2DG was found to inhibit cleavage and oxygen consumption of fertilized sea-urchin eggs. The developmental progress made varies inversely, and the inhibition of oxygen uptake varies directly, with the concentration of 2DG. The addition of an excess of glucose can overcome the effect of 2DG.
Publication:


ABILITY OF EGGS OF MARINE ANIMALS TO OXIDIZE CARBON MONOXIDE

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

Previously (Black, Epstein and Tyler, Biol. Bull., 115:153-161, 1958) it has been shown, by use of C-13 and mass spectrometry, that fertilized eggs of the marine worm Urechis caupo possessed the capacity to oxidize carbon monoxide both in the light and in the dark. Classically carbon monoxide is employed in studies of cellular respiration to reveal the functioning of the cytochrome oxidase system by virtue of a photo-reversible inhibition of oxygen uptake that it engenders. However, the literature indicates the occurrence of other examples of carbon monoxide oxidation by certain tissues of animals and plants. The operation of the cytochrome oxidase system may well be observed in cases in which undetected co-oxidation may occur. Since, also, this property was exhibited markedly by freshly fertilized eggs it was of interest to examine changes occurring with development and to explore further the pathway of oxidation of the carbon monoxide itself.

The rates of gas-uptake and oxidation of CO (labeled with C-13) have been determined for developing eggs of Urechis and for unfertilized eggs and developing embryos of the sea urchin, Strongylocentrotus purpuratus. In the light in 80% CO/20% O2, there is excess gas-uptake over that of the air-controls at all stages of development, but the percentage of excess uptake falls off as development proceeds. In the dark there is increasing inhibition of respiration by CO during development. The rate of CO-oxidation in the light increases less than two-fold in Urechis during 50 hours of development. In Strongylocentrotus the rate of CO-oxidation in the light nearly doubles after fertilization, but increases relatively little during later development. In the dark, in Urechis, the rate of CO-oxidation during early cleavage is about half that in the light, but the dark-rate decreases five-fold during the swimming stages. In the sea urchin there is only a slight decrease in rate of CO-oxidation during development. The developmental changes in the ratio of dark-to-light oxidation of CO are interpreted on the basis of an increasing degree of saturation of cytochrome oxidase with its reducing substrate.

The cytochrome oxidase activity and ability to oxidize CO (labeled with C-13) were determined for sedimented, washed granules obtained from unfertilized eggs of Strongylocentrotus. Both added cytochrome c and ascorbate were required for the rapid oxidation of CO by the particles, and it is concluded that cytochrome oxidase is in the pathway of oxidation. In the light, the rate of CO-oxidation was found to be greatest at the concentration of cytochrome c, which gave about one-half the maximum O2-uptake. When the uptake of oxygen was maximal, the CO inhibited both the O2-uptake and the CO-oxidation in the light. In the dark the rates of CO-oxidation by the particles were not greatly affected by changes in the level of cytochrome c. The results provide a basis for the interpretation of developmental changes in CO-oxidation, in embryos, in terms of degree of "saturation" of the cytochrome oxidase with reducing substrate.
Publications:


44. CHANGES IN RATES OF INFLUX AND EFFLUX OF K\textsuperscript{42} UPON FERTILIZATION IN SEA-URCHIN EGGS

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

In experiments with internal microelectrodes (Tyler, Monroy, Kao and Grundfest, 1956) the existence of a potential difference was demonstrated between the inside and outside of the echinoderm egg. Upon fertilization there was a temporary decrease in this potential. It was also shown that increase in concentration of the external potassium ion causes a reversible depolarization and that normally the concentration of this ion inside the egg is about 20 times that in the surrounding sea water while sodium ion concentrations are approximately the reverse. By analogy with other excitable tissues, such as nerve and muscle, this leads to the expectation that there should be a temporarily increased efflux of K\textsuperscript{+} from the eggs very soon after fertilization. The present experiments with radioactive potassium (K\textsuperscript{42}) give evidence for this and, also, for a change in exchange-ability of K\textsuperscript{+} upon fertilization.

Eggs of the sea-urchin Arbacia punctulata were loaded with K\textsuperscript{42}, a portion of the suspension was fertilized and the rate of release of the isotope from the two lots was followed at short intervals of time thereafter. The results show a greatly increased rate of release within the first minute after fertilization, the averages for 25 sets of experiments being approximately 2.3, 2.5, 2, 1.7 and 1.6 times that from the unfertilized eggs at 1/2, 1 1/2, 2 1/2, 4 and 6 minutes respectively. The results add another feature of resemblance of fertilization to the excitation process in muscle and nerve.

Measurements of uptake of K\textsuperscript{42} were made over longer periods of time and the rate was found to be considerably greater in fertilized than in unfertilized eggs. The unfertilized eggs fail to accumulate in a period of eight hours more than one-fourth of the amount of K\textsuperscript{42} that is to be expected at equilibrium on the basis of the relative concentrations of K inside and outside the cell. The fertilized eggs attain, or nearly attain, the expected value well within this period of time. It appears that, as reported for many other kinds of tissue, a large fraction of the K\textsuperscript{+} is not readily exchangeable in the unfertilized egg, but becomes so after fertilization.

Dr. Alberto Monroy of the University of Palermo, Italy, collaborated in this work.

Publication:

A THEORY OF CANCER DERIVED FROM CERTAIN DEVELOPMENTAL IMMUNOGENETIC STUDIES

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

This theory is derived primarily from a consideration of "runt" and other transplantation diseases. "Runt disease" was described by Billingham and Brent in experiments in which embryonic or new-born mice were injected with lymphoid cells of another strain so that upon later development the host would become immunologically tolerant to antigens (or skin grafts) from the donor strain. In most such experiments, however, the recipient dies, at an early age, of a wasting disease. This often occurs also when lethally irradiated mice are "protected" with foreign-strain hematopoietic cells, or when unirradiated F1 hybrid animals are injected with cells from one of the parent strains or when they are parabiosed to animals of one of the parent strains. The wasting disease that develops in these various experimental situations has many features (including the development of tumors in some cases) in common with the pathological syndromes of neoplastic diseases. They are interpreted on the basis of antibody formation by the graft against host tissues and tolerance of the graft tissue by the host.

The present theory proposes that a malignant tumor rises from a cell in which there has occurred a loss or inactivation of a gene (or both genes if homozygous) determining the presence of a specific histocompatibility-antigen. Since this is a "loss-mutation" no foreign antigen appears, and the new cell is tolerated by the "host" just as are parental cells by an F1 hybrid. The theory further proposes that the altered cells are immunologically competent cells or capable of becoming immunologically competent. By analogy with the proliferation of antibody-forming cells upon exposure to foreign antigen, the unrestricted growth of the tumor cells is interpreted, then, as due to the constant proliferative stimulus provided by the, now foreign, antigens of the "host." Further, the theory proposes that the pathological manifestations of cancer are due to the production of circulating and cell-fixed antibodies against "host" antigens. The theory suggests therapeutic measures based primarily on attempts at "allergic" injury or destruction of the neoplastic cells by administration of large doses of specific "host" antigens, and as more remote possibilities the administration to the tumor, of the appropriate DNA or RNA, to restore ability to produce the missing antigen.

Publication:


SITE OF ACTION OF ANTIBODIES THAT BLOCK CELL DIVISION IN SEA-URCHIN EGGS, EXAMINED BY FLUORESCEIN-LABELING

Investigators: Alberta Jones Seaton, Albert Tyler  
Support: National Institutes of Health, Public Health Service General Funds

Previous experiments have shown that antisera produced in rabbits against the fertilizins of sea-urchin eggs are highly effective in blocking cell division in the early cleavages of these eggs. In strong antisera blocking can occur within one quarter of a division cycle. It is not stage-specific. Nuclear as well as cytoplasmic division is blocked. The antisera against other materials of the eggs are much less effective in blocking cleavage. Those against sperm or adult blood
or other tissues have been generally ineffective.

For further investigation of the action of the antibodies produced against fertilizin, the antibody globulin and normal rabbit globulin (from pre-injection seras) were conjugated with fluorescein and the reaction with eggs examined by fluorescence microscopy. Retention of antibody activity in the conjugates was checked by the cell-division blocking action. The eggs were prepared for fluorescence microscopy either as whole mounts or as sections, following freezing or various fixatives. For the latter purpose alcohol-formol fixation proved satisfactory. The observations in two species of sea urchins (Strongylocentrotus purpuratus, Lytechinus pictus) showed the antibody to be taken up primarily by the surface of the egg. Reactions with internal material were generally weak and diffuse and not significantly different from that given by the normal globulin-conjugate. No reaction was obtained with the nucleus.

The results imply that the cell-division blocking action results simply from reaction of the antibodies with surface constituents of the egg, and that the antibodies need not penetrate the cell to produce their effect. These surface constituents of the fertilized egg must presumably possess antigenic determinants similar to those possessed by the fertilizin of the unfertilized egg. This is consistent with other evidence (e.g. re-fertilizability of de-membranated fertilized eggs) indicating that the surface (below the fertilization membrane) of the fertilized egg possesses a fertilizin-like substance for a considerable period of time after fertilization.

47. **PROPERTIES OF FERTILIZINS AND THEIR ROLE IN FERTILIZATION**

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

The chemical composition of fertilizins has been examined in additional species of animals among the echinoids, asteroids and gastropods in order to determine whether or not the glycoprotein nature, previously ascertained for certain sea urchins, holds generally. The evidence, so far, indicates that it does.

The fertilizins of certain echinoids (Arbacia punctulata and Echinorchinias paoma) was shown to have molecular weights in the neighborhood of 300,000 and to be highly elongate (axial ratio about 20:1) molecules. It has been possible by relatively mild procedures (brief exposure to heat or proteinares) to partially degrade the molecule so as to render the fertilizin non-agglutinating but still capable of specific combination with sperm. These "univalent" fertilizins are still of large molecular size. Experiments on more extensive degradation have been undertaken in attempts to obtain relatively small molecular fragments that possess specific reactivity for the sperm, but, so far, the more extensive hydrolyses have resulted in loss of activity.

The previous investigations assign to fertilizin on the surface of the egg and the complementary antifertilizin on the surface of the sperm the role of receptor substances by which sperm and egg are able initially to adhere to one another in the process of fertilization. In general the species-specificity of fertilizin-antifertilizin accords with the species-specificity of fertilization, as does the lack of these receptors in other tissues of the organism accord with the tissue-specificity of fertilization. Further analysis has indicated that besides their role in specific attachment, these substances may also be involved in the engulfment of the sperm by the egg, through a process of specific pinocytosis. Such a process can explain how the sperm "penetrates" the plasma membrane proper of the egg.
without leaving a hole on the surface; the sperm is simply encapsulated by the
surface, the capsule being formed by progressive fertilizin-antifertilizin inter-
action. The analysis leads to the inference that, in most species of animals, the
plasma membrane (bearing specific fertilizin-receptors) of the unfertilized egg
must protrude through pores in an overlying vitelline membrane. The latter
elevates as the fertilization membrane upon fertilization, and the accompanying
retraction of the processes of the plasma membrane would account for the block to
polyspermy and many other features of fertilization. Whether or not such protusions
of the plasma membrane occur generally in most animal eggs before fertilization is
not clear from the available electron microscopic information, and further
exploration of this point is planned.

Publications:

Albert Tyler, "Some Immunobiological Experiments on Fertilization and

Albert Tyler, "Immunobiological Aspects of Fertilization and Early
Development," Proc. Sixth Internat. Conf. on Planned
Parenthood, New Delhi, 1960.

Albert Tyler, "Some Experimental Features of Fertilization Common to
Mammals and Lower Animals," Proc. Sixth Internat. Conf. on Planned
Parenthood, New Delhi, 1960.

Albert Tyler and D. W. Bishop, Immunological Phenomena, Chapter VIII
in Physiological Mechanisms Concerned with Conception, ed. by

48. POLYSPERMIC RAT EGGS

Investigator: Lajos Piko
Support: Lalor Foundation

The possibility of polyspermic fertilization, i.e. the penetration into the
cytoplasm of the egg of two or more sperms, was demonstrated by Austin and
Braden (1953). They also showed that dispermy in the rat probably leads to triploidy.

In previous work (Piko 1958 and unpublished) factors influencing the in-
cidence of polyspermy in the rat were studied. Also evidence was obtained (Piko
and Bomsel-Helmreich, 1960) that dispermic eggs may give rise to advanced
triploid embryos most of which, however, apparently die at the time of mid-
gestation (10 to 12 days after copulation).

One unusual feature is the occurrence of a normal-appearing first
cleavage into two equal-sized cells on the part of dispermic eggs. In lower
animals (except for cases of physiological polyspermy, in birds, etc.) dispermy
usually results in cleavage into three or four cells at the first division. This is
to be explored further by egg culture methods along with experiments relating to
the mechanism of the block to polyspermy.

In a preliminary study during the past two months, the incidence of
dispermy in 15 Sherman females, mated by Wistar males towards the end of
estrus, was 3.5% (5 out of 143 fertilized eggs). Ninety-seven eggs in the 1- to 8-
cell stage were cultured in a balanced salt solution containing serum albumin,
autologous serum, Eagle's medium, or Puck's medium, after having been examined
by phase microscopy. Many eggs made one further division despite the flattening to which they were subjected for examination by phase microscopy. However, no more than one division was obtained in vitro in any case, from flattened or control eggs and the ones that divided were those that were put into culture shortly before the time of division.

References:


49. ATTEMPTS TO ACCLIMATIZE DIFFERENT ECOTYPES OF MUSSELS UNDER A SINGLE CLIMATIC CONDITION

Investigator: Peter Pickens *
Support: General Funds

The plan of this investigation was to obtain mussels from several localities up and down the West Coast and to try and acclimatize them to conditions at Corona Del Mar. The criterion for acclimatization was shift in rate-temperature curve of heart beat. Such changes were found but not in the direction expected. This matter is now being further investigated. A further point of interest is that there appears to have been sex changes in some of the mussel collections. Thus, collections from field populations of mussels of warmer habitats have high ratios of males to females. This is also true of mussels kept at Corona Del Mar.

* University of California
REDUNDANT GENETIC INFORMATION

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

It has long been known that chromosomes may have regions containing relatively non-essential genetic information. Such regions are known as "heterochromatin." Some chromosomes, such as the Y chromosome of man and other animals, are made up almost entirely of heterochromatin. One possibility is that heterochromatin consists largely of redundant genetic information. That is, it may be made up of multiple linear repetitions of a relatively small number of genes. If this is correct then it should be possible to create new heterochromatin, so to speak, by synthesizing multiple repetitions of some well known genetic region. Since it becomes increasingly difficult to count under the microscope the number of sections present in multiple linear repetitions, owing to the high confused pairing pattern of the repeated sections, it is desirable to select a region having in it some mutant whose effects will vary in proportion to the number of doses present. A region of the X chromosome of Drosophila carrying certain eye color mutants including the so-called ivory mutant ($w^1$) is in many ways ideally suited for such purposes. Thus, as the number of doses of ivory increases the eye color is expected to darken. Also, ivory undergoes rare but measureable reverse mutations to red so as to give, occasionally, flies with a variegated eye color: that is, an eye with an ivory background color containing a spot of red eye color. Presumably reversion rate as well as eye color should be correlated with the number of ivory mutants present. Starting with a tandem duplication (ABAB) containing the ivory mutant, a triplication (ABABAB) was first derived; from the triplication it has been possible to synthesize a probable quintuplication (ABABABABAB) type containing five doses of ivory. Males of the latter type have a decidedly pink eye color in contrast to the nearly white color of a single dose of the ivory mutant. Although quantitative studies are incomplete it also appears that the rate of ivory reversion increases in direct proportion to the number of ivory mutants present. The presumptive quintuplication for ivory is a starting point for the study of the reversion process itself as well as the properties of such regions of redundant genetic information.
51. MODIFYING GENES IN DROSOPHILA

Investigator: A. H. Sturtevant
Support: General Funds

A large series of experiments has been practically completed, in which modifiers for bristle-number were studied. Fourteen strains were established by selection and inbreeding, which differed in modifiers affecting the numbers of particular bristles in several mutant types (three alleles of scute, one of achaete, one of echinus). These sets of modifiers were then transferred between these mutant types, in an effort to find out something about the relative frequencies of modifiers specific, as to bristle or to mutant locus, in their effects. The data are voluminous, and their analysis will require much time; it is hoped, however, that the study may throw light on several general problems of gene action and development.

52. BAR EFFECT BY A TRANSLOCATION IN DROSOPHILA

Investigator: M. Ogaki
Support: General Funds

The Bar mutant of Drosophila causes a reduction in the number of facets of the compound eye. This effect of the mutant has long interested students of developmental genetics, particularly because it seemed to depend upon the presence of a specific kind of chromosomal rearrangement; namely, a small tandem duplication for a small segment of the sex chromosome. Also, certain chemicals, notably lactamide, are able to increase the facet number of Bar if fed to Bar larvae. How such chemicals tend to correct the morphological lesion of the eye is not understood. Nor has it been clear whether the presence of the duplication is a necessary condition for expressing the dominant Bar phenotype. Some progress in understanding the Bar effect has been achieved by analyzing a new Bar mutant known as Bar-581, which was placed at the author's disposal by E. B. Lewis. The mutant arose from an X-rayed wild-type stock (Canton S) and is independent in origin from the original Bar mutant. Examination of the giant salivary gland chromosomes by Ogaki and Lewis reveals that Bar-581 does not carry a duplication but instead is a reciprocal translocation having one breakage point in the Bar region (16A) and the other in the right arm of the third chromosome (88F). The mutant is remarkable also in having a very extreme reduction in facet number comparable to that seen only in the more extreme Bar derivatives having three or more sections of the Bar region in tandem repetition.

When reared on normal food at 25°C, females heterozygous for the new Bar mutant have eyes with only some 35 facets (on a narrow strip of fused facets), compared to about 780 facets for normal females reared under the same conditions. When females heterozygous for the new mutant are reared on food containing 2.5% lactamide, the facet number is found to increase to approximately 540 facets.

The Bar effect probably depends upon a position effect involving a specific new arrangement of the 16A region of the X chromosome, but a duplication for that region is evidently not necessary to produce the Bar effect nor to mediate the effect of lactamide.
53. RED FAT CELLS IN DROSOPHILA

Investigators: E. B. Lewis, Mihoko Ohnishi
Support: American Cancer Society
General Funds

With few exceptions, the blood of insects contains no pigmented cells. Some years ago in this laboratory a strain of Drosophila was found to have numerous red cells in the blood. This effect proved to be due, as shown by Dr. E. H. Grell, to the simultaneous presence of two recessive mutant genes in the second chromosome. The red cells appear to be fat cells and the red pigment is probably identical with that found normally in the eye.

An attempt has been made to try to enhance the expression of the red-cell trait by inducing new mutations with X-rays. Thus far, the X-ray experiments have failed to produce specific enhancers of the red-cell effect. However, two X-rayed induced mutations have been produced. These appear to be more extreme alleles of one of the two mutant genes needed to express the red-cell effect. It will be necessary to extract the new alleles and obtain them homozygous before it can be determined whether they are more effective than the spontaneous mutant in stimulating the production of red fat cells.

54. EFFECT OF FEEDING LACTAMIDE AND AMMONIUM LACTATE TO CERTAIN EYE-REDUCING MUTANTS OF DROSOPHILA

Investigator: Wilbur M. Luce
Support: General Funds

It had been found by Ogaki and others that feeding ammonium lactate to the sex-linked Bar-eyed mutant strain of Drosophila at critical periods during larval life increased the facet number of these reduced eye strains to a point where essentially the normal facet number was restored by the treatment.

Several autosomal mutants that had reduced eye facet numbers were similarly treated with both lactamide and ammonium lactate without any appreciable effect upon the facet number.

55. EFFECT OF FEEDING LACTAMIDE, AMMONIUM LACTATE AND SIMILAR COMPOUNDS ON THE FACET NUMBER OF OTHER STRAINS OF BAR MUTANTS

Investigator: Wilbur M. Luce
Support: General Funds

The effect of feeding lactamide and ammonium to forked Infrabar strains of Drosophila was similar to that in Bar. There was an increase in facet number but not as pronounced as in Bar. Several other chemicals were tried but none was as effective as the two mentioned above.

These same chemicals were fed to forked double Bar and to forked Infrabar-Bar strains. Both strains responded by a marked increase in facet number, the Infrabar-Bar strain responding to a considerably less extent than the double Bar.
56. MONOGRAPH ON BAR IN DROSOPHILA

Investigator: Wilbur M. Luce
Support: General Funds

Considerable progress was made on a projected monograph summarizing work on the Bar mutants. Professor Sturtevant's reprint collection was an invaluable aid here.
PART B. -- IMMUNOGENETICS

STAFF:
Professor: Ray D. Owen
Research Fellows: André Dubert, J. M. Dubert, John B. Loefer, Arne W. Nordskog, W. H. Stone
Graduate Students: Alan P. Roberts, Donald C. Shreffler
Research Assistants: Zeola Collins, Sondra F. Clearwaters
Student Assistants: Rolf F. Barth, David T. Denhardt
Laboratory Aide: Marilyn M. Moffat

57. BONE MARROW TRANSPLANTS IN IRRADIATED MAMMALS

Investigators: Ray D. Owen, Zeola Collins, Marilyn Moffat
Support: U.S. Atomic Energy Commission
Fleischmann Foundation of Nevada; General Funds

In continuing collaboration with the Experimental Biology and Medicine group of the Radiobiological Laboratory of the University of Texas and the United States Air Force, Balcones Research Center, Austin, Texas, several red-cell markers have been identified for individual differences among Rhesus monkeys. Several hundred monkeys have been tested with these reagents, following the techniques described in Biology 1959 (p. 39). The antigens, tentatively designated as A, C, and D, are distributed independently of each other in this large population and it is probable that they mark independent genetic loci. The distribution of antigen B suggests that it may be an allele of one of the other antigens, but B is so frequent in this population that the statistical tests require even larger numbers of monkeys than have so far been examined.

Donor-recipient pairs have been chosen for bone marrow transplantation experiments so that the progressive establishment of transplants and the correlated disappearance of host-type red blood cells could be followed. Two of the recipients in these experiments have survived for periods of several weeks after what should have been lethal irradiation, and have shown progressive transplants as judged by steady increases in numbers of red cells of donor type. However, the establishment of these transplants has not so far resulted in greatly prolonged survival of the recipients, and at present it seems probable that the critical irradiation damage in this primate species is not in tissues replaceable by healthy marrow transplants.

Further experimentation along this line is continuing, together with auxiliary studies such as a search for additional human red cell variation that might be identified by means of the rabbit-anti-Rhesus and chicken-anti-Rhesus antisera prepared for this study.

During the year under report, the results of a collaborative study with Dr. Joan W. Goodman of the Oak Ridge National Laboratory were published.

Publications:


58. EFFECTS OF WATER TEMPERATURE ON SURVIVAL OF IRRADIATED GOLDFISH

Investigator: Ray D. Owen
Support: U.S. Atomic Energy Commission

In collaboration with Dr. W. H. Hildemann of the University of California Medical School, Los Angeles, the reduction in the lethal effect of high levels of X-irradiation noted when goldfish are maintained in cooler water has been studied. Common goldfish averaging about 3 grams in weight were acclimatized to water temperatures of 15 ± 0.5°C and 25 ± 0.5°C, respectively, given 2,000 r total-body X-radiation, and maintained at the indicated temperatures along with non-irradiated controls. All eighteen irradiated fish kept at 25°C died by the 20th day post-irradiation, with median survival time (MST) = 12.8 ± 1.6 days (standard deviation [SD] = 3.6 days). Four of 36 fish maintained at 15°C showed weight recovery and greatly prolonged (indefinite) survival, indicating physiological recovery from radiation damage that would have been lethal in water 10° warmer. The MST of the 15°C group was 37 ± 6.8 days (SD = 19.3 days). Eighteen fish maintained at 15°C for two weeks after 2,000 r, then transferred to 25°C, gave MST 19.9 ± 1.3 days (SD = 2.8 days); one such fish survived indefinitely, returning to normal condition after showing general skin melanosis, hypoactivity, and slight weight loss. General X-ray effects and the course of radiation death, as functions of water temperature, have been described.

Publication:

59. GENETIC CONTROL OF SKIN GRAFT REJECTION IN MICE

Investigators: Rolf Barth, Zeola Collins, Andrée Dubert, Ray D. Owen
Support: U.S. Atomic Energy Commission
National Science Foundation
National Institutes of Health, Public Health Service
General Funds

Hybrids between two inbred lines (A x RF) were backcrossed reciprocally to the parental RF line, and the segregating backcross progeny were blood-typed for the H-2 characteristics. H-2 is a locus on the 9th chromosome of the mouse involved in the control of red-cell antigens and graft compatibility. The expected H-2 types occurred with the expected Mendelian frequency in the backcross generation — evidence that sperm populations segregating for this characteristic are not involved in selective sperm survival, fertilization, or zygote mortality, as appears to be true for certain other loci on the 9th chromosome. There was, however, a significant excess of males in one of the backcrosses. Out of a population of several hundred, a few mice gave atypical results with a battery of anti-H-2 reagents. These mice were selected for further matings, but to date they do not appear to have transmitted any unusual H-2 combinations.
Skin grafts from the non-recurrent parental line (A) were placed on more than 200 of the backcross progeny. Median survival times demonstrate clearly that the effect of H-2 incompatibility and of sex incompatibility (male→female) can be distinguished in terms of decreased graft survival time even against the background of numerous other histocompatibility characteristics distinguishing the two inbred lines and segregating in the backcross population. Only among donor-recipient combinations compatible for both H-2 and sex did prolonged graft survivals occur; in this group, predictability of relatively prolonged graft survival was fairly high. This finding supports the possibility that donor-recipient matching for major histocompatibility characteristics may permit relatively prolonged graft survival.

60. STUDIES ON PLANT HEMAGGLUTININS

Investigators: Ray D. Owen, Zeola Collins, Sondra Clearwaters
Support: U.S. Atomic Energy Commission
General Funds

The Brazilian legume Vicia graminea has been known for some time to contain in its seeds a principle that will agglutinate selectively human red cells with antigen N of the MN pair. Some time ago, it was noted in this laboratory that individual plants of this species lacked this agglutinin in their seeds, so that human N blood could be used to study a type of variation in the plant. Prof. Robert Allard of the Department of Agronomy, University of California, Davis, California, succeeded in crossing plants of the positive and negative types, and F2 seeds borne on F1 plants have now been tested. Seeds borne on negative plants are all negative, and seeds borne on positive plants have so far all been positive. No segregation was found for this characteristic among the F2 seeds, suggesting that this characteristic may be a function of the genotype of the plant bearing the seeds rather than of the embryos and cotyledons of the seeds. However, seed dissection indicates that the principle is in the internal rather than in the maternal seed coat part of the seed and is therefore probably a property of the cotyledon and/or the embryo. F2 plants are now being grown, and tests will be made to see whether or not they segregate for the original parental alternative. It is possible that the supposed hybrids were in fact accidental selfs or parthenocarpic, although the procedure used for emasculating the flowers before cross-pollination, in control tests, seemed to be completely effective.

Seeds from various wild legumes were collected casually on a summer vacation trip to Seattle, from areas including the Willapa Bay region and the Lassen Volcanic National Park region. One of the collections, tentatively identified as Vicia gigantea, gave a seed-extract of some interest; the specificity appears to be anti-human-H and can be used to distinguish between type O secretors and non-secretors. A quantity of these seeds has been obtained from Dr. Vance Tartar, near Oysterville, Washington, and is being subjected to further study.

61. THE RESISTANCE OF THE UNRESPONSIVE STATE TO X-IRRADIATION

Investigators: David T. Denhardt, Ray D. Owen
Support: American Cancer Society
U.S. Atomic Energy Commission
General Funds

If a process of continual differentiation of cells capable of forming different types of antibodies occurs during recovery from damage to this system by X-irradiation, one might expect that rabbits rendered unresponsive to a
soluble protein might regain their ability to respond during the recovery period after irradiation. This possibility was checked in rabbits rendered unresponsive to bovine serum albumin and given 300 r of X-ray. But unresponsive state was retained in these animals, as judged by immunochemical studies of clearance rates from the circulation of the injected rabbits and by the disappearance of a radioactive label after a second injection of $^{35}$S-bovine serum albumin. The hypothesis of randomized differentiation during recovery from irradiation under the conditions of this experiment was therefore not supported by the data. The antigen was effectively absent during the recovery phase; it seems possible that a different result might have been found were it arranged to have the antigen present during recovery.

Publication:


62. A STUDY OF MATERNAL-FETAL INTERACTION IN THE MOUSE

Investigators: Andrée Dubert, Ray D. Owen, Zeola Collins
Support: U.S. Atomic Energy Commission
National Institutes of Health, Public Health Service
General Funds

It has been reported by Hollander and Gowen that mice inheriting the normal allele of the recessive mutation for hairloss from their fathers are affected by some kind of incompatibility reaction if they develop in homozygous hairloss mothers. At first, this looks like an ordinary maternal-fetal incompatibility in which a maternal immune reaction is directed against some product of the normal allele in the heterozygous young. Hollander's and Gowen's observations have been confirmed and somewhat extended in this laboratory, but no evidence has been found for immunologic sensitization of the mother. No serum antibody could be detected with any of the techniques available, including cytotoxic tests on normal nucleated cells. Skin grafts to post-partum hairloss females whose normal young had shown the apparent incompatibility gave no indication of tissue sensitization of the females.

Radioactive strontium injected into these animals gave evidence that hairloss mice may maintain and perhaps require a considerably smaller pool of calcium than do mice heterozygous or homozygous for the normal allele. A model has been suggested in which the hairloss females, with this smaller calcium pool, have too little to contribute to their normal young, but enough for their hairloss young. In the reciprocal mating normal females would have a large enough pool so that all three genotypes progress satisfactorily—consistently with the experimental observations. The data are not fully persuasive, and do not at present suggest a primary relationship between the calcium pool and the growth aberration of the affected young. Furthermore, the failure to affect the course of this interaction by fostering the affected young on normal females suggests that the lesions occur during embryonic development, and are not modified by the milk calcium obtained through nursing. However, this model emphasizes that an apparent maternal-fetal incompatibility need not have an immunogenetic basis; it may be, for example, nutritional in nature.
63. **SEROLOGY OF TETRAHYMENA**

**Investigator:** John B. Loefer  
**Support:** National Institutes of Health, Public Health Service  
General Funds

Studies on comparative serology of Tetrahymenidae, and on amino acid composition of different serological types of T. pyriformis, as reviewed in Biology 1959, have continued. Earlier experimental work on three "wild" strains (WH6, WH14, and WH52) and one hybrid of variety 1 has been published.

Previous work had established the identity of several distinct immobilizing antigens, and several others not clearly distinguishable from one another. Strain WH52 in the range 20 to 35°C exhibited an antigen designated 52H. A clone derived from a cross of WH6 and WH14 was of serotype R. Strains WH6 and WH14 had antigens which seemingly crossreacted to a considerable degree. Cultures of the WH strains at 10°C revealed a serotype L, which could not be differentiated among the strains. Induced serotypes (by growth of cultures in presence of dilute homologous antisera) designated as I could not be clearly differentiated among the strains.

Recent work has extended the studies on T. pyriformis, var. 1, to include serotype differentiation of four additional "wild" strains, and 67 inbred strains derived from crosses of WH6, WH14, UM226 and ALP4 -- furnished by Dr. D. L. Nanney. Comparative tests on 75 strains of variety 1 were made with organisms grown at 15°, 25° and 35°C against diverse antisera prepared from selected strains of the 75. The results for tests at 15° and 35°C are incomplete, but extensive data have been collected on immobilization tests of 25°C cultures, and a manuscript is being prepared for publication. A summary of results indicates that:

1) Serotypes 6H and 14H are distinct.

2) A new serotype, 4H, was identified from "wild" strain UM226, and was detected in a number of crosses.

3) Of the inbreds at 25°C, none exhibited antigen I or 52H, but antigens 6H, 14H, R and 4H were noted among several families (designated by Dr. D. L. Nanney on the basis of the allele carried at the mating type locus).

   a) Of 27 strains derived from WH6 - WH14 crosses (families A and B), 15 were of serotype 14H, 6 of 6H, 3 of R, while 3 were "mixed."

   b) Of 17 strains derived from a cross of a member of family B and UM226 (family C), 2 were serotype R and 15 were 4H.

   c) Of 9 strains (family D) which originated from a cross of a ciliate from family B with one from ALP4, exhibiting serotype 14H, 3 exhibited R, 5 14H and l 4H.

   d) The 15 strains of family E, derived from a cross of strain No. 23 (family B) and No. 33 (of family D), all exhibited serotype R.
4) Preliminary results on 35°C cultures indicate that many, although not all, strains exhibit the same antigen as each did at 25°C.

5) Observations on cultures at 15°C indicate that most, although not all, strains produce an L antigen, seemingly comparable to that of WH6, WH14 and WH52.

Publication:

64. THE GENETICS OF THE H SEROTYPE SYSTEM IN VARIETY 1 OF TETRAHYMENA

Investigator: J. M. Dubert
Support: Rockefeller Foundation
General Funds

It has been shown that the available strains of variety 1 of Tetrahymena pyriformis can be classified into five distinct serological categories when grown at 25°C in an Aerobacter-Cerophyl medium. The present report is concerned with the genetic basis for this diversity of antigens.

Breeding analysis shows that the ability to produce these antigens is associated with a single genetic locus, the H locus. Four distinctive alleles, H_A, H_C, H_D, and H_E appear to be established in different inbred strains. Whether or not the fifth serotype, presumably H_B, is at the H locus cannot be ascertained as it exists only in a sterile strain.

Although the transmission of antigenic potentialities in sexual process is simple and uncomplicated, the expression of the H allele poses problems. Heterozygous clones initially express both antigenic specificities, but in the course of cellular proliferation they produce sublines expressing only one of the different antigens and others expressing only the other. The phenotypically "pure" lineages remain constant in further growth. The preferred explanation for this serotype "differentiation" is that one allele in each subnucleus of the compound macronucleus is suppressed, that the suppression becomes an hereditary character of the subnucleus and that the fixation process is the result of an assortment of differential subnuclei at macronuclear division.

The identity of the kinetics of antigen fixation with that of mating type fixation in clones of unstable mating type points to a common interpretation of both these phenomena.

65. GENETICALLY DETERMINED PLASMA PROTEIN VARIANTS IN THE MOUSE

Investigator: Donald C. Shreffler
Support: National Science Foundation
National Institutes of Health, Public Health Service
General Funds

In recent years progress in mammalian biochemical genetics has been greatly stimulated by genetic and chemical studies of hemoglobin variants in humans, cattle, sheep and goats. However, intensive genetic studies in these
species are difficult. Since the mouse is a much more favorable mammal for genetic study, an investigation has been initiated to detect and define genetically controlled protein variants in the serum of mice.

To date 13 inbred lines and a non-inbred stock of mice have been surveyed. Electrophoretically or immunologically distinguishable variants have been observed in four independent plasma protein components. One inbred line differs from all others tested in a $\beta$-globulin component, called $\beta_1$, which migrates in starch gel electrophoresis more rapidly than the analogous component, called $\beta_2$, in other lines. Serum from F$_1$'s of crosses between differing lines contains both $\beta_1$ and $\beta_2$ in approximately equal, but reduced, amounts. Tests of over 400 F$_2$ and backcross progeny indicate that this difference is controlled by a pair of codominant alleles. A second genetic difference, recognized by starch gel electrophoresis, has been observed in a complex group of minor $\alpha$- and $\beta$-globulin components in the stock of non-inbred mice. However, the precise nature of this difference has not yet been entirely resolved. A third electrophoretically recognizable difference has been observed in an $\alpha$-globulin (prealbumin) component in several inbred lines. Genetic studies of this difference are presently being carried out. A fourth difference is recognizable by an immunological technique utilizing the formation of precipitin bands when mouse serum and an antiserum against it are allowed to diffuse toward each other in an agar plate. When serum of certain inbred lines is compared in this manner with serum of other lines the position of one precipitin band is displaced in a way indicating a difference between these lines in the quantity of a specific protein. F$_1$'s between differing lines show a band in an intermediate position and results of preliminary tests of F$_2$'s and backcrosses indicate that this difference is controlled by a single pair of alleles.

66. THE CONTRIBUTION OF NORMALLY SUBLETHAL INFECTION TO THE SECONDARY DISEASE SYNDROME IN IRRADIATED MOUSE CHIMERAS

Investigator: Alan P. Roberts
Support: National Institutes of Health, Public Health Service

Secondary disease in lethally irradiated mice protected with transplants of homologous bone marrow is now generally considered to be an immunological reaction of the host against the graft, the graft against the host, or probably a combination of both reactions. The total syndrome of secondary disease may, however, be complicated by the presence of slightly pathogenic parasites in the population, and the role of these organisms in the syndrome should be clarified before a more complete description of the immunological contributions can be attempted.

It has now been shown that some differences in resistance mechanisms exist between mice with isologous transplants and mice transplanted with homologous marrow. The median lethal dosages with some slightly pathogenic bacteria are lower for homologous than for isologous mice, and are highest for untreated controls. However, this may be interpreted as being the result of the generally weaker physiological conditions of the animals due to, rather than a cause for, secondary disease as well as the response to irradiation. Slight, but as yet uncertain, differences have also been shown in the ability of these mice to clear bacteria from the circulation. It appears that the animals bearing a homologous transplant can effect a more rapid initial clearance. Other non-specific resistance mechanisms have been under study without clear-cut results to date.

The chimeras being used in this study are BAF$_1$/Jax -- (A/Jax x C57Bl) F$_1$ -- as hosts with Balb/c/Jax as the homologous donors. Studies of this system have shown that usually secondary disease appears quite early -- beginning in the second
to third week after treatment. Total repopulation by donor erythrocytes occurs usually five weeks or later. In a few long-term survivors that have been tested no reversion of the transplant to host-type has been seen. However there is considerable variation in the reaction of groups of mice treated at different times, probably more than can be explained by irradiation conditions or marrow cell dosages. Further work is planned to determine if this variation may be explained by the presence of differing bacterial flora and of subclinical infections present at the time of irradiation. Autopsies of animals dead from secondary disease have shown the presence of bacterial infection. Identification of these organisms will be attempted, and an effort will be made to trace their source by correlation with the organisms shown to be present in the normal flora or artificially injected before or at the time of irradiation.

67. A SEARCH FOR BLOOD GROUP FACTORS RESULTING FROM GENIC INTERACTION

Investigator: W. H. Stone
Support: National Institutes of Health, Public Health Service

With few exceptions, the blood groups of any animal species behave as though they were controlled by dominant genes. Consequently, a blood factor is not expected to appear on the red blood cells of an individual unless it was also present on the cells of one or both parents. Exceptions to this rule in birds, rabbits and cattle suggest that some kind of genic interaction may operate to produce "new" blood factors in heterozygous progeny only.

The object of these experiments is to determine whether or not this phenomenon also occurs in humans. To determine this, rabbits were immunized with blood from children in the hope that if the child's blood contained a "new" specificity the recipient rabbits would produce antibodies reactive with it. The rabbit antisera were absorbed with a pool of blood cells from both parents and the absorbed sera tested with the cells of the child. If a reaction remains after absorption with the parental cells, the assumption is that a "new" specificity appeared on the blood cells of the child that was absent on the blood cells of the parents. Only two of eleven families have been studied so far, both with negative results.

Acknowledgment is made of generous contributions of materials for this study by Dr. Phillip Sturgeon of the Los Angeles Children's Hospital.

68. THE PREPARATION OF HUMAN BLOOD TYPING FLUIDS FROM CATTLE IMMUNE SERA

Investigator: W. H. Stone
Support: National Institutes of Health, Public Health Service

One of the limitations in studying human blood groups is the scarce supply of certain typing fluids (reagents). Therefore, a source of large quantities of strong and specific typing fluids would be useful. These experiments were designed to determine if cattle could be used to produce immune sera against human cells. Large quantities of typing fluids could be prepared from such antisera.

Only one of several immune sera prepared at the University of Wisconsin has been analyzed. This serum yielded strong and specific anti-human M reagent. The hope is that these sera will yield other reagents, particularly those which at present are extremely scarce.
Acknowledgment is made of generous contributions of materials for this study by Hyland Laboratories.

69. THE EFFECTS OF IRRADIATION ON THE STATE OF IMMUNOLOGICAL SUPPRESSION

Investigators: W. H. Stone, Zeola Collins, Marilyn Moffat, Ray D. Owen
Support: National Institutes of Health, Public Health Service
U.S. Atomic Energy Commission
General Funds

Animals that fail to respond immunologically (do not produce antibodies, etc.) to a foreign substance (antigen) with which they have had contact during embryological development or during the newborn period are said to exhibit immunological suppression toward that antigen. The object of these experiments is to determine whether an animal suppressed for a given antigen may recover its ability to react to the antigen after irradiation.

One group of rats was injected with sheep red blood cells and another with saline during the first eight weeks of life. The latter group served as controls. Each of these groups was again divided into two groups, one of which was irradiated; the non-irradiated group serving as another control. All the rats were then injected with sheep cells. Following the injections, serum samples were obtained and stored for testing. These sera will be assayed to determine their antibody content and thus indicate what effect the irradiation had on the state of suppression.
PART C. -- CORN

STAFF:
Professor: E. G. Anderson
Research Associate: A. E. Longley
Research Assistants: Walter H. Hesse, F. D. Pettem
Laboratory Assistant: Ronald K. Burkholder

70. CHROMOSOMAL REARRANGEMENTS IN MAIZE

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

During the course of maize cytogenetic studies a large number of chromosomal rearrangements have been accumulated, all of which have valuable applications in economic maize improvement as well as in scientific studies. Many of these are already in use and form a valuable part of the framework of most of the experimental studies in maize genetics and to a lesser degree in the physiology, morphology and biochemistry of maize. Some use is being made in the field of practical corn improvement, but this is largely a field for the near future.

The number of translocations and inversions accumulated is about 900. These are being put in the form of stocks which will be maintained by the maize genetics cooperative group under the general supervision of Dr. Marcus M. Rhoades and Dr. Earl B. Patterson. The older series of translocations will be maintained at Purdue University by Dr. H. H. Kramer; the Crossroads material at Iowa State University by Dr. Donald S. Robertson; and the Eniwetok and later series at Illinois by Dr. Ritterson. Dr. Charles R. Burnham at Minnesota will maintain stocks of inversions and Dr. Edward Coe of Missouri will maintain a large series of translocations involving chromosome 9. The process of transferring the stocks to these investigators should be completed in the summer of 1961.

71. TRANSLOCATIONS AND INVERSIONS INDUCED BY IONIZING RADIATION

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

A cooperative project was started in June, 1947, to combine the interests of the United States Department of Agriculture and the California Institute of Technology in studying the effects of ionizing radiations on corn. During the thirteen years of study over 1,000 chromosome translocations and inversions were isolated and analyzed under the microscope. Dr. E. G. Anderson used pollen studies to detect the presence of chromosomal alterations caused by radiations such as X- and gamma-rays, Bikini and Eniwetok exposures of dry corn seed, and by X-ray treatments of corn pollen.

The breakage point positions were calculated from camera lucida drawings of mid-prophases of pollen-mother-cells for each chromosome abnormality isolated and propagated. At this phase pollen-mother-cells of heterozygous translocations show some opening out in the area of the cross configuration marking the exchange in the point by point pairing of homologous chromosome threads.
The variations in the calculated position of the breakage points in some translocations are due to two causes. The measurements for making calculation are made from a somewhat opened up cross at the intersection of the two chromosomes involved in a translocation. The mid-points of two of the single threads around the opening are assumed to be the breakage points. If the cross opens up more in one direction than in the other it causes an error in our assumption. The non-homologous pairing frequently reported for corn chromosomes is quite prevalent around the interchange area of some heterozygous translocations. This unorthodox pairing may place the breakage points some distance from the cross configuration, where their true position cannot be detected. This latter cause of variations in the calculated breakage point positions is particularly troublesome when the two points are near the centromeres.

72. GA FACTOR OF CHROMOSOME 5

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

An explanation for the occasionally observed transmission of chromosome 5 and its associated characters was attempted the last few seasons. The abnormal ratios encountered are due to a gametophytic factor (ga) that cannot compete with Ga carrying pollen when passing through certain types of female tissue to reach the eggs. This character of the female tissue that has the ability to eliminate ga pollen from a ga, Ga mixture may have one or more modifiers, one of which may be the environment.

The female tissue due to modifiers does not completely eliminate the transmission of ga carrying pollen to the egg; hence varying percentages of chromosome 5 that carry ga and its associated characters are found in the offspring when pollen from Ga ga plants is used. The ga character on chromosome 5 behaves like a two-factor-controlled character, one factor of which may be modified to give variable ga transmission.
PART D. -- NEUROSPORA

STAFF:
Professors: S. H. Emerson, N. H. Horowitz
Research Fellows: Rowland H. Davis, Marguerite Fling, Mary B. Mitchell, Eiji Ohnishi, Alfred S. Sussman, Yasushi Watanabe
Graduate Student: Harris Bernstein
Student Assistants: George S. Greenstein, Stephen F. Heineman
Research Assistants: Mary R. Emerson, Helen Macleod

73. "SLIME," A PLASMODIOID VARIANT OF NEUROSPORA

Investigators: S. Emerson, M. R. Emerson, George Greenstein
Support: National Science Foundation
Rockefeller Foundation
General Funds

Following ultraviolet irradiation of conidia of Neurospora strain 1207-IA (arginine-1, crisp, aurescent, osmotic) a variant was obtained which, on agar media, grows by plasmodial-like outflows.

Isolations from forced heterocaryons between "slime" and strain 1413-2A (albino-2, lysine-3, nicotinic-1, osmotic) indicate that there is a nuclear determiner for the plasmodial growth habit -- all plasmodial isolates required arginine for growth, and all arginine-requiring isolates were plasmodial. (Plasmodial colonies have never been obtained directly from conidia, but do occur following growth of heterocaryons in liquid medium.)

Genetic tests have so far been handicapped by poor ascospore germination -- only one ascus has been obtained from which more than two spore pairs germinated. Plasmodial types have not been recovered from outcrosses of "slime", though the five genetic markers carried by the parent of "slime" (1207-IA) segregated normally among those spores which did germinate.

74. PROTOPLAST-LIKE STRUCTURES OF NEUROSPORA

Investigator: Yasushi Watanabe, S. Emerson
Support: International Cooperation Administration
National Institutes of Health, Public Health Service
General Funds

Studies on the formation of protoplast-like structures of Neurospora by an enzyme obtained from an Actinomycete have been continued.

The culture technique for the Actinomycete has been improved by adding more KH₂PO₄ to maintain the neutrality of the medium, and the enzyme has been partially purified by fractionation with ammonium sulfate and alcohol. The enzyme preparations have been found to be satisfactory for the formation of spherical bodies from wild-type strains as well as from the osmotic mutant of Neurospora.

The protoplast-like bodies of wild type formed by this enzyme are quite similar in nature to those produced by the snail enzyme (Bachmann and Bonner, 1959), but differ from those of the osmotic mutant (Emerson and Emerson, 1958). When they are stored aerobically at 25°C in the incubation mixture in which they were formed, they vacuolate rapidly, grow irregularly, and finally burst, but do
not multiply. Spherical bodies of the osmotic mutant formed by the same enzyme grow and multiply. The protoplast-like bodies of both wild type and osmotic mutant regenerate to give, ultimately, normal hyphal growth when they are washed and transferred to a suitable liquid or solid medium containing 20% sucrose.

There are, however, some difficulties in separating the spherical bodies from intact conidia and hyphal fragments formed during digestion, so that they are still not very usable in biochemical studies, which was the primary purpose.

75. **AN ENZYMATIC DIFFERENCE AMONG PYR-3 MUTANTS OF NEUROSPORA**

Investigator: R. H. Davis  
Support: National Science Foundation  
General Funds

This research was designed to identify the enzymatic step or steps altered in the pyrimidine-3 mutants of Neurospora, which require pyrimidine supplementation in order to grow. Previous work had indicated that the mutant alleles were separable only infrequently by crossing over, and appeared to be grouped at a single complex locus. Certain physiological differences among the mutants led to the hope that these differences could be related to rather specific genetic effects on a single enzyme.

It has previously been indicated (see Biology 1959) that all pyr-3 mutants have all the enzymes necessary for the transformation of ureidosuccinic acid to uridylic acid, a stem pyrimidine compound. Therefore the study of the enzyme aspartic transcarbamylase, which catalyzes the formation of ureidosuccinic acid from aspartate and carbamyl phosphate (CAP), was undertaken to determine whether or not pyr-3 mutants were affected at this step.

It was found that one group of mutants, represented by 45502 (pyr-3d), lack activity for aspartic transcarbamylase, while another group, represented by 37301 (pyr-3a), display activity for this enzyme. Moreover, no differences in the properties of the enzyme from 37301 and that from wild type could be detected. The two groups of mutants defined on this criterion (activity or lack of it) were the same as in the classification based on physiological differences. Pyr-3a can be shown to complement in heterocaryons with pyr-3d.

These results have led to the suggestion that two enzymatic steps are controlled by the pyr-3 "locus," and that the step controlled by pyr-3a may be concerned with the availability of aspartic acid or of CAP. Both of these compounds, the substrates of the aspartic transcarbamylase reaction, are also required in the synthesis of arginine, and certain data given by M. B. and H. K. Mitchell indicate a competition between the arginine and pyrimidine synthetic pathways.

Publication:


References:


76. COMPLEMENTATION AMONG MUTANTS WITHIN THE iv-3 GROUP OF NEUROSPORA

Investigators: Harris Bernstein, Alexander Miller
Support: National Science Foundation
National Institutes of Health, Public Health Service

When nuclei from two different mutants, each lacking the same enzymatic activity, are allowed to share a common cytoplasm, the enzymatic activity is often partially restored. When many mutants are studied in all possible pairwise combinations for their ability to produce activity, as measured by growth, a matrix of positive and negative tests is obtained. Mutants can be placed in complementation subgroups (complements) on the basis of this pattern of responses, those having identical responses being placed in the same subgroup.

Four hundred and three mutants of the iv-3 group obtained by ultraviolet irradiation were tested extensively (about 20,000 tests). All mutants could be placed in only six subgroups. Furthermore these subgroups could be arranged in a linear array:

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  A
 B
 E  C
 F  D
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The convention followed is that non-complementing groups are drawn as overlaps on the map. Thus, A does not complement B, C, D, E or F, while D does complement B, E or F, but not A or C.

Further studies are in progress both with mutants obtained by mutagens other than UV, and with other techniques for detecting complementation. Thus far, the non-UV mutants can be placed in the previously defined subgroups. This suggests that the number and character of the subgroups at a genetic locus are properties of the locus and not of the mutagen used. It is hoped that the observed regularities in genetic interaction can be extended, by a study of the properties of the enzyme protein, to the chemical level of analysis.

77. THE CHARACTERIZATION OF ISOLEUCINE-VALINE MUTANTS OF NEUROSPORA

Investigators: Harris Bernstein, Alexander Miller
Support: National Science Foundation
National Institutes of Health, Public Health Service

The "inositol-less death" selection procedure of Lester and Gross was used to produce 518 mutants of Neurospora requiring valine and/or isoleucine. On the basis of extensive complementation testing these mutants fell into five distinct groups (cistrons). All tests between mutants from different cistrons were positive.
These groups have been designated iv-1, iv-2, iv-3, val-1, and val-2. Evidence from complementation and preliminary recombination data indicate that the previously known mutant 16117, blocked at the dehydrase reaction, is in the iv-1 group. Complementation evidence places 7110 in the iv-3 group, and data on its accumulation products put its block at the first step of isoleucine-valine biosynthesis. The mutant iv-2 is probably blocked at the isomerase-dehydrogenase reactions. The iv-1 and iv-2 mutants are 7 to 8 map units apart and neither is linked to iv-3. The val-1 and val-2 mutants will grow on valine alone, but their biosynthetic blocks are unknown.

Extreme variability in the proportion of mutants to wild type recovered, and in the group distribution of mutants, was observed in successive selection experiments for UV-induced mutants of the same parent strain. It appears that this variability is caused by rather subtle changes in technique which, as yet, are uncontrolled.

78. SPORE-PAIR ORDER IN TWIN ASCI OF NEUROSPORA

Investigator: M. B. Mitchell
Support: U.S. Atomic Energy Commission
National Institutes of Health, Public Health Service

As a continuation of the investigation of genetic relationships between adjacent, twin ascis (Genetics, 44:847-856, 1959) an unusually fertile cross was so analyzed as to allow, to some extent, recording of locations of ascis relative to one another in the fruiting bodies. Also, larger than average samples of ascis per fruiting body were tested to increase the likelihood of recovering twins.

The observations made indicate that, with respect to the two markers followed, twin ascis contain equal numbers of the same types of segregants. That is, if one twin is recombinant, the other is recombinant to the same degree, and so forth. (Although exceptions are found, ascis from a two-marker cross are, in general, non-recombinant, half recombinant or totally recombinant.) It also appeared that twins may often be identical with respect to order of the four spore pairs. Consideration of the latter point is complicated, however, by indications that rearrangement of spore pairs within the ascus may occur more often than formerly suspected and may be a good deal more frequent in some crosses than in others.

The relationships of twin ascis are of interest in connection with considerations of the manner and time of determination of ascus phenotypes and of the genetic significance of spore pair order.

Publication:

M. B. Mitchell, "Evidence of non-random Distribution of Ascus Classes in Fruiting Bodies of Neurospora crassa," Genetics, in press.

79. AN IRREGULARLY SEGREGATING MODIFIER OF A MUTANT CHARACTER IN NEUROSPORA

Investigator: M. B. Mitchell
Support: U.S. Atomic Energy Commission
National Institutes of Health, Public Health Service

The character to be considered is a modification of the phenotypic
expression of a familiar genetic marker. Its pattern of segregation is unusual in two respects. Although it has been observed in a number of crosses it has so far appeared in only a small fraction of the susceptible progeny. Also, its segregation in asci is unusual in that it has so often appeared in only one member of a spore pair. (The eight-spored ascus is thought to consist of four pairs of spores, the two members of a pair having identical nuclei.) This atypical segregation may be illustrated by the results from two crosses which will be considered again below. Only 43 among 571 susceptible spore pairs showed the modification and in 19 of the 43 only one member of the pair was modified.

Its tendency to appear erratically suggests classification of the modifier as a mutation or as a non-Mendelian or non-nuclear character. There are, however, indications that its appearance is not independent of segregation of more typically Mendelian traits. The relationship indicated is illustrated by two crosses of an unmodified isolate of the susceptible marker, m, one to a marker, 1, linked to m and the other to an unlinked marker, nl. These crosses produced two classes of asci containing m segregants (those in which the modification can be detected). One class was non-recombinant with respect to the markers and therefore contained four m segregants per ascus. The second was a half-recombinant class, the ascus containing only two m segregants each. If the modification were equally likely in all m segregants, then it would be expected to appear about twice as frequently in non-recombinant asci since these contain twice as many susceptible segregants as those of the recombinant class. In m x nl this expectation was realized. The modification appeared in 30% of the non-recombinant asci and in 16% of the recombinant class. With respect to the latter class the result from m x 1 was much the same. The modifier appeared in 19% of these asci but in only 5% of the non-recombinant class. Thus failure of the linked markers, m and 1, to recombine seems to be associated with a decreased likelihood of appearance of the modification.

This situation does not appear as a simple case of linkage of a modifying factor to 1. In recombinant as well as non-recombinant asci the segregants scored for modification were non-recombinant with respect to m and 1. Elaboration of alternative explanations will be delayed, however, pending more detailed investigation of the modifier’s behavior.

80. ISOLATION AND PROPERTIES OF NEUROSPORA TYROSINASE

Investigators: Marguerite Fling, Helen Macleod, N. H. Horowitz
Support: National Science Foundation

Tyrosinase has been isolated from the T^S strain of Neurospora in electrophoretically and ultracentrifugally homogeneous form. The isolation procedure involves fractionation by salts, organic solvents, and column chromatography. Improvements in culture methods now make it possible to obtain Neurospora in which tyrosinase forms 5% of the extractable protein. Higher concentrations are sometimes found. The molecular weight of the enzyme is in the range 50,000 to 100,000, the turnover number is of the order of 10^4 per minute with dopa as substrate, and the isoelectric point by free boundary electrophoresis is near 6. Copper is present in excess of one atom per molecule of enzyme, suggesting the possibility that the enzyme is composed of subunits.
Tyrosinase is induced in Neurospora by aromatic D-amino acids. The L-stereoisomers of the same amino acids have only a weak inductive effect (see Biology 1959). The stereochemical preference is being investigated.

The possibility that D-amino acids are preferentially taken up by Neurospora was studied, using the D- and L-isomers of dopa and phenylalanine. There is a rapid uptake of both stereoisomers from the medium by non-growing mycelium. No significant preference for either stereoisomer could be found in short-term experiments. In long-term experiments, complications may arise owing to enzymatic induction.

The possibility that aromatic D-amino acids are inverted to their L-enantiomorphs inside the cell before induction occurs was investigated by use of the oxD-1 mutation. This mutation prevents the inversion of D-amino acids (see the following report of Ohnishi et al.). It was found that the oxD-1 mutation does not prevent the induction of tyrosinase by D-amino acids. It is concluded that the D-stereoisomers are active in the D-form.

The very weak inductive action of L-amino acids suggests that the effect may be due to contamination of L-amino acid preparations by the D-isomers. This possibility has been excluded by the finding that 5x recrystallized L-phenylalanine shows the same low inductive effect as the unrecrystallized amino acid. It had previously been shown, by an isotopic labeling experiment, that recrystallization from water is a very effective method for optically purifying L-phenylalanine.

Two means for obtaining strong inductive effects with L-aromatic amino acids have recently been found. The first consists in utilizing L-fluorophenylalanine instead of L-phenylalanine. The fluorophenylalanines (both meta- and para- have been tested) are all good inducers, with little difference between optical antipodes. The second consists in exposing non-growing Neurospora to a natural L-aromatic amino acid (only L-dopa has been tested so far) for a brief period and then transferring the mycelium to buffer devoid of inducer. Tyrosinase activity appears in the mycelium after the transfer. The initial exposure to L-dopa can be for 10 minutes or for 48 hours, but the result is the same -- i.e., little or no induction of enzyme activity until the mycelium is transferred to a dopa-free medium. Untreated controls form little or no enzyme. These findings suggest that tyrosinase induction involves two steps. Step I (induction proper) requires an aromatic amino acid. Step II (expression) is inhibited by L-dopa, but not by D-dopa.

Publication:

82. D-AMINO ACID METABOLISM IN NEUROSPORA

Investigators: Yasushi Watanabe, N. H. Horowitz
Support: International/Cooperation Administration
National Institutes of Health, Public Health Service
Arthur McCallum Fund, General Funds

Taking advantage of the D-amino acid oxidaseless mutant (oxD-1) of Neurospora, in which D-amino acids are not deaminated (Ohnishi and Horowitz, Biology 1959), the fate of labeled D-amino acids is being studied.

As a first step, the possible occurrence of D-amino acids in the cell wall of Neurospora has been investigated. A washed cell-wall preparation from wild type was hydrolyzed in 6N HCl and chromatographed on a Dowex-50 column. Peaks corresponding to alanine, valine + proline, and leucine + isoleucine were collected, oxidized with D-amino acid oxidase, and the ketonic acids converted to their dinitrophenylhydrazones. The latter were subjected to hydrogenolysis and the products chromatographed on paper. A small quantity of alanine, amounting to 3 to 4% of the total cell-wall alanine, was found.

Control experiments in which L-alanine was subjected to the same hydrolysis treatment showed that sufficient racemization took place to account for most of the D-alanine detected in the above experiment. It is therefore unlikely that more than a trace of D-alanine occurs in the cell wall of wild-type Neurospora.

When the same procedure was carried out with the cell wall of the oxD-1 mutant, essentially the same result was obtained. It is concluded that D-alanine does not accumulate in the cell wall of the mutant.

Further indication of the non-participation of D-alanine in the cell wall structure of Neurospora comes from experiments in which wild type and oxD-1 were grown in the presence of C-14-D-alanine. Hydrolysates of the cell walls showed insignificant incorporation of radioactive alanine.

83. GENETIC STUDIES ON D-AMINO ACID OXIDASE IN NEUROSPORA

Investigators: Eiji Ohnishi, Helen Macleod, N. H. Horowitz
Support: National Science Foundation
General Funds

As a continuation of studies reported last year, attempts have been made to clarify the genetic and biochemical characteristics of the D-amino acid oxidaseless mutant (designated oxD-1). In this mutant, the D-amino acid oxidaseless character is accompanied by another biochemical defect -- inability to utilize sulfate as the sole source of sulfur. The question is whether the D-amino acid oxidase is involved in the biological reduction of sulfate, or the two defects result from coincident mutations in the same organism. Attempts to separate the two characters by crossing over have been unsuccessful; if two genes are involved, they are very closely linked. Attempts to separate them by reversion of the oxD-1 mutation have also failed; the D-oxidaseless character appears to be stable to reversion by ultraviolet. A search for new occurrences of the D-oxidaseless mutation is now under way. Three independent sulfiteless mutants have been tested for D-amino acid oxidase activity; all show normal activity.

As reported last year, the oxD-1 gene prevents the inversion of D-amino acids in vivo. It has also been found that strains carrying this mutation are more sensitive to inhibition by D-phenylalanine and D-tyrosine than is wild-type.
Neurospora. In addition, they are unable to utilize D-methionine as a sole source of sulfur. These effects provide further evidence that the enzyme is active in vivo, as well as in vitro, in oxidizing D-amino acids and provide additional clues to its possible metabolic function.

Publication:


84. L- AND D-HOMOSERINE DEAMINATION IN NEUROSPORA

Investigators: Eiji Ohnishi, N. H. Horowitz
Support: National Science Foundation
General Funds

It has been shown in rat liver that the enzyme cystathionase, which cleaves cystathionine to cysteine and a-ketobutyric acid, also deaminates L-homoserine. Another enzyme, cystathionine synthetase, which forms cystathionine from serine and homocystein, is identical with serine deaminase (Greenberg et al., 1958, 1959). It was of interest to know whether or not the dual nature of these enzymes is also observable in Neurospora, where several mutants have been known in connection with cystathionine metabolism.

The results of studies on mycelial extracts of Neurospora showed no correlation in activity between cystathionase I (corresponding to cystathionase of rat liver) and L-homoserine deaminase and also no correlation in activity between cystathionase II (corresponding to cystathionine synthetase of rat liver) and L-serine deaminase.

In the course of this study an enzyme which deaminates D-homoserine was found.
PART E. -- OTHER

STAFF:
Research Fellows: Alexander Miller, David Norman

85. ELECTRON MICROSCOPY OF NEUROSPORA

Investigators: Alexander Miller, Elizabeth Crawford
Support: National Institutes of Health, Public Health Service

In a previous report (see Biology 1958, page 17), various aspects of the cytology at the level of electron microscopy of Neurospora were delineated. This work has now been elaborated with respect to several of the structures previously described.

The cell wall of both conidia and mycelia, especially after uranium staining, appears "woven" or composed of many layers.

The large number of "mitochondrial-like" bodies, previously described in conidia, in some aspects appear to be connected to each other and/or to nuclei.

In mycelia, besides the usual structures, two unusual structures have been found. First, scattered throughout the mycelium are uniformly dense bodies, about the size of nuclei (ca. 1 µ), which in some aspects appear as perfect hexagons. These bodies are found in the "mitochondrial" fraction after differential centrifugation. The other unusual structures are found along the cell membrane and in most aspects appear to be target-like bodies, but in occasional sections appear separated from the cell and may represent bodies involved in pinocytosis or extrusion of material from the cell.

86. ULTRATHIN SECTIONING OF VITRIFIED TISSUE FOR ELECTRON MICROSCOPY

Investigator: David Norman
Support: American Diabetes Association
General Funds

A new technique for the examination of biological material in the electron microscope which bypasses the need for tissue fixation and water removal is currently being investigated. Since the formation of ice crystals disrupts much of the fine structural organization within a cell, it is necessary to bring the specimen through the temperature range in which ice crystal formation takes place at a rate which exceeds that of ice crystal growth. Using liquid nitrogen as the coolant, liquid propane (-185°C) as the immediate surrounding liquid and specimen material having dimensions of 1 cu. mm or less, the liquid water of the specimen can be transformed to vitreous ice without the formation of crystalline ice. Since the growth of ice crystals takes place from 0° to -130°C, it is necessary to keep the vitrified specimen outside of this temperature range during all operations prior to and including examination in the electron microscope.

The problem of sectioning a specimen while maintaining its water in the vitreous state becomes a matter of designing some apparatus which permits ultrathin sectioning to take place at -150°C or lower. This has been achieved by the construction of a specimen holder and a section well, both of which are cooled with a continuous liquid nitrogen flow system. Many difficulties were encountered in the proper design of these two devices. Such difficulties have for the most part been
overcome and the requisite for sectioning below -150°C has been satisfied.

In the electron microscope the path through which the electron beam passes is essentially a vacuum tube and for this reason it is necessary to encapsulate the ultrathin vitreous section with a material that is relatively impervious to water. This encapsulating material must, in addition to its ultrathin requirement, have a scattering cross section and intensity constant which readily permit transmittance of the incident electron beam to the image plane without any appreciable interference. Certain fluorocarbon plastics are being presently considered for use as the encapsulating material.

The one remaining problem which must be dealt with in order for this technique to be exploited to the fullest is the matter of specimen contrast.
87. OPTICAL ROTATORY DISPERSION AND SECONDARY STRUCTURE IN NUCLEIC ACIDS

Investigators: Paul O. P. Ts'o, G. K. Helmkamp, Christian Sander
Support: National Institutes of Health, Public Health Service

In a typical melting experiment, calf thymus DNA in aqueous buffer at pH 5.5 has a transition temperature of about 85°C, and exhibits an increase in optical density of about 35 to 40% between 25°C and 95°C. The curve relating optical density to temperature is similar to that relating optical rotatory power to temperature. However, from the slope of the curve at the highest temperature used for measurement, the maximum optical density or minimum specific rotation has not yet been attained at the highest practicable temperature. Consequently, the interpretation has been made that some helical regions still exist, and that, in the case of optical rotation, the highly positive rotatory contribution of helical regions is the controlling factor in a solution of structures of mixed conformation. During a study of the effect of organic solvents on the conformation of calf thymus DNA and pea microsomal RNA, it has been found that solutions of these materials in formamide or dimethylsulfoxide have specific optical rotations which were independent of temperature in the range normally considered. This has led to the use of rotatory dispersion as a tool in determining secondary structure.

The chromophore in purine and pyrimidine nucleosides, nucleotides, and nucleotide polymers which is primarily responsible for optical activity appears to be that which absorbs in the ultraviolet spectral region near 260 μm. Most data show a moderately good fit to a single term Drude equation, with...
in the vicinity of 240 m\(\mu\). We have shown that the positive simple dispersion curve (increasing specific rotation with decreasing wavelength) of DNA does not disappear in aqueous solvents, even with "random coil" material at elevated temperatures. However, a distinct change does occur with the same solute in formamide or dimethylsulfoxide. In the former organic material the magnitude of rotations is so low that it is difficult to carry out calculations for the Drude equation, but in the latter the simple dispersion curve is negative. Calculations of \(\kappa_0\) indicate the chromophore is in about the same region as that for the positive dispersion curves. Thus, since the position of the chromophore is established as equivalent for DNA in organic and in aqueous solvents, the difference in the sign of the simple dispersion curves must be the result of a change in the asymmetric environment of that chromophore.

If specific solvent effects were responsible for changes in dispersion, it would be expected that similar behavior would be found for nucleotides. This, however, is not the case. A change in solvent system from aqueous to organic has no effect on sign, and little effect on magnitude, of simple dispersion curves.

Further pertinent information concerning the relationship between structure and dispersion has been gained from the base-homogeneous polyribonucleotides. For poly A, the chromophore position calculated from a single term Drude equation is 237 m\(\mu\) for aqueous solution and 253 m\(\mu\) for formamide solution. The dispersion curve of the latter, however, has a negative sign, while the former is positive. Furthermore, the dispersion curve in organic solvent is quite comparable in sign and order of magnitude to that of 5'-AMP. Poly C shows similar behavior. The dispersion of its formamide solution is positive, and compares closely with that of 5'-GMP. In these instances, the asymmetric environment of the purine or pyrimidine chromophore appears to be a function of the parent nucleotide and not of any secondary structure resulting from helical regions. The same interpretation, when applied to RNA and to DNA, is consistent with all present observations. Both nucleic acids exhibit practically zero rotation in formamide as does a mixture of nucleotides of equivalent composition.

88. EFFECT OF ORGANIC SOLVENTS ON NUCLEIC ACIDS

Investigators: Paul O. P. Ts'lo, G. K. Helmkamp, Inglore Melvin
Support: National Institutes of Health, Public Health Service

Formamide and dimethylsulfoxide have been found to be effective solvents for nucleic acids. Solutions of calf thymus DNA and pea microsomal RNA may be prepared directly in these solvents, and the polymeric material can be recovered by precipitation with benzene or alcohol-ether.

The specific optical rotation of nucleic acid in the organic solvent is low, or even negative, and there is essentially no dependence of rotation on temperature within the normal range of measurement, 25 to 90\(^\circ\)C. This is interpreted in terms of loss of formal secondary structure by the polymers, for the strong dependence of rotation on temperature of aqueous nucleic acid solutions is associated with loss of intramolecular hydrogen bonding and change in molecular conformation.

Further verification of such solvent-induced denaturation is based on physical data obtained from DNA and RNA possessed of a history of solution in formamide or dimethylsulfoxide. When precipitated nucleic acid is dissolved in aqueous buffer, it behaves much like heat-denatured material. DNA loses the sharp transition (melting) temperature typical of a two-stranded helix, and now
shows a hyperchromic or temperature-rotation effect comparable to that of the random coil product. RNA is altered from a two-component system with sedimentation values of about 17S and 27S to a single component system with a very broad sedimentation band of about 7-10S.

When potassium chloride is added to a solution of DNA or RNA in formamide there is partial restoration of positive optical rotation. The same rotational effect can be observed when nucleic acids are dissolved directly in formamide containing the salt. In either situation, the dependence of rotation on temperature is reminiscent of that of nucleic acid with random coil conformation in aqueous solvents. DNA reaches a minimum in rotation at about 45°, and no effects above this temperature have been observed.

Titration of aqueous solutions of the nucleic acids with formamide or dimethylsulfoxide, followed by observation of changes in specific rotation, shows that DNA possesses a sharp transition at about 51 and 28 mole percent organic solvents respectively. Similar titration with RNA yields a broad curve of profile similar to that of a hyperchromicity curve.

Publications:


89. THE NUCLEOTIDE COMPOSITION OF MICROSOMAL RNA

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

Studies are continuing on the comparison of base ratios of microsomes which differ by virtue of ability to synthesize slightly different protein species, namely, different hemoglobins. It has already been found that the molar percent composition of the four nucleotides of RNA of pea stem microsomes differs considerably from that of rabbit reticulocyte microsomes. The averages of triplicate analyses for microsomes of rabbit and sheep reticulocytes are listed below:

<table>
<thead>
<tr>
<th></th>
<th>Rabbit Reticulocytes</th>
<th>Sheep Reticulocytes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Proportion in microsomal RNA: molar percent</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adenylic Acid</td>
<td>18.5 ± 0.2</td>
<td>18.0 ± 0.5</td>
</tr>
<tr>
<td>Uridylic Acid</td>
<td>18.5 ± 0.2</td>
<td>17.2 ± 0.2</td>
</tr>
<tr>
<td>Guanylic Acid</td>
<td>34.4 ± 0.2</td>
<td>34.4 ± 0.3</td>
</tr>
<tr>
<td>Cytidylic Acid</td>
<td>28.6 ± 0.1</td>
<td>30.3 ± 0.3</td>
</tr>
<tr>
<td>6 Amino Base/6 Keto Base</td>
<td>.89</td>
<td>.93</td>
</tr>
</tbody>
</table>
The adenylic/uridylic ratio is 1 for rabbit and close to 1 for sheep reticulocyte microsomes, but the 6 amino base/6 keto base ratios are considerably below unity. Guanylic acid constitutes about one-third of the total base composition of reticulocyte microsomes, a result similar to that reported previously for pea microsomes.

The reticulocyte base ratios are quite similar, a fact which suggests that the nucleotide compositions of microsomes which synthesize similar proteins may be similar, although those microsomes which synthesize proteins of vastly different composition (e.g. pea stem microsomes) have different base ratios. Under investigation is one of the base compositions of reticulocyte microsomes of sheep possessing two genetically different hemoglobins.

90. ON THE SEQUENCE OF NUCLEOTIDES IN RNA FROM RABBIT RETICULOCYTE RIBOSOMES

Investigator: Richard F. Squires
Support: National Institutes of Health, Public Health Service

The genetic information required to determine the unique sequence of amino acids in a specific protein molecule is believed to be encoded in the nucleotide composition and sequence of ribosomal RNA.

It is therefore of interest to look for a possible correlation between the nucleotide composition on ribosomal RNA and the amino acid composition of the protein whose synthesis may be mediated by the ribosome. Most of the ribosomes in the reticulocyte are assumed to contain the information for synthesizing a single protein, hemoglobin.

General information about the nucleotide sequences in RNA can be obtained by quantitatively examining the pancreatic ribonuclease digestion products of the RNA. Pancreatic ribonuclease is an enzyme which selectively cleaves the RNA chain adjacent to every pyrimidine nucleotide residue. This gives rise to oligonucleotide fragments composed of a variable number of purine nucleotide residues terminating in a pyrimidine nucleotide residue.

Ribosomal RNA was prepared by the detergent-chloroform procedure (see Biology 1959) and digested to completion (24 to 36 hours) with ribonuclease in a pH stat. The digestion products were partially separated by chromatography on Dowex-1 columns. All of the mono- and di-nucleotides anticipated were quantitatively isolated and estimated.

There are a variety of techniques for the fractionation of the oligonucleotide digestion products of ribonuclease described in the literature. These involve paper electrophoresis and chromatography, or column chromatography on DEAE cellulose or Dowex-1. These methods have the disadvantage of either giving incomplete resolution of the components, or of being somewhat unquantitative. The present technique utilizes a preliminary chromatographic fractionation of the enzymatic digest into mono, di, tri, tetra, etc., nucleotides followed by re-chromatography of the individual fractions.

The following results are expressed as mole percents of total mono-nucleotide residues.
<table>
<thead>
<tr>
<th>Digestion product</th>
<th>Found</th>
<th>Calculated</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>15.6</td>
<td>13.9</td>
</tr>
<tr>
<td>U</td>
<td>8.3</td>
<td>8.5</td>
</tr>
<tr>
<td>AC</td>
<td>4.1</td>
<td>5.3</td>
</tr>
<tr>
<td>GC</td>
<td>9.6</td>
<td>9.3</td>
</tr>
<tr>
<td>AU</td>
<td>2.4</td>
<td>3.2</td>
</tr>
<tr>
<td>GU</td>
<td>4.8</td>
<td>5.7</td>
</tr>
</tbody>
</table>

The mole percents found are compared to values calculated from the previously determined base ratios of the RNA (see Wallace, Biology 1960) assuming a random distribution of the nucleotides. It appears that cytidylic acid occurs more frequently, and three of the di-nucleotides occur less frequently than would be expected if the constituent nucleotide residues were randomly distributed along the RNA chain. The excess of cytidylic acid indicates some clustering of this nucleotide in the RNA chain.

91. STUDIES OF RIBOSOMES AND RIBOSOMAL RNA

Support: National Institutes of Health, Public Health Service
National Science Foundation

In the past year the properties and the structure of ribosomes and ribosomal RNA have been examined in greater detail. Preliminary experiments on the chemistry of nucleic acids in organic solvents were also done. These experiments were performed with the purpose of chemical modification of nucleic acids, and removal of the secondary structure of the nucleic acids due to hydrogen bond formation by gentle and specific manner.

I. The binding of magnesium to microsomal nucleoprotein and RNA.

Mg$^{28}$ was used as a tracer in studying the Mg binding by microsomal particles and RNA isolated from rabbit reticulocytes. Methods for following the exchange of Mg by dialysis with Mg$^{28}$, for precipitating RNA or nucleoprotein with its complement of bound Mg, and for extracting the bound Mg were established. The combination of these methods provides appropriate material for chemical and radioisotopic studies of the binding of Mg to polynucleotides.

Data have been presented to indicate that almost all, if not all, the Mg of the RNA-protein is bound by the RNA moiety. Removal of the protein component from RNAP uncovers about 30% more Mg binding sites.

Preparations of RNAP isolated from buffer of low K:Mg ratio contain mostly intact 80S particles and have a Mg:P molar ratio of 0.3, similar to the ratio in intact pea microsomal particles. Dissociation of the 80S particles occurs when the Mg:P molar ratio of RNAP is lowered to about 0.2 by isolating the microsomal particles in buffer of high K:Mg ratio.
II. Properties of ribosomes from reticulocytes.

An extensive investigation of the dissociation and the properties of subunits from rabbit reticulocytes, and to a lesser extent from human and sheep reticulocytes, has been done, in association with Dr. Jerome Vinograd, C. I. T.

These ribosomes dissociate into subunits upon removal of Mg ions, and these subunits may be reassociated to ribosomes when the Mg ions are replaced. The first dissociation products appear to be two large nucleoprotein subunits, one being roughly twice the mass of the other. In these respects, the particles from reticulocytes are similar to those from pea, liver and microorganisms. However, unlike the ribosomal subunits from pea seedling, the reticulocyte ribosomal subunits are unstable in the presence of dilute electrolytes. Thus, the investigation had to be carried out at low values of ionic strength. This leads to the usual difficulties which arise from electrical charge effects.

The Mg ion controlled dissociation and reassociation is substantially reversible. Thus, are the subunits and the ribosomes present in a given system at a given Mg level in mobile equilibrium with each other in a process governed by one equilibrium constant? Experimental investigation shows that at a given level of Mg, the remaining ribosomes and the dissociated subunits are not in mobile equilibrium. It appears, then, that the populations of ribosomes are heterogeneous with respect to their dissociation constants. At a given level of Mg, certain classes of ribosomes are dissociated and others are not.

III. The secondary structure of nucleic acids in organic solvents

Calf thymus DNA and pea microsomal RNA lose all of their formal secondary structure due to hydrogen bonding when dissolved in formamide or dimethylsulfoxide. The following observations on such solutions are used as evidence for this conclusion: Low specific rotation, independence of rotation on temperature, similarity of the optical and hydrodynamic properties of nucleic acids once dissolved in organic solvents at room temperature to those of nucleic acids denatured by heat, and finally, the opposite signs of simple rotatory dispersion curves of nucleic acids, but not of the nucleotides, in aqueous and organic systems.

Thus, it is very interesting to know that after solution in formamide, the TMV-RNA does not change its molecular weight and is still equally infective, while the molecular weight of pea microsomal RNA after solution in formamide decreases to about 1.5 to 2.5 x 10^5 from its original value of 1.2 x 10^6.

92. THE SITE OF AMINO ACID ASSEMBLY IN MICROSMAL PARTICLES

Investigators: Joan Wallace, Richard F. Squires, Paul O. P. Ts'o
Support: National Institutes of Health, Public Health Service

Earlier studies have demonstrated that microsomes can be dissociated into two classes of ribonucleoprotein subunits. It is of interest to know whether either or both kinds of subunits become labeled when C-14-labeled amino acids are incubated with reticulocytes under conditions appropriate for amino acid incorporation. The two kinds of subunits can be separated from one another for such analysis by sucrose density gradient centrifugation. By this procedure it has been found that the specific activities of the 38S and 60S subunits of microsomes (dissociated by dialysis against 0.05M KCl solution after incubation) are within the same range. This indicates that the incorporation of valine-C-14 occurs equally in the two fractions of the intact microsome.
There also appear to be slowly sedimenting components (of less than 10S) of high specific activity which are present among the dissociation products of microsomes, but which are not found in non-dissociated microsomes. The nature of this fraction is still under investigation.

93. **PROTEIN SYNTHESIS BY PEA RIBOSOMES**

Investigator: Max L. Birnstiel  
Support: National Science Foundation

Pea ribosomes as prepared in this laboratory are capable of substantial protein synthesis in vitro.

In the present investigation, the conditions which influence yield of protein by isolated ribosomes have been systematically studied. Optimal conditions with respect to ATP, GTP and sucrose concentration in the incubation mixture have been established. Phosphoglyceric acid and creatine phosphate are equally efficient as ATP regenerating systems. Addition of pH 4.5 enzymes exerts but little effect in increasing the rate of protein synthesis as measured by C-14-leucine incorporation into soluble (non-ribosomal) protein.

Ribosomes of pea roots were found to be more active than those of pea stems. The systems studied show higher activity in incorporating amino acid into soluble RNA than into protein. The factor which limits rate of protein synthesis must therefore involve reaction steps which are dependent on the presence of ribosomes.

94. **EFFECT OF RED RADIATION ON YIELD OF MICROSONES FROM PEA SEEDLINGS**

Investigators: Gordon Alderton, Ingelore Melvin, James A. Lockhart  
Support: U.S. Department of Agriculture, Frasch Foundation

Observations of day-to-day variation in yield of pea microsomes led to an investigation of the influence of red light irradiation on pea microsome yield. Four-day-old, dark-grown Alaska pea seedlings were irradiated for 2 hours with red light of 150 ergs·cm⁻²·sec⁻¹ intensity. Irradiated and dark-grown seedlings were harvested 16 hours later and yields of crude microsome fractions from the stem tips compared. In the first experiment irradiated plants yielded approximately 1.0 mg microsomes per gm fresh weight compared with 0.5 mg per gm for dark-grown plants.

A second experiment was conducted to determine the optimum time of irradiation for maximum yield and the minimum time for an observable effect. Two-hour irradiations were given to separate lots 2, 4, 8, 16 and 32 hours prior to harvest. Maximum microsome yields were found when plants were irradiated 16 and 32 hours prior to harvest. No effect of irradiations 2 and 4 hours prior to harvest could be observed.

Red light inhibits stem elongation but promotes developmental changes in the pea plant. The increased yield of microsomes may well be a reflection of these developmental changes. Since the first observable growth response to red light occurs within two hours following the onset of irradiation, the observed change in total microsomes is, presumably, a result rather than a cause of the initiation of developmental changes.
For isolation of pea microsomes it has become standard practice to expose the seedlings to red radiation about 24 hours prior to harvest.

95. PROTEIN SYNTHESIS

Investigators: Clifford S. Sato, Hedi Imfeld
Support: National Institutes of Health, Public Health Service

The purpose of this investigation is the study of the direction and manner of growth of the peptide chain during protein synthesis. Methods have been devised for the separation of the peptide chains of the globin of rabbit hemoglobin. Depending on the types of hemoglobin present in the rabbit, 2 to 5 major components have been obtained by column chromatography.

Approximately 48 valine molecules are present in rabbit hemoglobin; 4 of these are present at the amino ends of the 4 peptide chains. An immediate comparison of incorporation of labeled valine into the N-terminal position and incorporation into subterminal positions can therefore be made.

Valine-C-14 was incorporated into hemoglobin by incubating rabbit reticulocytes with Borsook's incubation mixture. After removal of the heme, chain separation was accomplished by chromatography. The N-terminal valine was separated from internal valine of globin by reacting the separated chain with 2,4-dinitrofluorobenzene followed by acid hydrolysis and chromatography. When the total radioactivities were determined, the internal valine contained 14 times as much radioactivity as the N-terminal valine. Although it appears that hemoglobin approaches uniform labeling within 12 minutes, the N-terminal valine has less than the average radioactivity of the internal valine. Although this result does not prove sequential protein synthesis, it does suggest it and suggests in addition that the N-terminal end of the peptide chain is the first to be completed.

96. ISOLATION OF NUCLEI OF CHLORELLA

Investigator: Joon H. Rho
Support: International Cooperation Administration
National Institutes of Health, Public Health Service
General Funds

Present data indicate that microsomes are contained in the nucleus, but the question whether or not they are wholly synthesized within the nucleus remains open. It has been known for some years that the nucleus is the seat of active RNA synthesis and that much of the RNA of the cell is made there.

One way to investigate the formation of the microsomal particles would be the study of the microsomal RNA synthesis within isolated nuclei. Chlorella, a unicellular organism which grows rapidly and in which all cells of a population can be synchronized as to stage of life cycle, was chosen for the present study. Optimum growth conditions of the desired Chlorella strain have been established and techniques have been worked out for breaking the cell membrane by means of an Omnimagizer with the aid of 200 µ glass beads. Isolation of the nuclei had been carried out by the use of millipore filter paper and differential centrifugation procedures.

When radioactive phosphorus is fed to Chlorella, the greater proportion is converted to acid-labile inorganic phosphorus polymers and little of it is used for nucleic acid synthesis. In addition Chlorella nuclei are small, and it is rather
difficult to check the purity of the isolated nuclear preparations with ordinary optical apparatus. It has therefore been concluded that Chlorella is not a suitable organism for the study of RNA synthesis by isolated nuclei even though this organism has many attractive features.

SYNTHESIS OF RNA BY ISOLATED NUCLEI

Investigators: Joon H. Rho, Margaret I. H. Chipchase
Support: International Cooperation Administration
National Institutes of Health, Public Health Service

Numerous lines of evidence indicate that the cell nucleus may play an important part in the synthesis of microsomal RNA and nucleoprotein. The present work was undertaken to find out whether isolated nuclei can carry out steps assumed to be important in the formation of microsomes, namely synthesis of RNA. For this work it is necessary to obtain isolated nuclei in highly purified form. Conventional methods of grinding tissues result in the disruption of many nuclei, and produce large quantities of cell debris which is difficult to remove. A new technique has therefore been developed in which the tissue is crushed between rollers and the juice collected in buffered sucrose containing calcium ions. The nuclei are centrifuged down at low speed. The tips of etiolated pea epicotyls have proven a rich source of nuclei.

Nucleic acid precursors C-14-labeled orotic acid and C-14-adenosine diphosphate are incorporated into RNA by such isolated nuclei. This incorporation is increased by addition of other nucleic acid precursors.

It is therefore clear that isolated pea nuclei can synthesize RNA. It remains to be discovered whether or not this RNA is packaged into microsomes by the nucleus.

POLYNUCLEOTIDE PHOSPHORYLASE

Investigator: Gerhard Richter
Support: National Institutes of Health, Public Health Service

The enzyme polynucleotide phosphorylase which catalyzes the synthesis of polyribonucleotides from the nucleoside phosphates ADP, GDP, CDP and UDP was originally isolated from extracts of bacteria. An attempt has been made to detect this enzyme in various plant materials. Particle-free extracts from etiolated pea seedlings, green oat seedlings, and Chlorella were partly purified by ammonium sulfate fractionation. Activity was tested by incubating with labeled ADP-C-14 and cold ADP alone or together with the other nucleoside diphosphates. Incorporation of ADP-C-14 and/or the amount of acid-insoluble polymer formed was measured. The fraction with the highest activity was then used for further purification by means of gel adsorption, ammonium sulfate and acetone precipitation. The preparations from the seedlings were always low in specific activity even after additional purification steps. From Chlorella, however, partly purified fractions with high specific activity were isolated.

In other investigations, the localization of the enzyme in plant cells was determined. Extracts of isolated pea nuclei were tested, but found to contain little activity.
A SYNTHETIC MEDIUM FOR ACETABULARIA

Investigator: Gerhard Richter
Support: National Institutes of Health, Public Health Service

The unicellular and uninucleate marine alga Acetabularia has already been successfully used to study the synthetic capacities of surviving non-nucleated cells. This organism can be cultivated in sea water supplied with soil extract. For some experiments, however, it is desirable to work with a well defined artificial culture medium which can be easily maintained in aseptic condition. A medium has therefore been developed which is composed of natural sea water plus phosphate, iron, and a selection of trace elements (Mn, B, Co, Zn, Mo). Soil extract is replaced by versene. The mixture can be autoclaved without precipitate formation. Comparative studies have shown that cell growth, differentiation and sexual reproduction occur as well in this medium as in natural sea water supplied with soil extract.

CAROTENOGENESIS

Investigators: Guy A. Thompson, Jr., Albert E. Purcell
U.S. Department of Agriculture
General Funds

The incorporation of 2-C-14-mevalonate into tomato carotenoids has been described in previous work. Although mevalonate is the best carotene precursor so far found, more of its radioactivity is incorporated into a series of unidentified compounds than into purified carotenes.

Other workers have shown that mevalonate is synthesized from acetic acid and the results of the incorporation of labeled 1-C-14-acetate into carotenes is in agreement with expectations. Glucose-C-14-labeled uniformly or in the 1, 2, or 6 position, is incorporated into the carotenes several times as effectively as acetate, with practically no label being incorporated into the unknown compounds that become so highly labeled with 2-C-14-mevalonate.

For the present it is postulated that only the chromatoplasts (intracellular bodies which contain practically all the carotenes) can utilize glucose for carotenogenesis, while mevalonate or acetate can be used for making all isoprenoids, some of which may be synthesized more rapidly than carotenoids but at some site other than chromatoplasts. This hypothesis is in agreement with all present information.

Attempts have been made to develop cell-free systems for carotenogenesis. Several preparations of tomato chromatoplasts have demonstrated limited net synthesis (10% of total) within 6 hours but none of the preparations have incorporated labeled mevalonate, indicating perhaps that the systems are incomplete.

It was found that gaseous radioactivity was given off by tomatoes treated with 2-C-14-mevalonate. The gas was trapped in mercuric-perchlorate and identified by gas chromatography. The principal labeled materials are acetaldehyde and acetone. (See report of Nimmo, Pollard and Haagen-Smit.)

Publication:

A. Purcell and G. A. Thompson, Jr., "Glucose as a Carotene Precursor in Tomatoes," Arch. Biochem. and Biophys., in press.
101. BIOSYNTHESIS OF DIOSGENIN

Investigators: Erich Heitmann, James Bonner
Support: National Institutes of Health, Public Health Service

While the sequence of reactions involved in the biogenesis of various animal steroids has been thoroughly studied, nothing is known about the origin of typical plant steroids. Diosgenin, a steroidal sapogenin, occurs in the tubers of the Mexican "yams" belonging to the Dioscoreaceae.

On the assumption that diosgenin, like cholesterol, is formed from acetate via mevalonic acid, slices of Dioscorea floribunda were incubated with radioactive acetate or mevalonate. A number of radioactive products, not identical with diosgenin, were isolated by chromatography. Only homogenates and fortified centrifugates of the tubers produced diosgenin of rather low specific activity.

102. BIOGENESIS OF VOLATILE PLANT MATERIALS

Investigators: C. C. Nimmo, C. J. Pollard, A. J. Haagen-Smit, James Bonner
Support: U.S. Department of Agriculture
U.S. Atomic Energy Commission
General Funds

Plant volatile substances, including ethylene, are being studied to determine biogenetic relationships between these and certain nonvolatile plant substances. Carbon-14-labeled materials have been vacuum-infiltrated into whole tomatoes, the volatiles trapped, separated by gas chromatography, and specific activity values determined. The findings of previous investigators (Burg, Arch. Biochem and Biophys., 84:543, 1959) that carboxyl-labeled acetate and uniformly labeled sucrose are effective in labeling ethylene from apple tissue have been substantiated, using tomatoes, and it has now been shown that both carbons of acetate, and both atoms 1 and 6 of glucose transfer the label to ethylene. On the basis of present experiments, the specific activity transfer is of the same order for both compounds, the ethylene being about 10⁻⁴ times the specific activity of the source. However, considering the probable pool size of glucose in the tomato, it is possible that the label transfer from glucose to ethylene is undiminished in specific activity. This is supported by the low specific activity levels in ethanol and acetaldehyde from the labeled glucose experiments. These facts, however, supply no immediately useful clues about precursor relationships.

Although glucose-1- or -6-C-14 and acetate transfer some labeling to ethylene, lactate-2-C-14 transfers, if any, less than the above two. Other materials evaluated increase lactic-acid-1-C-14, mevalonate-2-C-14, and ethanol-2-C-14. All are ineffective in labeling ethylene, but otherwise performed as expected. The last-named labels acetaldehyde so directly and efficiently as to rule it out also as a possible ethylene precursor.

Carbon dioxide labeling values were as expected for the individual compounds, and this supplies further evidence that Krebs-cycle acids are not effective as ethylene precursors.
THE BIOSYNTHESIS OF TERPENOIDS

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

Terpenoids are universal constituents of higher plants. Important representatives are: the vitamin A precursors -- carotenoids, vitamins E and K and components of the essential oils. Several lines of evidence indicate that mevalonic acid is a precursor of some of these isoprenoids. The elucidation of the metabolic pathways whereby mevalonic acid is converted into these diverse compounds is contingent on the development of cell-free enzyme systems that are able to effect these reactions. This research has been directed to that end.

A cell-free system has been found in mature green peas that efficiently incorporates mevalonic-2-C-14 acid into neutral lipids. The enzymatic activity resides in the soluble fraction. Known cofactors for the reaction are adenosine triphosphate and magnesium chloride. In addition, thiamine pyrophosphate significantly enhances the incorporation; pyridine nucleotides and coenzyme A have no effect. Known inhibitors are sodium arsenate (but not arsenite), hydroxylamine, manganese sulfate, sodium fluoride and iodoacetamide.

The probable complexity of the reactions is indicated by the variety of compounds formed. When mevalonic acid is incubated with acetone powder preparations of the soluble fraction, in the presence of cofactors, at least four water soluble metabolites may be demonstrated by paper chromatography. Three of these compounds contain phosphorus. The predominant compound has properties similar to a monophosphate derivative of mevalonic acid. The compound that does not contain phosphorus is also present in significant quantities. When mevalonic acid is incubated with the soluble fraction as many as seven neutral lipid compounds may be extracted into fat solvents and detected by paper chromatography. This cell-free system suggests that it may be related to similar systems studied by Witting and Porter (J. Biol. Chem. 234:2843, 1959) and Popjak (Tetrahedron Letters No. 19:19, 1959).

INDUCTION OF INSECT GALLS BY INSECTS AND INSECT-PRODUCED SUBSTANCES

Investigators: William Hovanitz, Dennis R. McGalla, Elizabeth Peterli, Margaret K. Genthe
Support: American Cancer Society
          National Research Council of Canada

A method of bioassay has been developed for testing substances for ability to promote the growth of willow galls. This has been used to follow purification of the active substances from whole and fractionated accessory glands of either willow leaf- or stem-gall wasps ("sawflies").
The active factor of the glands of the leaf-gall wasps was found to be water soluble, dialyzable, resistant to mild acid and alkali, and stable for at least 15 minutes in boiling water. The active material is absorbed on charcoal from aqueous solutions and can be eluted slowly with 50% aqueous ethanol or rapidly with 5% aqueous phenol. Attempts to purify the material by the use of ion exchange resins have so far been unsuccessful.

Examination of the wasp gland extract by paper chromatography in a butanol, acetic acid, water solvent has shown that most of the activity is associated with a zone of Rf 0.25. This zone strongly absorbs ultraviolet light. An additional zone at Rf 0.8 possesses weaker activity.

Preliminary experiments with extracts of stem-gall wasp glands have shown that similar active compounds are present in this material also.

Kinetin, N-furfuryl adenine, has been found, in a single experiment, to simulate the effect of the native material in inducing gall growth.

105. PHYSIOLOGICAL STUDIES ON A PROGRESSIVE NECROTIC MUTANT OF CORN

Investigator: Dennis R. McCalla
Support: National Research Council of Canada
General Funds

This work concerns the physiology of a single gene necrotic mutant of corn. The mutant grows normally as a seedling but later becomes necrotic. The findings that etiolated plants do not become necrotic and that the rate of photosynthesis in normal tissue (i.e. before visible necrotic symptoms appear) from plants homozygous for the necrotic gene is much lower than that of tissue from normal corn (Biology, 1959) have been extended by studies on light intensity and photosynthetic rate. Plants become necrotic under light intensities of only 100 foot candles and under moderate intensities of red, blue, green, and white plus ultraviolet light. It is therefore concluded that neither the quantity nor the quality of the light is critical for the production of necrotic plants.

Comparative studies of the rate of photosynthesis at various light intensities show that the large differences between normal and the normal tissues of necrotic plants at high light intensity decrease as the light intensity is reduced and disappear at from 100 to 500 foot candles depending upon the material. This finding suggests that some factor severely limits photosynthesis in the necrotic plants at high light intensity.

The plastid pigments of the two tissues have been examined by means of column chromatography and spectrophotometry and are essentially identical in the two tissues.

The ability of chloroplast suspensions prepared from normal and from necrotic plants to carry out the Hill reaction (reduction of ferricyanide dyes, etc. in the light) and to photosynthetically phosphorylate ADP was examined. The first reaction was easily demonstrated and found to occur at nearly the same rate in necrotic as in normal corn. It has been, however, impossible to detect any photosynthetic phosphorylation even in chloroplasts isolated from normal corn, even though spinach chloroplasts, under the same conditions, gave rates nearly comparable with the literature values. It has been further shown that the lack of phosphorylation by corn chloroplasts is not due to the presence of ATPase in the
preparations. It is concluded that either corn chloroplasts are more delicate than those of spinach or that corn contains materials which are absent from spinach and which inactivate the phosphorylating system in the chloroplasts. Since it was not possible to demonstrate phosphorylation directly, studies of phosphorous metabolism in intact seedlings and in detached shoots were carried out. The same compounds become labeled to about the same extent in both tissues, labeled ATP being rapidly formed in both. It thus appears that photosynthesis in the necrotic is not limited by lack of phosphorylation.

Tracer experiments have been carried out with C-14-labeled acetate and glucose. The water soluble compounds which become radioactive in the necrotic and in normal plants appear to be identical.

106. KINETIC ANALYSIS OF GROWTH FACTOR INTERACTIONS

Investigator: James A. Lockhart
Support: Frasch Foundation

The recent discovery of the gibberellin hormones, capable of markedly promoting growth of intact plants, has emphasized the problem of interpreting results of plant growth experiments. A much greater understanding of the mechanisms of growth, and its environmental and hormonal control, can be achieved through proper interpretation of interactions of the combined effects of two or more growth promoting and/or inhibiting treatments (environmental and chemical). A kinetic analysis of growth factor interactions has, therefore, been undertaken.

Simple model chain reactions have been set up and the appropriate differential equations have been solved for steady-state process rate as a function of original substrates and specific reaction rate constants. The effect (on steady-state process rate) of varying the level of each substrate and reaction rate constant at different levels of each of the other variables has been examined.

It must be recognized immediately that only systems in the steady-state can be adequately analyzed. Thus, the practice of measuring growth of excised sections or individual stem internodes not in steady-state growth is not useful because the data cannot be analyzed for growth factor interactions.

The following model reaction systems have been examined in this manner for the effect of interactions. In general, each reaction step has been made reversible except for a final irreversible step.

1) Simple catenary system:

\[ A \rightleftharpoons B \rightleftharpoons C \rightarrow P \]

2) Simple zero-order system

\[ A \rightleftharpoons S; \quad S + E \rightleftharpoons C \rightleftharpoons I \rightarrow P \]

3) Two-step substrate zero-order system

\[ A \rightleftharpoons B \rightleftharpoons S; \quad S + E \rightleftharpoons C \rightarrow P \]
4) Two-step substrate system made zero-order by limiting a second substrate (instead of catalyst)
\[ A \rightleftharpoons B \rightleftharpoons S; \quad S + E \rightleftharpoons C \rightarrow P \]

5) Same as 4) except that second substrate enters reaction by diffusion rather than by a reversible reaction
\[ D \]
\[ A \rightleftharpoons B \rightleftharpoons S; \quad S + E \rightleftharpoons C \rightarrow P \]

6) Alternative sources of substrate
\[ A' \]
\[ A \rightleftharpoons B \rightleftharpoons S + E \rightleftharpoons C \rightarrow P \]

The following general conclusions have emerged from these analyses:

1) Varying the rate of any reaction step at different levels of a second reaction step results in diverging process rates as the rate of the first is increased.

2) Varying the concentration of either substrate at different levels of the other substrate (or amount of catalyst) gives diverging process rates as concentration is increased.

3) Varying the concentration of a substrate at different reaction rate constants (of a reaction which provides substrate for the zero-order step) results in process rates which converge to the same maximum as substrate concentration is increased. The converse is not true. If the rate constant is varied at two levels of substrate, process rates diverge as rate constant is increased.

4) If substrate level is varied at different rate constants of a reaction which is a part of, or follows, the zero-order step, process rate curves are parallel and approach the same maximum rate as substrate level is increased, but at different substrate concentrations.

5) If two separate treatments affect the same reaction rate constant, process rates will converge to a maximum as either is increased at different levels of the other.

Thus, four types of interactions can be distinguished using model kinetic analyses. These may be described as:

1) Independent action. Demonstrated by diverging process rates as either factor is increased.

2) Second factor affects activity of the substrate factor. Demonstrated by parallel process rates, saturating to the same maximum at different substrate levels.

3) Second factor affects availability or level of substrate factor. Demonstrated by process rates converging to same maximum.

4) Interchangeable factors. Demonstrated by process rates converging to same maximum rate with either factor as substrate.
These analyses should be applicable to any growth system in which the reactions follow the mass action law and in which the variables tested influence only one reaction. Kinetic analyses in the biological literature and by the author yield results consistent with those predicted here.

This work demonstrates the potentialities of kinetic analyses when applied to growth studies. At least some of the relationships described here are not intuitively obvious. It is hoped that this work, when made available, will be of value in directing and evaluating many growth studies.

107. SENESCENCE IN THE ALASKA PEA

Investigators: James A. Lockhart, Virginia Gottschall, Susan Sherbet
Support: Rockefeller Foundation
Frasch Foundation

Investigations on the physiology of senescence in the Alaska pea have been continued. It has previously been found that:

1) Removal of the flowers -- preventing fruit development -- will markedly delay senescence of the plant.

2) Eventual senescence, which occurs even when flowers are removed, is caused by a degeneration of the stem apex.

Recent experiments have demonstrated that the developing seeds are principally responsible for fruit-induced senescence. This has been shown by surgically detaching the undeveloped seeds from the carpel wall. When seed development is prevented in this manner vegetative growth response is nearly equal to that of plants on which no fruit had been allowed to develop.

Experiments have also been conducted to determine whether a senescence factor -- or a juvenile factor -- is translocated from the stem apex. Juvenile stem tips were side-grafted to old but not yet senescent plants, and old stem tips were side-grafted to young plants. In neither case did the grafted tip influence the fate of the stem apex to which it had been grafted. This and other experiments make it unlikely that the stem apex controls senescence in the rest of the plant.

A second question investigated was whether flowering itself affects the duration of the plant's life exclusive of the effects of the fruit. In other words, do the physiological conditions which lead to flower initiation also influence aging in the plant. It was found that daily far-red irradiation would substantially delay flowering in Alaska and in Alderman Improved varieties of peas. In certain cases flowering could be completely prevented but senescence occurred at the same time whether or not any flowering took place. Apparently, then, senescence is independent of the flowering condition.

108. THE INFLUENCE OF HIGH LIGHT INTENSITIES ON STEM GROWTH

Investigators: James A. Lockhart, Susan Sherbet
Support: Rockefeller Foundation
Frasch Foundation

Stem growth of many plant species is most rapid at light intensities less than that of full sun (full sun gives 8,000 to 12,000 foot candles). Pinto beans have been found to elongate 2 to 3 times more rapidly when shaded to approximately 50%
of the intensity of full sun. Heavier shading results in a progressive decrease in growth rate, undoubtedly due to a limitation in photosynthetic products. Periodic sucrose applications prevent most of the decrease in growth rate caused by heavy shading.

On the other hand, the decrease in growth rate at high light intensities is apparently another function of endogenous gibberellin. When plants are saturated with gibberellin by external application, those growing in full sun are tallest, those in 50% shade are nearly as tall, while those growing in heavier shade are much shorter. Thus, applied gibberellin can prevent the high light intensity inhibition of stem growth, but it cannot prevent the decrease in growth at very low light intensities. Low intensity inhibition, on the other hand, can be at least partially prevented by sucrose treatments.

The pinto bean is among the many plants which respond markedly to applied gibberellin. Other plants, for example, Alaska peas, respond little to applied gibberellin. Furthermore, the Alaska pea shows very little promotion of stem growth at reduced light intensities. It may be suggested that certain types of plants grow at a maximum rate in full sun because they are able to synthesize adequate gibberellin even at high light intensities, while plants whose capacity to synthesize gibberellin is relatively limited might be expected to be much more sensitive to light intensity.

When pinto beans, grown in full sun, are irradiated with far-red light at the beginning of the daily dark period, stem growth is promoted to the same extent as when plants are grown in 50% shade. A combination of 50% shade and far-red irradiation has no greater effect than either alone. Further, red irradiation at the beginning of the dark period will not reverse growth promotion elicited by 50% shade. Thus, both partial shade and appropriate far-red irradiations influence stem growth through an effect on the same limiting system, undoubtedly gibberellin synthesis.

109. REGION OF LIGHT SENSITIVITY FOR STEM GROWTH INHIBITION

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

Visible radiation, particularly the red region of the spectrum, markedly inhibits stem growth, while far-red radiation promotes growth. Evidence is strong that red light acts by reducing the amount of available gibberellin within the plant. It is of considerable interest to know in what part of the plant light exerts its effect. Particularly, one would like to know whether the influence of light is translocated within the plant.

Greenhouse-grown pinto bean plants, whose growth is sensitive to red and far-red light, were irradiated with red light at the beginning of the daily dark period, then specific portions of the plants were irradiated with far-red to reverse the effect of red irradiation.

It was found that light affected the growth of only those internodes which received direct irradiation. Irradiation of leaves was without effect. It appears that no transmission of the influence of light between nodes occurs under these conditions. Thus, the growth response cannot be spatially separated from the photochemical and immediately subsequent chemical processes which ultimately regulate growth.
110. GIBBERELLIC ACID TREATMENT OF BASE OF PEA EPICOTYLS AND GROWTH OF SECTIONS SUBSEQUENTLY EXCISED FROM THE TIP REGION OF THE TREATED EPICOTYLS

Investigators: Paul H. Deal, James A. Lockhart
Support: National Science Foundation
Frasch Foundation

It has been reported that a short pre-treatment of the base of excised Alaska pea epicotyls with gibberellic acid prior to cutting sections for growth in culture causes a considerable increase in growth of these sections, and, in particular, such growth is synergistic with that caused by auxin added directly to the culture medium. Gibberellic acid added to the culture medium reportedly had no such synergistic effect with auxin. It was determined to verify this phenomenon and to investigate it more fully.

Experiments revealed the presence of a definite growth promotion in sections excised from the gibberellic acid pre-treated epicotyls, but this effect was not found to be synergistic with that of auxin added to the medium, and in fact was approximately equivalent to the effect of gibberellic acid added directly to the growth medium.

It was concluded that, while gibberellic acid is conveyed very rapidly through the pea epicotyls, its passage through a length of stem prior to reaching the reactive tissue does not enhance its ability to promote growth of such tissue.

111. GIBBERELLIN REVERSAL OF LIGHT-INHIBITION OF STEM GROWTH IN GREENHOUSE-GROWN PLANTS

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

It has previously been found that the light-inhibition of growth of etiolated plants may be completely prevented by treatment with gibberellin. Stem growth of greenhouse-grown plants may also be markedly influenced by light. When plants are irradiated with red light immediately prior to the beginning of the dark period, stem growth is relatively inhibited, while far-red irradiation at this time causes a marked promotion of stem growth. It has been found that gibberellin treatment of such plants results in an equal growth rate of red and far-red irradiated plants. When growth rates of red and far-red irradiated plants are plotted against dose of added gibberellin, the two curves converge at saturating doses of gibberellin.

These responses to gibberellin and red/far-red irradiations demonstrate that visible radiation affects endogenous gibberellin at the substrate level. That is, radiation affects synthesis or subsequent conversion of the hormone rather than the factor on which it acts or some independent growth process. Thus, in greenhouse-grown just as in dark-grown plants, visible radiation exerts its effect on stem growth through the gibberellin hormone system.
112. **PREVENTION OF LIGHT INHIBITION OF STEM GROWTH BY GIBBERELLIN A₄**

Investigators: James A. Lockhart, Paul H. Deal  
Support: Frasch Foundation  
National Science Foundation

During the course of previous work (Biology, 1957, et seq.) it was found that gibberellic acid (gibberellin A₃) failed to completely prevent photoinhibition of stem growth in certain plant species. Principal species which showed this lack of response were in the Cucubitaceae. It has recently been reported that members of the Cucubitaceae will respond to a much greater extent to gibberellin A₄ than to gibberellin A₃. Following this report gibberellin A₄ was tested for its effect on light inhibition in species of Cucubitaceae which had been previously investigated. It was found that gibberellin A₄, in contrast to A₃, would completely reverse red light inhibition of stem growth in species of Cucubitaceae.

Publication:  

113. **EXTRACTION OF GIBBERELLIN-LIKE MATERIAL FROM CUCUMBERS**

Investigators: Paul H. Deal, James A. Lockhart  
Support: National Science Foundation  
Frasch Foundation

Gibberellin-like factors have been extracted from many higher plants. Because cucumbers are more sensitive to gibberellin A₄ than to gibberellic acid (GA₃), in contrast to most other higher plants, it was decided to investigate the natural gibberellins from these plants.

Extractions have thus far revealed the presence of substances having gibberellin activity in immature and mature cucumber seeds and in germinating seeds at least through the first 72 hours. The immature seeds have been found to contain vastly greater gibberellin activity than older seeds or seedling plants. Gibberellin from immature seeds appears to be similar physiologically to GA₃ rather than the more effective GA₄. Qualitative and quantitative investigations of these substances and extraction of older plants are being carried out.

114. **GIBBERELLIN IN HIGHER PLANTS IN RELATION TO DEVELOPMENT**

Investigators: A. Lang, E. Reinhard, J. Kato  
Support: National Institutes of Health, Public Health Service  
National Science Foundation  
General Funds

Application of gibberellin induces flower formation in some cold-requiring plants not given any low-temperature treatment, and in many long-day plants continuously kept on short days. Gibberellin thus "substitutes" for the normal, flower-inducing environmental conditions. The same effect can be obtained with gibberellin-like substances derived from other, higher plants, and preliminary studies suggested that the gibberellin content of flower-induced and non-induced (vegetative) plants of the same species shows quantitative as well as qualitative differences.
However, the methods used to extract gibberellins from higher plants -- our own as well as those of other investigators -- were not satisfactory for quantitative work; they were quite laborious and accomplished insufficient purification so that the results were at the best semiquantitative. Efforts have therefore been concentrated on developing a simpler and more reliable method. The plant material is lyophilized and extracted with methanol; the extract is dried and treated with petrol ether and then chromatographed on a long cellulose column, using an acetone-butanol-tertiary-amyl alcohol-ammonia mixture as solvent. The active fractions are subjected to further purification by ion exchange. Preliminary activity tests are done with an improved dwarf-pea test, peas being more readily available than dwarf mutants of corn and being in fact more sensitive than the latter. Final tests, however, should be made using a series of gibberellin-responsive dwarf corn mutants as these permit recognition not only of quantitative but also of certain qualitative differences between gibberellins.

With these procedures it has been possible to separate four of the known gibberellins (A1 to A4) in a single partition step, a result which had not been accomplished so far, and to demonstrate the presence of unknown, native gibberellins in plant extracts. It is now proposed to study the gibberellin content of selected plants in relation to specific developmental events, such as transition from rosette to caulescent growth, vegetative development to flower inception, etc., in order to obtain a clearer correlation between gibberellin content and developmental stages as a premise for a more precise understanding of the role of gibberellins in the control of plant development.

115. CONTROL OF DIRECTION OF CELL DIVISION IN THE HYOSCYAMUS SHOOT

Investigators: M. Negbi, A. Lang
Support: National Institutes of Health, Public Health Service
National Science Foundation
General Funds

This study concerns the factors controlling the direction (plane) of cell division in the subapical meristematic region of the shoot of biennial Hyoscyamus niger. Unless given a cold treatment this plant grows in the habit of a rosette, i.e. without an elongate stem, and exhibits a characteristic, low mitotic activity in the region immediately below the apical shoot meristem. Application of gibberellin causes an increase of the mitotic activity in this region, and thus results in the growth of an elongate stem. The majority of the divisions thus induced are "transverse" divisions, i.e. their spindle axis is more or less parallel to the longitudinal axis of the plant and the newly-formed cell walls are at right angles to this axis. It is clear that this orientation of cell division is an essential factor in normal shoot development since cells have to be added in the longitudinal direction.

Application of 50 µg gibberellin per plant, fixation 24 hours after treatment and microscopic examination revealed the following facts. Gibberellin increased the number of cell divisions in the subapical region of both intact and defoliated plants by a factor of 5 to 10. In intact plants 80% of the divisions were transverse, but in defoliated plants this number was reduced to 40%, that is, the direction of divisions was essentially at random, as in untreated plants. If leaves not longer than 3 mm were left on the plant, the results were as in completely defoliated plants; if leaves 10 mm long and down were left, the divisions were oriented as in the intact, gibberellin-treated plant. No difference in the response was found if gibberellin was applied immediately, 24 hours or 48 hours after defoliation.
116. INTERACTION BETWEEN GIBBERELLIN AND AMO-1618

Investigator: J. Kato
Support: National Science Foundation
National Institutes of Health, Public Health Service

Amo-1618 ([4-hydroxy, 5-isopropyl, 2-methylphenyl] trimethylammonium chloride) acts as "dwarfing" agent in plants and has been reported to be an anti-gibberellin. Sachs, Lang et al. (1960) showed that Amo suppresses selectively cell division activity in the subapical meristematic region of the shoot, an effect which accounts for the dwarfing action. This effect is reversed by simultaneous or subsequent application of gibberellin.

In the present experiments, the effect of combinations of Amo and gibberellin on dwarf-corn and dwarf-pea seedlings was investigated. On corn (d-1) no effect of Amo on the growth promotion by gibberellin was noted, even at concentrations which caused considerable injury to the treated parts of the plant. In peas, the results have been variable: no effect in some experiments and a decrease of the promotive action of gibberellin in others.

Since the gibberellin effect in corn dwarfs is mainly due to promotion of cell elongation, it appears possible that Amo affects specifically the effect of gibberellin on cell division. Further work is directed at elucidating the behavior of dwarf peas and at comparing the action of Amo with that of other reported "anti-gibberellins" (quaternary ammonium derivatives).

117. THE ROLE OF ENDOGENOUS PERIODICITY IN PLANT GROWTH

Investigator: H. J. Ketellapper
Support: Rockefeller Foundation

It has been shown earlier that plants possess an endogenous, time-measuring element which plays an important function in growth and development. For the further analysis of the role of this time-measuring element, it is necessary to find elementary physiological processes which are under its control. An effort has therefore been made to establish whether or not there is a daily periodicity in the respiration rate of tomato plants. Although some indications for the presence of such a periodicity have been found, it is neither very clear nor very persistent. Differences in rate of respiration measured at various times during a 24-hour period are small. So far the efforts to discover rhythmic processes in the tomato variety which has been used previously in growth experiments have been unsuccessful. It has been found, however, that some tomato varieties are more sensitive to changes in the external cycle than others.

Publication:


118. THE CHEMICAL CURE OF CLIMATIC LESIONS

Investigator: H. J. Ketellapper
Support: Rockefeller Foundation

A variety of substances have been applied to plants grown under unfavorable climatic conditions with the objective of therapy of the climatic lesions thus induced.
The substances tested include vitamins of the B group, vitamin C, ribosides and amino acids (applied in the form of casein hydrolysate). Aqueous solutions of these substances were sprayed regularly on the leaves of plants growing either under optimal or under non-optimal temperature conditions. Both too high and too low temperatures were tested. So far 18 different plant species have been tested for their response to applied organic substances.

Increases in dry weight of up to 40% were obtained by application of vitamin C, of vitamin B or of ribosides to peas grown in high temperature conditions (30/23°C) in a greenhouse. Application of the vitamin B mixture to Cosmos grown in cool temperature (17/10 or 20/14°C) resulted in increases in dry weight of approximately 30%. In one experiment an increase of 64% was obtained at the lowest temperature. These results are similar to those of Bonner (1943). Application of vitamin C increased the growth of Cosmos at slightly higher temperatures (20/14, 23/17°C). It is concluded that in some instances at least, climatic lesions can be cured by application of an appropriate chemical.

119. THE COMPARATIVE PHYSIOLOGY OF GEOGRAPHIC STRAINS OF HARDING GRASS (PHALARIS TUBEROSE L.)

Investigator: H. J. Ketellapper
Support: Rockefeller Foundation

The cold requirement of geographic strains of Harding grass has been studied previously. It was found that the amount of cold required for the induction of flowering differs between strains. However, when the strains are grown in the field in a common environment where the cold requirement of all strains can be completely met, differences in flowering time still occur. Other factors must play a part. Further experiments were carried out with the aim of identifying such factors and studying interactions between them.

It was found that genetically determined differences in the rate of development of the inflorescences exist between strains. When fully induced plants are brought under conditions optimal for flowering (sufficiently long days, suitable temperature), the inflorescences in the various strains develop at different rates.

The rate of development of the inflorescence is strongly influenced by temperature: the lower the temperature, the slower the rate. This holds over a temperature range from 7 to 20°C. Some strains are more sensitive to changes in temperature than others. Therefore, the temperature during the development of the inflorescence is a factor determining relative flowering time.

Strains differ in critical daylength. The differences may be as much as 1/2 to 1 hour. The critical daylength changes with the temperature under which the plants are grown. Thus when the temperature is increased from 15 to 29°C, critical daylength increases by 1 hour. The relation between critical daylength and temperature is not the same for all strains. Furthermore, the relationship between daylength and rate of development of the inflorescence varies between strains.

The regulation of flowering time in Harding grass is complicated, achieved as it is by interaction of a relatively large number of factors: cold requirement, rate of development of the inflorescence, daylength, critical daylength, and temperature. Properties of the plant and characteristics of the environment both play a part.
Publications:


120. CHEMICAL STUDY OF PHOTOPERIODIC INDUCTION

Investigators: James Bonner, Osvaldo Caso, Jan A. D. Zeevaart
Support: National Science Foundation
National Institutes of Health, Public Health Service
Netherlands Organization for the Advancement of Pure Research
General Funds

The vegetative apical bud of the cocklebur may be induced to turn into a flower bud by exposure of one leaf of the plant to a night longer than 16 hours. It has further been shown that such induction by the leaf is inhibited by application of fluorouracil (5-FU) to the bud. The present studies are concerned with the mechanism of action of 5-FU.

Synthesis of RNA in the bud is inhibited by 5-FU, as measured either by increase in net RNA or by incorporation of label from orotic acid. At the same time, 5-FU is itself incorporated into the bud RNA, thus forming fraudulent RNA. Studies of the time course of RNA and DNA synthesis in the bud reveal no changes in over-all rate related to the act of induction.

It is concluded that synthesis of particular species of nucleic acid is an essential feature of the act of induction and that so far as the flowering cocklebur is concerned, such synthesis takes place solely in the bud.

121. EFFECT OF 5-FLUOROURACIL ON FLOWERING IN THE JAPANESE MORNING GLORY

Investigator: Jan A. D. Zeevaart
Support: Netherlands Organization for the Advancement of Pure Research
National Science Foundation
National Institutes of Health, Public Health Service

It is known that photoperiodic induction of Xanthium may be inhibited by application of 5-fluorouracil (5-FU) to the bud. Pharbitis nil, the Japanese morning glory, is, like Xanthium, a short-day plant and is likewise induced by a single long night applied to the leaves or cotyledons. It has been found that applications of 5-FU strongly inhibit photoperiodic induction in Pharbitis, provided that the application is made at the beginning of the long dark period. Application may be either to bud or to cotyledon. The effectiveness of 5-FU is reversed by simultaneous application of thymidine, but not by application of uridine. The inhibitory effect of 5-FU appears therefore to involve suppression of DNA synthesis.
122. EFFECT OF ENVIRONMENTAL FACTORS ON THE FLOWERING OF CARROTS

Investigator: Osvaldo Caso
Support: National Council for Scientific Research (Argentina)
National Science Foundation
General Funds

Carrots, as most biennial plants, must reach a minimum size or age before cold treatment (vernalization) is effective in inducing flower formation. In Argentina, winter sowing of seeds imported from Europe will produce typical biennial plants. However, seeds produced on plants grown from imported seeds and planted again in winter, produce plants which bolt (flower) in the following summer. It thus seems that they may pass their vernalization -- as many annuals do -- in the early seedling stage.

It was found that at least part of the change in floral behavior in carrots is caused by high temperatures, and possibly short days during vegetative development prior to the cold treatment which induces flowering. Thus, plants grown from imported seeds which received high temperatures (30°C during the day and 26°C at night) flowered earlier than those receiving low temperatures (17/14°C). When the plants were kept in a medium temperature (20/17°C), short photoperiods (8 hours) resulted in more flower formation upon subsequent vernalization than longer ones (16 hours).

In another experiment the minimum and optimum ages for vernalization of plants from imported (from France) and locally grown (Argentina) seeds were established. Plants grown from local seeds and receiving 6 weeks of vernalization (4°C) when they were 8 weeks old flowered in 30 days after the cold period. Only 40% of plants grown from imported seeds flowered when given the same cold treatment at the same age. The highest percentage of flowering in these plants was attained when 12-week-old plants were vernalized for a period of 9 weeks.

123. EFFECT OF GIBBERELLIC ACID ON FLOWER DIFFERENTIATION IN PETKUS WINTER RYE

Investigators: H. R. Highkin, O. H. Caso
Support: National Science Foundation
National Council for Scientific Research (Argentina)

It is known that application of gibberellic acid (GA) causes flowering of many biennials (Hyoscyamus, Apium, Brassica, Daucus). In these cases GA seems to substitute for cold (vernalization) treatment.

Treatment of winter cereals (Petkus rye, barley, winter wheats) has on the contrary been shown to have either no effect or to actually inhibit flowering.

Experiments have shown that the lack of response to or inhibition of Petkus winter rye by GA is due to treatment with the hormone at too early a stage in the development of the plant. Treatment of plants having ten leaves or less is indeed inhibitory or ineffective. However, if the plants are treated with GA after the ten leaf stage they can be induced to flower normally without a cold treatment.

This work was done in collaboration with Dr. D. Koller.
124. EFFECT OF ABNORMAL LIGHT REGIMES ON THE GROWTH OF PEAS

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

It has been shown for both the tomato and the pea that growth is abnormal when the plants are kept in constant temperature and in a light regime consisting of 24 hours of light and 24 hours of darkness (a 48 hour cycle). On the basis of these results it could be assumed that a 24 hour dark period was excessively long. Pea plants (variety L5) were therefore grown on a 48 hour cycle with a main light period of 24 hours and a supplementary light period of 2 hours given either early, in the middle of, or late in the dark period. They were compared with other plants grown on 48 hour cycles with uninterrupted dark period.

Those plants which received the supplementary light early in the dark period were greatly reduced in growth when compared to plants receiving the supplementary light in the middle, or at the end of the dark period, as well as those in which the dark period was uninterrupted.

The plants receiving the supplementary light late in the dark period were superior to all other treatments.

The fact that those plants in which a supplementary light period was introduced early in the dark period were inhibited in growth suggests that the 24 hour uninterrupted dark period as such is not excessively long. On the other hand the fact that interruption late in the dark period results in superior plants suggests that either a critical uninterrupted night length is required for optimal growth of the plants, or the plants pass through periods in which light is favorable or unfavorable for growth and which may be phases of an endogenous clock.

125. EFFECT OF CONTINUOUS LIGHT ON THE GROWTH BEHAVIOR OF PEAS

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

Studies of the effect of continuous light on the growth of tomato plants have shown that this environment results in abnormal, necrotic plants. Went has also shown that continuous light is detrimental for the growth of some varieties of peas. However, in at least one variety (L5) growth as well as maturation rate is markedly increased when the plants are grown on a continuous light regime as compared to plants grown on a more normal 16 hour photoperiod. Furthermore it was found that seeds formed on the plants grown in continuous light result in superior plants (when compared to those formed on plants grown in 16 hours) when they are grown in either a 16-hour photoperiod or in continuous light. There is thus a carryover of an environmental effect through the seed which could, at least in part, be attributed to a carryover of storage material giving the continuous-light-grown plants an advantage at either environment. Further experiments will be done to determine whether a carryover effect is also evident in later generations, as had been found in plants grown on a constant-temperature regime.
126. GENETIC VARIATION IN DAY AND NIGHT TEMPERATURE REQUIREMENTS IN FOREST TREE SPECIES

Investigator: Thomas O. Perry
Support: National Science Foundation

Do trees vary genetically in day and night temperature requirements for optimal growth? Open pollinated seedlings from single mother trees of different geographic origins were grown under varying day and night temperature treatments in the Earhart Laboratory. The seed from these experiments was collected from different points in the range of red maple and loblolly pine.

Red maple from the Everglades of Florida required relatively high day and night temperatures for optimal growth. Red maple seeds from Canada grew best at lower temperatures. Seedlings from intermediate latitudes and climates had intermediate responses.

Seedlings from different geographic origins in the range of loblolly pine showed variation in temperature requirements that parallel those of red maple.

These exploratory studies indicate that there is a common pattern of genetic variation within different tree species such that tree races have developed which are adapted to day and night temperature regimes that prevail at different parts of the species' range.

127. STUDIES ON SEEDLING GROWTH OF CONIFERS

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

Work to date shows that temperature requirements for optimum growth of forest tree seedlings vary with the species. Temperature controlled experiments conducted in the Earhart Plant Research Laboratory demonstrated the differences between forest tree species. Based upon the heat requirements for optimum growth of the seedling, species can be placed in one of four categories:

1) a day-night temperature differential represented by loblolly pine and Douglas fir; 2) the day temperature alone represented by coastal redwood and sugar pine; 3) the night temperature alone represented by digger pine; 4) the total heat during the 24-hour period represented by Jeffrey pine and erectcone pine.

Work is continuing on other species.

Publication:

128. ROOT AND TOP GROWTH OF REDWOOD SEEDLINGS IN RELATION TO SOIL AND AIR TEMPERATURES

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

Forest trees growing in the field usually have their tops and roots at different temperatures. In experiments on the growth of plants under controlled temperatures tops and roots are commonly at the same temperature. A study is therefore being made to determine the separate effects of soil and air temperature on the growth of forest seedlings.

Tree seedlings were planted in crocks in which the soil temperature was maintained by cooling or heating coils at 8°C, 18°C, or 28°C. Sets of crocks at each temperature were placed in the warmest (30°C day) and coolest (17°C day) greenhouses of the Earhart Plant Research Laboratory under controlled air temperature conditions.

Results with redwood, after nine months, showed that root temperature has a greater effect if the air temperature is low than if it is high. In the cool greenhouse only the tops of the plants with the 28°C root temperature grew rapidly. The roots under all three temperature conditions were of almost equal weight but there were large differences in their appearance. The roots grown in the 8°C soil temperature condition were short, about 12 cm long, and thick. In contrast the roots grown in the 18°C and 28°C root temperature conditions were thin and over 60 cm long. In the warm greenhouse all plants grew large tops with relatively small roots. The roots were almost of equal weight and size under all three root temperature conditions.

129. ROOT DORMANCY

Investigator: John A. Romberger
Support: U.S. Forest Service
General Funds

The physiology of periodic growth and dormancy in roots has never been thoroughly investigated. Yet as early as 1910 the anatomy of dormant root tips of many plants was known to be quite different from that of growing tips. The dormant tip is characterized by layers of suberized cells arranged in such a manner that translocation of water and solutes between the promeristem and the external medium is greatly inhibited. The physiology of development of the suberized layers and the bursting of them when a new period of growth begins has received little attention since 1920. An extensive review and analysis of the literature on root dormancy has been made as a prerequisite to experimental work.

130. PHYSIOLOGY OF MERISTEMS OF WOODY PLANTS

Investigator: John A. Romberger
Support: U.S. Forest Service
General Funds

This project concerns activity and dormancy of shoot, root and lateral meristems of woody plants, especially the roots of coniferous trees. Attempts will be made to determine the physiological basis for the existence of a quiescent center within the active root meristem. Present evidence indicates that cells
of the quiescent center are inactive only because of their position deep within a mass of active tissue. Suppression of metabolic activity within the center may be related to lowering of respiratory rates within large masses of storage tissue by the apparent production of metabolic inhibitors faster than they can diffuse away.

131. TEMPERATURE TOLERANCE IN TOMATOES

Investigator: John H. Curme
Support: Campbell Soup Company

The study of response of tomato plants to low night temperatures is being continued in an effort to understand the limitations imposed by cool, short growing seasons. Earlier reports have indicated that night air temperatures of 7° and 10°C do not interfere with normal fruit fertilization in some lines of L. esculentum where daily solar radiation intensities are above 2500 foot candles. This becomes a problem of maturity where low air and soil temperatures delay the development of plant tissues before flowering. This delay in developmental sequence is passed on through the rest of the life cycle of the plant. Hence interest is focused on rates of development at low air and soil temperature during the early stages of growth and on the influence of some remedial foliar sprays on these development rates. To date sucrose sprays have been used at several concentrations at low temperatures and normal solar light intensities. Results show that while sucrose is effective at higher temperatures and at low light intensities (Alex M. M. Berrie, 1954) there is no positive response from these treatments at low temperatures and normal light levels. Other materials will be used to determine whether a nutritional or metabolic deficiency can be established for low temperature control of development in the tomato plant.
PSYCHOBIOLOGY

STAFF:
Professor: R. W. Sperry
Research Fellows: Harbans L. Arora, Domenica G. Attardi, Mitchell E. Glickstein,
               John Steiner, Theodore J. Voneida
Graduate Students: Charles R. Hamilton, Colwyn B. Trevarthen, Ivan Jeanne Weiler
Research Aides: Janet O. Chapple, Octavia G. Chin, B. Denise Cleveland, Lois E.
               MacBird, Michael Saxlund
Laboratory Assistants: Ruth T. Johnson, John Sedat, Katherine Van Dyke
Student Assistants: James T. Geddis, Leroy B. Hood, John B. Trenholme

132. INTERMANUAL TRANSFER IN SPLIT-BRAIN MONKEYS AFTER SOMATIC CORTICAL ABLATION

Investigators: M. Glickstein, R. W. Sperry
Support: National Institutes of Health, Public Health Service

Although callosum-section tends to block the normally strong intermanual transfer of somesthetic discriminations in monkeys, good transfer has been observed in an occasional animal with accidental unilateral damage to the somatic cortex or where the tactile test objects looked alike, and the monkeys were sophisticated in pairing either hand with either eye. Therefore it seemed probable that transfer might be favored by deliberate surgical removal from the arm cortex on one side. Two monkeys were studied that had previously exhibited no intermanual transfer, even after extensive overtraining. In both cases the bulk of somatic arm area I was ablated unilaterally. After recovery subjects were trained on new tactile problems and tested for transfer. Both monkeys now exhibited high level transfer from the hand contralateral to the lesion to the unaffected hand in a variety of problems. This contrasts with the complete absence of transfer observed prior to cortical lesion. The results confirm the capacity for intermanual transfer in split-brain monkeys, and suggest that the recovery following unilateral cortical lesion may be effected to some extent via the ipsilateral projection system.

133. DELAYED RESPONSE PERFORMANCE OF SPLIT-BRAIN MONKEYS

Investigators: M. Glickstein, H. Arora, R. W. Sperry
Support: National Institutes of Health, Public Health Service

Bilateral (but not unilateral) lesions of prefrontal cortex in monkeys markedly impair performance on tests of immediate memory. Many authors have suggested that this impairment may be secondary to other classical frontal symptoms of hypermotility and distractability. A number of animals are being tested on the delayed response following seriatim section of one optic tract, ablation of one prefrontal area, and section of corpus callosum, along with anterior and hippocampal commissures. Of three cases completed to date, two with prefrontal lesions ipsilateral to the cut optic tract were relatively unaffected by commissural section. One case with prefrontal ablation contralateral to the cut tract showed marked impairment of delayed response performance after commissurotomy. Moreover, the latter deficit appeared to be unaccompanied by the classical frontal symptoms of hypermotility and distractability found after bilateral ablations. Results suggest that the impairment of the capacity to perform the delayed response is a genuine direct effect on immediate memory that may occur without hyperactivity and distractability.
134. CONDITIONAL VISUAL-TACTILE INTEGRATION IN SPLIT- BRAIN MONKEYS

Investigator: R. W. Sperry
Support: National Institutes of Health, Public Health Service
Frank P. Hixon Fund

Monkeys trained in a discrimination problem that requires close integration of both visual plus tactile cues are given various types of brain surgery. The aim is to learn more about the cerebral centers and pathways involved in this type of perceptual integration. The monkeys learn to pull the correct (rewarded) one of a pair of levers that remain out of sight and must be distinguished on the basis of touch: one rough, the other smooth. The rough one is rewarded in association with a certain black-white visual pattern, the smooth one with a different black-white pattern, the two patterns being equated for brightness.

Two monkeys having one optic tract sectioned to restrict the visual inflow to one hemisphere have learned to perform the task with either hand. Section of the corpus callosum did not prevent performance with either hand. Subsequent excision of the somato-sensory arm area from the hemisphere on the side of the visual inflow abolished performance with the affected (contralateral) hand, but not with the hand the sensory and motor cortical areas for which lie in the hemisphere not receiving the visual input. This latter performance was not impaired by extension of the midline sections to include habenular and posterior commissures plus the anterior half of the quadrigeminal plate. This surprising result, involving manual tactile stimuli, confirms an earlier finding obtained with proprioceptive arm and shoulder cues. It indicates that interhemispheric integration of this sort can be carried out despite the separation of the hemispheres at the cortical and thalamic level.

135. INTERACTIONS BETWEEN VISUAL DISCRIMINATION PROCESSES AND MOTOR FUNCTIONS IN EYE-HAND COORDINATION LEARNING OF SPLIT-BRAIN MONKEYS

Investigator: Colwyn B. Trevarthen
Support: National Science Foundation

Further experiments with split-brain monkeys, in which the optic chiasma, anterior comissure and corpus callosum were transected, have gained new information concerning the mechanism of an eye-hand coordination performance. These experiments have been made possible by use of an apparatus in which both eyes may be used at one time for different discrimination tasks, and which employs projection of plane-polarized visual stimuli as described in Biology 1959.

Preference for use of one limb develops when either limb is free to perform the task which is common to the two visual discriminations. This preference becomes stronger with practice on a series of tasks, and is accompanied by bias for the use of one eye -- that contralateral to the hand preferred. Thus a strong monocular attention, determining the course of learning, appears to develop spontaneously. However, there are cases where learning is only slightly behind in the other unpreferred eye and a balance can be reached quickly by monocular training.

Forced monocular training often results in change of limb use in favor of the contralateral combination, but sometimes a strong set for use of a limb may result in voluntary performance with an ipsilateral combination of eye and limb.
Differences in response-time and efficiency of visual guidance suggest that the ipsilateral eye-hand combination may function by a process substantially different from that governing contralateral coordinations, and tests have been devised to investigate this hypothesis.

Recent training in which the left hand was forced to push a left response plaque, and the right hand was obliged to push the right plaque, has resulted in a rebalancing of performance for a subject sophisticated in the sense of having a strongly developed learning set and limb preference. Changed behavior may indicate that the forced situation reinstates a more strenuous type of learning, and is comparable to that of naive subjects. Perfect performance in this training, and with both eyes open, has been followed immediately by monocular performance with either eye at near the 75% correct level. Since each batch of 10 trials contained 5 requiring left-hand responses and 5 requiring right-hand responses, it would appear as if the performance may have been perfect for one eye-hand combination (apparently the contralateral in each case), and defective (about 50% correct) when the other combination was in use.

That visual attention in the split-brain monkey does oscillate between the two eyes when the limb bias is balanced out is to be the hypothesis tested in experiments involving brief presentation of the visual cues. This precaution would eliminate any corrective shift of attention within a trial. At this stage it is still uncertain if simultaneous binocular visual attention and simultaneous learning of two different discriminations can in fact occur at any moment in the brain of a monkey with interhemispheric commissures sectioned, even when the limb use and over-all learning performance is balanced on the two sides.

136. INTERHEMISPHERIC TRANSFER OF VISUAL DISCRIMINATION LEARNING IN SPLIT-BRAIN MONKEYS

Investigator: Colwyn B. Trevarthen
Support: National Science Foundation

In the course of the above experiments, cases were obtained in which there was interaction between the two contradictory visual discriminations. In some tests there was strong indication of interference with the discrimination with the less preferred eye, due apparently to transfer of the dominant visual learning from the other side. Decisions made with use of both eyes open, and those made with the preferred eye (i.e. contralateral to the preferred limb), when both kinds were nearly perfectly correct, were immediately followed by runs of trials with the other eye in which the choices were predominantly wrong.

It is of interest that the strongest cases of this transfer concern brightness and color discriminations, while differences between figures with strong "object reference," such as "cross vs. circle," showed no signs of transfer between the hemispheres. It is known that animals with the visual cortex removed can make brightness discriminations, and experiments with split-brain cats have revealed some transfer between the hemispheres of habits based on a brightness difference. The possibility that this transfer occurs through mid-brain structures receiving visual information is to be investigated by use of more extensive mid-line surgery in the brain.
137. A BEHAVIORAL APPROACH TO THE PROBLEM OF NEURAL PATHWAYS INVOLVED IN LEARNING

Investigators: Theodore Voneida, R. W. Sperry
Support: National Institutes of Health, Public Health Service

The aim here is to learn more about the cerebral pathways and centers involved in a simple conditioned reflex. Adult, callosal-sectioned cats with the visual inflow restricted to the right hemisphere and the remaining motor centers confined to the left hemisphere have been conditioned to flex the right forepaw to a flashing light signal. Controls are now being tested to ascertain whether or not the cerebral cortex is utilized in establishing the conditioned response under these conditions.

As a continuation of the above experiment, an apparatus has been designed to test similarly operated animals on a learning problem that involves visual discrimination and hence probably depends on the visual cortex. In this situation, the cat is presented with two identical levers. Slight pressure or contact with either lever illuminates one of two different visual patterns directly in front of the cat's head. The animal, in order to achieve a food reward, must then learn to depress that lever which illuminates the positive visual cue. This technique combines operant conditioning with discrimination learning, and provides a means for investigation of cerebral mechanisms involved.

138. AN ANATOMICAL STUDY OF THE COURSE AND DESTINATION OF FIBERS ARISING IN THE SUBSTANTIA NIGRA

Investigator: Theodore Voneida
Support: National Institutes of Health, Public Health Service

An investigation is under way designed to study the efferent fiber system of the substantia nigra, a well-defined and relatively large neural center in the ventral portion of the midbrain. Following the placement of small electrolytic lesions in this nucleus, the degenerated axons will be traced to their destinations, which at present are for the most part largely unknown.

Previous attempts to place lesions in this area have also destroyed large portions of adjacent neural tissue, thereby obscuring the results. A stereotaxic approach is being tested that will more accurately confine the lesion to this nucleus and will eliminate extraneous damage.

139. UNILATERAL TEMPORAL LOBE ABLATION IN SPLIT-BRAIN MONKEYS

Investigator: John Steiner
Support: National Institutes of Health, Public Health Service

Severe impairment of recent memory has been observed after bilateral removal of medial temporal lobe structures, in particular the hippocampus, in human subjects. To investigate this as yet unexplained effect, the present study plans to combine ablation of the hippocampus and related limbic structures with various forms and degrees of cerebral commissurotomy.

The first step is to test memory for monocularly trained habits, including pattern discrimination and delayed alternation, comparing the directly trained operated hemisphere with the untrained intact one, both before and after section of parts or all of the corpus callosum. One eye is occluded with a
translucent plastic contact lens that later can be transferred to the other eye, and retention is tested, first on the intact and then on the operated side, both before and after section of the remaining remnants of the corpus callosum.

The first operated monkey showed, for six days postoperatively, some of the features previously described for bilateral lesions, when the eye leading to the normal hemisphere was occluded. The animal showed an increased tameness, and failed to discriminate food from non-food, placing all objects which came into his field of view in his mouth. This and a second case with extensive commissurotomy showed placidity to tactile stimulation on the side of the body opposite the temporal removal, and the usual alarm reaction when touched on the other side.

140. CENTRAL ROUTES TAKEN BY REGENERATING OPTIC FIBERS

Investigators: Domenica G. Attardi, R. W. Sperry
Support: National Institutes of Health, Public Health Service

There is good physiological evidence that regenerating optic fibers of fishes and amphibians find their original place of termination in the optic lobe. Whether the course taken by the growing fibers in regaining their central terminals is a random one or is selective was investigated. Histological studies were made in which section and regeneration of the optic nerve was combined with retinal lesions in goldfish and other species. The results suggest definite preferential selection by the fibers for their original central pathways: evident in the choice of medial or lateral tracts, in the point of entrance into the tectum, and later, the point of entrance into the plexiform layer within the tectum. Neuronal specificities thus appear to operate in the patterning of fiber pathways of the central nervous system as well as in the details of the synaptic interconnections.

141. NEURONAL SPECIFICITY IN THE OCULOMOTOR SYSTEM IN THE CICHLID FISH

Investigator: Harbans L. Arora
Support: National Institutes of Health, Public Health Service

It has been reported previously that section and regeneration of the main trunk of the oculomotor nerve in the cichlid fish Astronotus ocellatus leads to complete recovery of normal function. However, when individual muscle branches of this nerve are cut and inserted into foreign muscles within the same system, the nerves regenerate and establish transmissive connections with the new muscles but do not acquire the timing of discharge appropriate to those muscles. In the present study smaller fishes were selected (ranging in size from 3 to 4 cm) in the hope that there might be greater plasticity in the respecification of oculomotor muscles in these very young fish. Nerve branches to the superior and inferior rectus muscles were cut and crossed in one series; nerve branch of inferior oblique muscle was cut and united with superior rectus muscle in the second series and to medial rectus muscle in the third series. In all cases, the nerves regenerated and established connections with the new muscles but retained their original incidence of discharge. It would, therefore, appear that the individual nerves in the oculomotor system are already highly irreversibly specified at this stage. The excellent motor recovery that follows section and regeneration of the whole oculomotor trunk may be ascribed to unknown factors that favor normal over abnormal reconnections in the musculature.
Publications:


INCORPORATION OF C-14-LEUCINE INTO PROTEIN BY CELL-FREE PREPARATIONS FROM NORMAL HeLa CELLS AND FROM HeLa CELLS INFECTED WITH POLIOVIRUS

Investigator: G. Attardi
Support: American Cancer Society

Experiments are being made with the aim of developing a cell-free system capable of synthesizing the poliovirus specific protein. It is hoped that such a system may eventually be useful for an analysis of the mechanism by which the information carried by the virus ribonucleic acid (RNA) is translated into the synthesis of the virus specific protein.

HeLa cells, strain S3, grown in suspension, and poliovirus type 1, strain R68, are used for such studies.

Homogenates of non-infected HeLa cells were found to incorporate C-14-leucine into protein and the conditions for such incorporation were investigated. Most experiments were carried out with the total cytoplasmic fraction. In the few experiments made with the whole homogenate the level of amino acid incorporating activity was equal to or lower than that in the total cytoplasmic fraction. The incorporation of C-14-leucine into protein was found to be strongly energy dependent and completely sensitive to ribonuclease.

The kinetics of the amino acid incorporation into protein shows that the reaction is most rapid during the first 10 minutes, but continues at a decreasing rate up to 30 minutes.

Assuming that there is no dilution of isotope by intracellular leucine, then the minimum rate of incorporation of leucine into cytoplasmic protein, as calculated from the initial 10 minutes' incorporation, is, under optimal conditions, of the order of 1 mg per mg protein per hour. From the known content of leucine in the whole HeLa cell protein (about 9%) it can be calculated that the rate of this incorporation, tentatively taken as a measure of actual protein synthesis, is at least 3% of that required to account for the known generation time of HeLa cells in vivo (about 24 hours).
The conditions used with homogenates of non-infected HeLa cells were found to be favorable also for amino acid incorporation into protein in homogenates of HeLa cells infected with poliovirus at a high multiplicity.

The level of incorporating activity in homogenates prepared 7 hours after infection, that is 2 to 3 hours after the beginning of virus maturation, is as high as in homogenates prepared from non-infected cells.

The incorporation of labeled leucine into the poliovirus specific protein in homogenates of infected cells is at present being investigated by immunological methods. Preliminary experiments have shown the presence of radioactivity in the specific precipitate formed by addition of anti-type I poliovirus serum and carrier virus to the supernatant after sedimentation at 15,000 x g for 15 minutes of the sonicated incubation mixture. The nature of this labeling is now the object of a detailed study.

143. STUDY OF MUTABILITY OF POLIOVIRUS

Investigators: W. D. McBride, R. Dulbecco, M. Vogt
Support: The National Foundation
American Cancer Society
General Funds

These studies have been continued to clarify further the interrelations among mutants affecting different characters, of which mention had been made in the previous report. The characters investigated include neurovirulence (nv), sensitivity to inactivation by heat (t), the requirement for bicarbonate (d), the requirement for high cystine concentration during multiplication (cyr), inhibition by cystine (cyi), ability to plate on MS cells (ms), ability to multiply at 30°C (rt/30) and at 40°C (rt/40), and resistance to bovine (bo) and equine (ho) serum inhibitor.

A result of general validity and possibly of general significance obtained with these mutants is that when a mutation affects one of these characters, the state of the virus in respect to other mutant characters is modified. This phenomenon, which is indicated as covariation, can occur in two ways. One aspect of covariation is exclusion, in which a mutation of character A prevents the expression of an allelic type of character B; the other aspect of covariation is pleiotropism, in which a mutation of character A causes what appears to be the simultaneous mutation of character B.

Covariation does not arise from trivial causes, as for instance simultaneous selection of two mutant types; it does not derive either from the occurrence of mutations involving an extended segment of the genetic material of the virus (see report no. 151 on density of the virus mutants); in general the mutations involved can be considered as point mutations, as is also suggested by studies of the induction of one such mutation by nitrous acid, reported last year. The hypothesis presented to explain covariation is that these mutations affect closely located sites of the genetic material of the virus, a hypothesis suggested by the results obtained in the study of intragenic suppression in bacteriophages.

The results obtained in the study of covariation have significance from two possible points of view. One is the structure of the virus and the correlation between this structure and the genetic function. In this respect the following picture emerges: Covariation may be a consequence of the structure of the virus. The genome of poliovirus is constituted by either one or a few genes (as required by its short length), and correspondingly it has information for the synthesis of one or a few specific viral proteins. As of now, one protein is known, that of the
viral coat; all genetic properties of the virus could be explained as the function of this one protein. The various characters can be considered the expression of the function of different active groups present on this protein. For instance, the \( J_\ldots \) character appears to result from the special properties of an \( SH \) group; the \( nv \) character may result from the function of another group allowing attachment of the virus on primate nerve cells. Covariation would then result from interrelation among the functions of these groups.

144. NUCLEIC ACID OF POLYOMA VIRUS

Investigator: J. D. Smith
Support: National Institutes of Health, Public Health Service

A preliminary study of polyoma virus nucleic acid was made using radiocchemical techniques. Virus labeled with P-32 was purified by centrifugation in a cesium chloride gradient. A single band of radioactive and infective material was obtained. Virtually all the radioactivity in the purified virus could be recovered as deoxyribonucleotides after enzymic hydrolysis of the virus nucleic acid. The virus contained no detectable amounts of ribonucleic acid.

Publication:


145. TERMINAL GROUPS OF RIBONUCLEIC ACIDS

Investigator: J. D. Smith
Support: National Institutes of Health, Public Health Service

Methods for the identification of terminal nucleotide residues in polyribonucleotides are being investigated for the purpose of determining chain length and terminal sequences in ribosome and virus ribonucleic acids.

A method in which a terminal nucleoside residue in a polyribonucleotide chain is oxidized and coupled with radioactive thiosemicarbazide has been further developed to enable quantitative determination of terminal residues of this type. The method gives satisfactory results with ribonucleic acids of known chain length and terminal residue such as E. coli soluble ribonucleic acid.

Publication:


146. INFECTION OF CHICK IRIS EPITHELIUM WITH ROUS SARCOMA VIRUS

Investigators: Boris Ephrussi, Howard M. Temin
Support: Rockefeller Foundation
American Cancer Society
General Funds

Cells of chicken iris epithelium have been grown in vitro for over four months. During this period they have maintained the characteristics of the tissue of origin (formation of epithelial membranes and production of pigment).
The ability of these cells of ectodermal origin to be infected with the Rous sarcoma virus and to support its multiplication has been demonstrated.

Under the influence of the infecting virus, the cells of iris epithelium undergo morphological changes similar to those exhibited by infected fibroblasts, the type of change depending upon the genotype of the infecting virus.

Publication:


147. FURTHER STUDIES WITH MORPHOLOGICAL MUTANTS OF ROUS SARCOMA VIRUS

Investigator: Howard M. Temin
Support: American Cancer Society

The two Rous sarcoma virus types, described in Biology 1959, differed in the changes they induced in chick cells infected in vitro. These viruses were similar in altering growth properties of cells in vitro, forming sarcomas in vivo, having similar neutralization constants with antiserum and having normal chromosome complements.

148. VIRUS-CELL COMPLEX IN ROUS SARCOMA

Investigator: Howard M. Temin
Support: American Cancer Society

Rous sarcoma virus has been shown to convert chicken cells in a specific manner controlled by the virus genome (Biology 1959). The infected cells continue both to release virus and to divide. Experiments were performed to determine the mode of inheritance of the virus. It was found that some infected cells could be superinfected and then stably carry and release two different viruses for many cell generations. Such cells release about equal numbers of both viruses. The morphological type of such cells is controlled by one of the two viruses. These data suggest that all cells have two sites for virus replication and inheritance, one of which is dominant in control of cell morphology.

149. STUDY OF GENETIC RECOMBINATION WITH POLIOVIRUS

Investigators: W. D. McBride, R. Dulbecco, M. Vogt
Support: The National Foundation

In the last report certain encouraging results were described. These results were subjected to critical scrutiny during this year; it appears now that they were not due to genetic recombination.

Many other experiments were carried out in an attempt to discover recombinants of poliovirus. In total, twelve different types of crosses, involving lines derived from the same plaque-purified virus by selection of spontaneous mutants, have been carried out. Ten of these crosses involved lines marked with different markers; the other two involved various pairs of independently occurring mutants of the same character \((cy^7\) and \(d)\). In some cases one of the parents was inactivated by UV light; in some other cases both parents were inactivated.
Non-parental types were frequently isolated from these crosses. However on careful checking, these types could never be attributed to true recombinants.

The very extensive amount of work carried out in this field demonstrates that the frequency of recombinants in poliovirus crosses, as carried out so far, is very small. Inability to detect recombination is attributed to the relatively high mutation frequency, which is present in all cases. In certain crosses a frequency of recombinants greater than $10^{-4}$ would have been clearly detected; in most crosses a frequency of about 1% would have been detected.

On the basis of the frequencies observed in bacteriophages, the low recombination frequency in poliovirus is not a surprise, in view of the very short length of RNA of this virus. These results, however, differ markedly from those obtained in influenza, where recombination frequencies appear to be high, although the RNA is of similar length.

The discrepancy may arise from the profound structural difference between the two viruses; or from differences in their mode of reproduction. This point is worthy of being investigated further.

150. PURIFICATION OF ROUS SARCOMA VIRUS

Investigator: Lionel V. Crawford
Support: American Cancer Society
Rockefeller Foundation

The purification of the Rous sarcoma virus was undertaken as a preliminary to biochemical studies on the virus particle. Methods were developed whereby preparations with high purity and infectivity could be obtained. The purity and specific infectivity (physical particles per infectious unit) of the preparation were determined by electron microscopy.

The nucleic acid of the particle appears to be ribonucleic acid, present to the extent of 2 to 3 million molecular weight units per particle. The base composition of the nucleic acid is being investigated.

Publication:

151. THE DENSITY OF SOME POLIOVIRUS MUTANTS

Investigator: R. Dulbecco
Support: The National Foundation
National Institutes of Health, Public Health Service
James G. Boswell Foundation

It has been shown in the previous report that poliovirus is concentrated in a narrow, nearly symmetric band, when subjected to equilibrium density gradient sedimentation in cesium chloride. In the gradient the density of the virus is 1.34.

The work here described was undertaken to examine some aspects of the nature of the molecular changes present in poliovirus mutants. In many mutants several virus properties are changed at the same time, as described in another
section of this report; this phenomenon may suggest that these mutations are caused by large structural changes of the genetic material of the virus, the RNA, perhaps involving several genes. If so, the mutants may differ in density from the wild-type virus.

Comparison of densities of various mutant lines was carried out by centrifuging mixtures of two mutants which can be differentially assayed and determining their relative proportions in successive fractions collected from the centrifuge tube. In this way very small density differences can be detected.

The results showed that several virus lines carrying either a mutation to resistance to inactivation by heat, or d, or of attenuated neurovirulence, did not differ measurably from the wild type. A d mutant, the R30 line isolated in this laboratory from type I Brunhilde virus, was found to be somewhat lighter than the wild-type virus, the difference in density being about 0.0014 density units. A d + reversion obtained from this d line was found to have again the density of the wild-type, type I virus.

Wild-type viruses of type 1 and type 3 showed density differences, of the order of 0.0015 density units.

The significance of these findings can be evaluated on the basis of the following considerations. Assuming the density of the RNA to be 2.05, and that of the protein 1.30, the virus containing a proportion of 30% RNA and 70% protein in weight, should have a density of 1.45. The observed density of 1.34 can be attributed to the presence, in the virus, of a volume filled with water inaccessible to the Cs+ ions. The proportion of such water in the virus would then be 0.19 of the total weight. The minimal density difference that could have been demonstrated in these experiments is of the order of 0.0005. A difference of this magnitude, if due solely to a different proportion of RNA per particle, would correspond to a difference of 0.0017 in the proportion of RNA in weight, i.e. about 12 nucleotides.

The fact that in most cases of mutation a difference of this order was not found shows that no important structural changes were present in these mutations. A similar conclusion is also probably valid for that d mutation which had a different density, since its d + reversions had the density of the wild-type virus. This mutation perhaps causes in the virus structural changes which alter the proportion of water inaccessible to the Cs+ ions.

152. STUDIES WITH POLYOMA VIRUS

Investigators: M. Vogt, R. Dulbecco
Support: American Cancer Society
The National Foundation
National Institutes of Health, Public Health Service
James G. Boswell Foundation

Earlier work with the polyoma virus, summarized in the previous progress report, centered around a study of the cytocidal (cell-killing) property of polyoma virus and led to the development of a plaque-assay method for the virus on mouse-embryo cells as host. During the last year, the moderate virus-cell interaction leading to the oncogenic (tumor-producing) effect of the virus has been studied in greater detail. It has been possible to obtain the oncogenic effect in vitro in cultures of both mouse and hamster embryo cells. In hamster-embryo cultures, the moderate virus-cell interaction occurs with a high probability, as shown by the rapid replacement (within a few weeks after infection) of the normal cell population.
by neoplastic cells. In mouse embryo cultures, in which the cytocidal virus-cell interaction is predominant, the probability for a moderate virus-cell interaction is low and accounts for the late (4 to 8 weeks after infection) transformation of the mouse cultures into tumor cultures. The neoplastic cells are recognized by their needle-like shape and their tendency to grow into multilayered net-like structures. Upon subcutaneous transplantation into adult hamsters, the transformed hamster cultures give rise to fibromatous neoplasias.

The state of the virus in the transformed cells is being investigated. Certain properties of the transformed cells are equal to those of lysogenic bacteria and suggest that the virus may be present in the form of a non-infectious provirus. These properties are: 1) resistance of the transformed cells to superinfection; 2) absence (hamster) or low level (mouse and rarely hamster) of virus production per transformed culture; 3) persistence of the potentiality to produce virus after 60 cell generations in the presence of antiserum -- the experiment was then discontinued -- and 4) discontinuous production of virus. Discontinuous production was shown in the following way: 47 microdrops were each seeded with 10 transformed mouse-embryo cells and incubated for four days. At the end of this period the average number of cells per drop was 65. The contents of the drops were assayed for virus: 11 drops showed virus yields varying from 60 to 220 pfu; 34 drops contained no virus; and 2 drops contained a few particles (background virus). The probability of virus production per cell, per cell generation, was thus about $5 \times 10^{-3}$. The large virus yields per cell suggest furthermore that the virus produced by transformed cultures is released by cells which die.

Another interesting feature of the stable virus-cell association between transformed cells and polyoma virus is the fact that all virogenic cultures so far studied were virogenic for a small-plaque mutant of polyoma virus, independent of whether the cultures had originally been infected with the large-plaque (wild-type) virus or a small-plaque mutant (compare also Medina and Sachs, Virology, 10:387, 1960). Experiments are in progress to clarify the origin of the small-plaque mutant as well as the meaning of the association between the mutant and virogeny.
PART B. -- BACTERIAL VIRUSES

STAFF:
Professor: Max Delbrück
Research Associate: J. J. Weigle
Assistant Professor: R. S. Edgar
Instructor: Charles M. Steinberg
Senior Research Fellow: Matthew S. Meselson
Cossney Research Fellow: Harriett Ephrussi-Taylor
Research Fellows: Sidney Brenner, Richard H. Epstein, Francois Jacob, John L. Jinks, Ulrich Winkler
Graduate Students: Juan P. Bozzini, John R. Menninger, Ann Roller, Millard Susman
Student Assistants: Andrew D. Bacher, Stanley Klein
Research Assistant: Ilga Lielausis
Research Aides: Diana J. Burkhardt, Lois G. Edgar
Laboratory Assistants: Johanna Feyen, Betty A. Haskell, Bertha E. Jones

153. THE MECHANISM OF GENETIC RECOMBINATION IN BACTERIOPHAGE

Investigators: M. Meselson, J. J. Weigle
Support: National Institutes of Health, Public Health Service
American Cancer Society
The National Foundation

If phages with several different hereditary traits are allowed to propagate in the same host cell, the progeny are found to include genetically recombinant types. It is the purpose of this investigation to determine the molecular mechanism by which these recombinants are produced. The general procedure is to infect cells with two sorts of phage. One type carries several genetic markers; the other carries the corresponding wild-type alleles and is labeled with the heavy isotopes C-13 and N-15. Among the progeny of this mixed infection are found phages which have received various amounts of heavy isotopic label. They may be fractioned according to their content of heavy isotope by means of density gradient centrifugation. Their genetic constitution may then be determined and correlated with the amount of heavy isotope they have inherited from the wild-type parent. The several current hypotheses for genetic recombination in bacteriophage make quite different predictions for the outcome of this experiment.

Bacteriophage labeled with C-13 and N-15 have been prepared and the various crosses and control experiments have been performed. The phages have been fractionated by means of density gradient centrifugation and the required genetic analyses have been carried out. The results indicate that the observed genetic recombination is a result of chromosome breakage.

154. A CRITICAL TEST OF A CURRENT THEORY OF GENETIC RECOMBINATION IN PHAGE

Investigators: C. M. Steinberg, R. S. Edgar
Support: The National Foundation

Results of experiments reported last year indicated that recombinants for very closely linked markers arise as segregation products of heterozygotes. Older
work of C. Levinthal had indicated that heterozygotes are recombinant for markers external to the heterozygous region. These two notions taken together suggest that selected recombinants for very closely linked markers should be recombinant for loosely linked unselected outside markers, independently of the number of exchanges required to obtain the selected recombinants. When this number of exchanges is odd, the prediction is trivial, but when it is even, the prediction amounts to a reversal of normal linkage relations. The experimental design for a critical test of the above notions is the following cross:

\[
\begin{array}{cccc}
\text{Parent 1} & \text{Parent 2} \\
\text{Interval} & \text{Interval} \\
\text{I} & \text{I} \\
\text{II} & \text{II} \\
\text{III} & \text{III} \\
+r+ & +r+ \\
+ & + \\
\end{array}
\]

The cross progeny were collected by premature lysis in order to minimize the randomizing effects of multiple matings. The \( r^+ \) recombinants were selected by plating on an appropriate bacterial strain and scored for the remaining three markers. Unexpected asymmetries were found in various recombinant classes depending upon the arrangement of the outside markers in the cross. This asymmetry is easily interpreted as the result of an appreciable fraction of heterozygotes at the \( ti \) and \( ac \) loci; the scoring technique is such that heterozygotes are phenotypically wild type for both of these loci.

The major result is that the majority of the \( r^+ \) recombinants are parental (\( ti \text{ ac}^+\text{ tu}^+ \) for the cross diagrammed above) rather than recombinant in genotype for the outside markers. Other observations were:

1) About 15\% of the selected recombinants were heterozygous for the \( ti \) marker; and 15\% are heterozygous for the \( ac \) locus.

2) The frequency of recombination in intervals I and II was greatly increased over the values found in control crosses without selection for exchanges in the \( r \) intervals.

3) The frequency of recombination in interval III was increased somewhat, but only to the extent expected from pool kinetics.

These results suggest that a completely new interpretation of heterozygosis and its relationship to recombinant formation is needed.

155. A NEW CLASS OF SELECTIVE MUTANTS OF PHAGE T4D

Investigators: R. H. Epstein, C. M. Steinberg, H. Bernstein

Support: National Institutes of Health, Public Health Service
The National Foundation

A number of mutants of phage T4D have been isolated which grow normally in some K-12 strains of E. coli but are unable to grow in strain B. These mutants have been designated amber (am). In all cases where the test can be made, the mutants are capable of reverting to "wild type." In those cases which have been examined, infection of strain B with an am mutant results in normal lysis of the bacteria, but only a few (\( 10^{-2}-10^{-4} \)) of the cells produce any infective particles. A comparison of the infectivity and bacterial killing ability has been made with one of the mutants and the two measures are in good agreement.
Five of the am mutants have been crossed to each other and to several known morphology markers. In all crosses, each behaved as a single point mutant. Four of the mutants occur at well separated sites in the third linkage group. The fifth mutant is not linked to any of the other am mutants or to any of the known morphology markers tested. In one am by am cross, the double-am recombinant type was isolated and shown to be am in phenotype. In this cross, the double-mutant and wild-type recombinants were not unequal in frequency.

Mixed infection of strain B with an am mutant and wild type leads to productive infection with a normal yield of progeny phage. This shows that the wild type supplies the function which the am mutant lacks. Complementation tests have been made with the five mutants used in the above crosses. In all cases, mixed infection of strain B with two different am mutants resulted in a normal yield of infective progeny. This result is not surprising since none of the mutants are closely linked to one another.

The inactivation of the am\(^+\) phenotype by ultraviolet (UV) light has been studied with three of the mutants. In these experiments, strain B is mixedly infected with an unirradiated am mutant and wild-type phage which have been exposed to various doses of UV. At each UV dose, the fraction of infected bacteria capable of yielding any infective phage is measured by plating (before lysis) on a K-12 strain; this is a measure of the survival of the am\(^+\) function. The fraction of infected bacteria liberating at least one am\(^+\) phage is measured by plating on strain B. This is a measure of function and incorporation of the am\(^+\) genes into a viable genome. The results with two of the mutants are similar to those in the previously studied \(\text{rII}-K(\lambda)\) system:

1) The measure of functional survival decreases in a simple exponential manner with increasing UV dose.

2) The cross-section for this inactivation is a small fraction (0.08, 0.10) of the cross-section for phage survival.

3) Among those infected bacteria in which the am\(^+\) function survived, the fraction liberating at least one wild-type phage decreases, reaching a limiting value (0.10, 0.15) at high UV doses.

The third mutant appears to behave somewhat differently. The fraction of infected bacteria yielding infective phage decreases much more rapidly with increasing UV dose, and this decrease is not exponential. This mutant requires further study.

156. DISTRIBUTION OF r MUTANTS OF PHAGE T4D

Investigator: I. Lielausis
Support: The National Foundation

As reported last year, a large number of T4D r mutants were isolated. This set of mutants was screened by spot tests on bacterial strain K-12 (\(\lambda\)). Mutants not growing on this strain are of the rII type. Such rII mutants all lie in a small region of the second linkage group and were mapped by a selective technique. Seventy-five of the remaining mutants have been crossed to known r-markers in the hope of finding new loci. The majority (64) are closely linked to r48 in the first linkage group and are designated rI mutants. The rI mutants are of two easily phenotypically distinguishable classes: one type gives standard r-plaques; plaques of the other type have a fuzzy border.
Eight mutants were closely linked to \(\text{rb50}\), a standard \(\text{rII}\) mutant, and are thus very "leaky" \(\text{rII}\) mutants. One mutant is closely linked to \(\text{r67}\) in the third linkage group. Two mutants are not linked to any known \(\text{r}\) mutant, and their plaque morphology is quite distinct from other \(\text{r}\)-type mutants.

157. GENETIC FACTORS INFLUENCING ACRIDINE DYE INHIBITION OF T4 DEVELOPMENT

Investigator: Millard Susman
Support: National Institutes of Health, Public Health Service
The National Foundation; Arthur McCallum Fund

The production of plaque-forming phage by T4-infected bacteria is prevented by the presence of various acridine dyes in the growth medium. The concentration of dye at which such inhibition is observed is a function of the bacterium used as host, of environmental factors such as temperature and pH, and of the genotype of the phage being studied. The results reported here concern the influence of phage genetic factors.

Two acridine-resistant mutants of T4D have been used in these experiments. The first of these, designated \(\text{ac41}\), was selected for ability to form plaques on agar plates containing 0.25 \(\mu\text{g/ml}\) of neutral acriflavine. A second-step mutant capable of forming plaques on 0.75 \(\mu\text{g/ml}\) of acriflavine was isolated from T4D\(\text{ac41}\). This second-step mutant was the source of the second mutant, \(\text{g41}\), so named because it proved to be resistant to quinacrine inhibition. The \(\text{ac41}\) locus is in the second linkage group, about six map units from \(\text{r73}\); the \(\text{g41}\) locus is in the third linkage group, about eight map units from \(\text{tu43}\).

The relative resistance of cells infected with \(\text{ac}\), \(\text{ac}\;\text{g}\), \(\text{g}\), and wild-type (+) phage to three acridine dyes has been determined in liquid culture and on gradient plates. The results may be summarized as follows (resistance decreasing from left to right):

- **Acriflavine:** \(\text{ac}\;\text{g}\;\text{ac}\;\text{g}+\)
- **Proflavine:** \(\text{ac}\;\text{g}\;\text{g}\;\text{ac}+\)
- **9-Amino acridine:** \(\text{g}\;\text{ac}\;\text{g} = +\;\text{ac}\)

Bacteria mixedly infected with phage of \(\text{ac}\) and \(\text{ac}^+\) genotypes do not yield viable phage at concentrations of acriflavine or proflavine at which bacteria infected with \(\text{ac}\) alone will produce viable phage. In this sense, \(\text{ac}^+\) is dominant to \(\text{ac}\). Bacteria mixedly infected with \(\text{g}\) and \(\text{g}^+\) phage do yield phage at concentrations of proflavine and 9-amino acridine which totally inhibit \(\text{g}^+\) development. In this sense \(\text{g}\) is "dominant" to \(\text{g}^+\). Furthermore, \(\text{g}\) is "epistatic" to \(\text{ac}\) in the sense that bacteria mixedly infected with \(\text{ac}\;\text{g}^+\) and \(\text{ac}^+\;\text{g}\) phage will yield phage at concentrations of proflavine lethal to \(\text{ac}^-\;\text{ac}^+\) mixed infections but not to \(\text{g}^-\;\text{g}^+\) complexes.

Gradient plate studies, however, indicate that the dominance and epistasis are not all-or-none phenomena. Mixed infections with \(\text{ac}\) and \(\text{g}\) in coupling out repulsion give quantitatively as well as qualitatively identical results.
158. RECIPROCAL SUPPRESSION BY MUTANTS WITHIN ONE CISTRON

Investigator: R. P. Feynman*
Support: The National Foundation
General Funds

The rII mutant r43, in the B cistron of phage T4D, like other mutants in this cistron, is unable to grow on a number of strains of E. coli (e.g., F(λ), C600 (λ), 112-12 (λ)) which support the growth of wild-type T4, and forms r-type plaques on bacterial strain S/6. When large numbers of this mutant are plated on F(λ), it is found that a small fraction (about 4 x 10^-6) forms plaques. These are usually interpreted as "back mutations" in which the r43 site reverts back to the wild-type state, but results here show that this is not invariably the case. About 5% of such apparent revertants are demonstrably not wild type in one or more characteristics: their plaques on S/6 are not wild type (usually r or intermediate between r and wild); their plaques on F(λ) differ in morphology from wild type; they fail to plate (or plate feebly) on C600 (λ) and/or 112-12 (λ). Three cases have been investigated in detail. Backcrosses to wild type show that they are double mutants, containing a suppressor mutation in addition to the original r43 mutation; all three suppressors are within 0.5 map units of the r43 site. Furthermore, the "suppressors" are themselves otherwise typical rII B mutants. Thus we have a situation in which two closely linked mutants within a cistron are able to reciprocally suppress each other so that the double mutant has a less extreme mutant phenotype than either single mutant. That the suppression is specific is shown by the fact that crosses of the suppressors to a number of other rII mutants (and to each other) yield double mutants which are unable to grow on F(λ).

Aside from the intrinsic interest of the phenomenon of symmetric suppression, the results also indicate that caution is required in mutagenesis studies where it is assumed that reversions are true back mutants if no loosely linked suppressors are demonstrable. The same caution applies to recombination studies where it is assumed that double mutant recombinants are mutant in phenotype.

The function of r43 revertants which could be shown to be different from wild type by the criteria outlined above is only 5%, which is quantitatively negligible. But this is not so with the new suppressors: over 99% of their revertants are not wild type by the same criteria. (It should be pointed out, however, that this is a deliberately selected case, for there is a high spontaneous rate of forward mutation to r at the r43 site.)

159. GENETIC FINE STRUCTURE OF THE hIII REGION OF PHAGE T4D

Investigator: J. L. Jinks
Support: Commonwealth Fund
The National Foundation

Wild-type T4 adsorbs very poorly to bacterial strain K/4 and gives turbid plaques on a mixed indicator of strains K/4 and S/6. It is possible to isolate phage mutants, designated h, which adsorb more readily to K/4 and which give clear plaques on the mixed indicator. Most of these mutants are located within a region of about 12 map units on the third linkage group between m41 and tu45.

Mapping experiments have allowed 10 of these mutants to be unambiguously ordered relative to each other and to tu45. Phenotypically h⁺ recombinants from

* Division of Physics, Mathematics and Astronomy, California Institute of Technology
each of three crosses between pairs of \( h \)'s were backcrossed to wild type. The results of the backcrosses exclude the possibility that the double mutant is phenotypically \( h^+ \). For two crosses, the double mutant was actually isolated and shown to be \( h \) in phenotype. Accordingly, map distances for \( h \times h \) crosses are twice the frequency of the \( h^+ \) recombinants.

All of the \( h \) mutants and the closely linked \( tu45 \) mutants are inactivated exponentially at \( 45^\circ C \) with rates ranging from 2 to 10 times that of wild type (see report #160). Heat stable reversions have been obtained from all of these mutants with a frequency of approximately \( 10^{-6} \) by strongly inactivating stocks of the mutants. Of 129 independent reversions examined, all are due to linked suppressor mutations rather than back-mutations. None of the suppressors have been isolated alone. In two cases it has been determined that the phenotype of the suppressor is not \( h \), while in another case the suppressor has been shown to be lethal.

The suppressor mutations may be classified into three types:

1) Reversions containing this type of suppressor are very much less leaky on \( K/4 \) than wild type. All the suppressors of this type are very closely linked to each other, occupying positions near the center of the \( hIII \) region. Crosses of different reverted \( h \)'s provide indirect but cogent evidence that the type 1) suppressors non-specifically suppress all \( h \)'s. This view was supported by finding several instances of allelic suppressors isolated against different \( h \)'s. Type 1) suppressors do not suppress either the plaque morphology or the heat sensitivity of the \( tu \) mutants.

2) Reversions containing this type of suppressor are about as leaky as wild type on \( K/4 \). The suppressor mutations all map internally to the \( hIII \) region, and they tend to occur close to the \( h \) marker against which they were isolated. This distribution of map positions argues strongly for some degree of specificity for the type 2) suppressors; on the other hand, a small fraction suppress even the \( tu \) mutants.

3) This type of suppressor suppresses both the plaque morphology and heat sensitivity of \( tu45 \), but does not act as a suppressor in the case of the \( h \)'s.

### 160. COMPLEMENTATION STUDIES OF THE \( hIII \) REGION OF PHAGE T4D

Investigator: J. L. Jinks  
Support: Commonwealth Fund  
The National Foundation

At \( 45^\circ C \), mutants of the \( hIII \) region (including the \( tu45 \) cluster) are exponentially inactivated at rates from 2 to 10 times that of wild type. This property can be used to test whether the same or different functions are affected in the various mutants.

Basically the test consists of making a mixed infection with a pair of mutants and examining the progeny for the existence of a heat-stable (i.e., phenotypically wild type) fraction. If the two mutations involve different functions, such a heat-stable fraction should exist; otherwise, not. An independent expectation for the size of the heat-stable fraction can be obtained by examining the progeny from a mixed infection of each mutant by wild type. Denote by \( S_1(t) \) the fraction surviving (as a function of time at \( 45^\circ C \)) of the progeny from a mixed infection of a mutant by wild type and by \( S_2(t) \) the corresponding survival curve for a second mutant; then if independent functions are affected by the two mutants, the heat-survival curve for the progeny from a mixed infection of the two mutants should be simply the product
If the mutants affect the same function and do not interact, the survival of the progeny should be the same as the parents. In principle, one could distinguish degrees of "partial complementation," but the precision of the data does not justify this.

Another test involves a comparison of the heat survival curves of progeny from mixed infections with markers in cis and trans configurations. For mutants involving different functions, the curves should be essentially identical; otherwise the survival should be much greater with the cis configuration. This test has been applied in a few cases and the results agree with those obtained using the test outlined above.

The results are summarized in the following table. A plus sign indicates that the pair of mutants complement (i.e., apparently involve different functions); a minus sign, that they do not complement. The mutants are linked in the order given.

<table>
<thead>
<tr>
<th></th>
<th>h41</th>
<th>h49</th>
<th>h52</th>
<th>h50</th>
<th>h43</th>
<th>tu45a</th>
<th>tu45c</th>
</tr>
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<tbody>
<tr>
<td>h41</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>h49</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>h52</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>h50</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>h43</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>tu45a</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

From an inspection of this data, it is easily seen that the mutants cannot be grouped into functional units or cistrons. It is possible to draw a linear "complementation map" of the type used in presenting complementation data from enzymatic mutants of Neurospora (see report # 76), but such a complementation map would not be colinear with the genetic map.

161. PROPERTIES OF A "DENSITY"-MUTANT OF PHAGE λ

Investigator: Juan P. Bozzini, University of Buenos Aires
Support: The National Foundation

The standard of reference strain of phage λ has a buoyant density in cesium chloride of 1.508 gm/cm³. A less dense variant is known, the density being controlled by a single genetic factor called b2.

The alteration in density may affect other properties of the phage λb2. It was found in particular that λb2 does not establish stable lysogenicity.
162. IDENTIFICATION OF THE CONSERVED SUBUNITS OF DNA FROM E. coli

Investigators: J. R. Menninger
Support: National Science Foundation

It has previously been shown that DNA molecules from E. coli are composed of two subunits which, upon replication of the DNA molecule, separate from each other and become associated with newly synthesized subunits. This result is to be expected from the model of Watson and Crick for DNA replication if it is assumed that the subunits are single polynucleotide chains. In order to substantiate this assumption, an attempt is being made to determine the number of parallel strands in coli DNA by means of low-angle X-ray diffraction. Over a broad concentration range DNA in solution forms an ordered phase in which the cylindrical molecules are closely packed. Values for the DNA concentration and intermolecular repeat distance in this phase may be used to calculate the mass per unit length of the molecules. Knowledge of this parameter should allow a specification of the number of parallel chains in the DNA molecule. At present the diffraction data are being collected.

163. THE RELATIONSHIP BETWEEN DNA COMPOSITION AND TRANSFORMATION IN Hemophilus

Investigators: Ronald Rolte*, R. S. Edgar
Support: National Institutes of Health, Public Health Service

The ability of a given DNA sample to transform Hemophilus influenzae depends upon 1) the efficiency of adsorption of the DNA by the bacterium, and 2) the efficiency with which markers in the adsorbed DNA are incorporated into the bacterial genome. Preparations of DNA from Hemophilus strains A61 and Fid transform the strain Rd with lower efficiency than homologous DNA, but their adsorption efficiency is the same as that of homologous DNA.

The buoyant densities of DNA from the strains Rd, Fid and A61, as well as buoyant densities and adsorption efficiencies of DNA's from organisms closely related and unrelated to Hemophilus have been determined. The following conclusions may be drawn from the data collected so far (see table):

1) Preparations of DNA from taxonomically related bacteria may be highly dissimilar in nucleotide composition.

2) Transformation is possible between bacterial species whose DNA's differ in composition.

3) Adsorption efficiency is unrelated to DNA composition.

4) At a given adsorption efficiency, transformation efficiency appears to be correlated with DNA composition.

This work was done in collaboration with Pierre Schaeffer, Institut Pasteur, Paris.

* Department of Chemistry and Chemical Engineering, California Institute of Technology
### Adsorption and Transformation Efficiencies and DNA Composition

<table>
<thead>
<tr>
<th>DNA Source</th>
<th>Transformation Efficiency</th>
<th>Adsorption Efficiency</th>
<th>%GC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hemophilus influenzae, strain Rd</td>
<td>1</td>
<td>1</td>
<td>38</td>
</tr>
<tr>
<td>Hemophilus sp., strain Fid</td>
<td>$3 \times 10^{-3}$</td>
<td>1</td>
<td>39</td>
</tr>
<tr>
<td>Hemophilus sp., strain A61</td>
<td>$10^{-4}$</td>
<td>1</td>
<td>41</td>
</tr>
<tr>
<td>Bacteria related to Hemophilus:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pasteurella bovisenteus</td>
<td>-</td>
<td>1/3</td>
<td>38</td>
</tr>
<tr>
<td>Actinobacillus equuli</td>
<td>-</td>
<td>-</td>
<td>41</td>
</tr>
<tr>
<td>Brucella abortus</td>
<td>-</td>
<td>-</td>
<td>56</td>
</tr>
<tr>
<td>Bordetella bronchisepticus</td>
<td>0</td>
<td>1/15</td>
<td>68</td>
</tr>
<tr>
<td>Organisms unrelated to Hemophilus:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Staphylococcus sp.</td>
<td>-</td>
<td>1/7</td>
<td>34</td>
</tr>
<tr>
<td>Bacillus megatherium</td>
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</tr>
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</tr>
<tr>
<td>Bacteriophage T2</td>
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<td>35</td>
</tr>
<tr>
<td>Calf thymus</td>
<td>0</td>
<td>1/100</td>
<td>44</td>
</tr>
</tbody>
</table>

* determined on different specimen
+ value obtained from literature

### 164. DENSITY DIFFERENCES BETWEEN GENETIC MARKERS IN PNEUMOCOCCAL TRANSFORMING PRINCIPLE

Investigators: Ronald Rolfe*, Harriet Ephrussi-Taylor
Support: National Institutes of Health, Public Health Service
The National Foundation
Gosney Fund

If the sequence of purine and pyrimidine bases in a DNA molecule is responsible for its content of genetic information, it might be expected that molecules of transforming principle differing in nucleotide composition would contain different genetic markers. Since the buoyant density of bacterial DNA depends on its content of guanine-cytosine base pairs, density gradient centrifugation provides a means of studying the biological activity of transforming principle as a function of nucleotide composition.

Pneumococcal DNA containing the markers streptomycin resistance ($S^r$), optochin resistance ($Q^p$), and bryamycin resistance ($B^y$) has been studied in the density gradient. The density distribution of biological activity for each marker is approximately gaussian, and indicates a molecular weight not less than $13 \times 10^6$

* Department of Chemistry and Chemical Engineering, California Institute of Technology
for the biologically active molecules.

The existence of compositional heterogeneity in the DNA is indicated by the following observations:

1) The density distribution of biological activity for a single marker is narrower than the DNA band width obtained from ultraviolet absorption photography.

2) The mean densities of the distributions for the different markers do not coincide.

3) Transformation frequency ratios for pairs of markers change exponentially with changing density.

4) In an experiment involving P-32 labeled transforming DNA, the density distribution of biological activity for a single marker occupied only a portion of the density distribution of the radioactivity.

Assuming that the densities of the molecules are determined exclusively by their nucleotide composition, we may calculate the magnitude of the compositional heterogeneity, expressed as the standard deviation of the distribution of compositions among the molecules. If there are 15,000 nucleotide pairs per molecule

\[ \sigma_{GC} = 1.2\% = 180 \text{ guanine cytosine pairs per molecule} \]

which may be compared with a standard deviation of 60 GC pairs for molecules of the same size, but composed of random sequences of AT and GC pairs. Molecules with the streptomycin resistance marker have 180 more GC pairs than those with the optochin marker, and 150 more than those with the bryamycin marker.

Since a genetic marker occupies only a small portion of a DNA molecule, the density at which it is found depends on the compositions of neighboring, genetically linked markers. These experiments demonstrate that a marker is associated with molecules more homogeneous in nucleotide composition than the bulk of the DNA. Such a result would not be expected if genetic information is carried in structural features which are independent of nucleotide sequences. Thus these observations are consistent with the hypothesis that 1) genetic information is contained in sequences of nucleotides rather than in other structural features of DNA and 2) the sequence of nucleotides determines the genetic map. They also suggest that the AT and GC base pairs cannot be used interchangeably in the construction of biologically active molecules.

165. STUDIES ON CROSS-REACTIVATION IN PHAGE T4D

Investigator: R. H. Epstein
Support: National Institutes of Health, Public Health Service
The National Foundation

Bacteria infected with unirradiated active phage and UV-irradiated inactive phage yield some progeny phage with genetic markers derived from the irradiated parent. This phenomenon of cross-reactivation (CR) or "marker rescue" appears to be accomplished by the recombination of a marker away from surrounding UV damages.

Studies on recombination between closely linked markers in phage T4 have shown that particles heterozygous for the markers in the cross are often
intermediates in recombinant production. As a part of a study of CR using phage T4D, the relation of the heterozygote to the rescue event was investigated. In these experiments, bacteria were mixedly infected with rII mutant and wild-type phage, the wild-type particles having been irradiated prior to adsorption to the bacterial host. The phage yield from these infected bacteria was examined for particles with an r+ marker derived from the irradiated wild-type parent and for particles heterozygous at the r locus. The use of the rII locus is technically advantageous since it permits the selective determination of heterozygosis at the r locus among those particles with an r+ allele. In addition to the analysis of progeny phage obtained after normal lysis of the infected bacteria, early progeny phage, obtained by premature lysis of the infected bacteria, were examined. Such an analysis shows that the fraction of particles heterozygous for a given rII locus, among those particles with an r+ allele derived from the irradiated parent, is 18% when the average phage yield per productive bacterium is 2 to 3 particles. This fraction falls to a value of 6 to 8% at the time of normal lysis. Appropriate control experiments with unirradiated phage provide a value for the normal fraction of heterozygotes at the r locus among those progeny with an r+ allele. This value is not greater than 4%, whether one examines the progeny obtained after normal lysis or early progeny obtained by premature lysis.

Since only 4% of the observed heterozygotes can be accounted for by matings between r and r+ particles which occur after the rescue event, these observations indicate that the heterozygote is often an intermediate in the rescue event. The decrease in the frequency of heterozygotes as the time of normal lysis is approached is compatible with the hypothesis that the heterozygotes formed in the rescue event segregate upon multiplication.

In the premature lysis experiments it was also observed that the time of appearance of r+ recombinant particles is later than that for the unirradiated r genotype. This finding suggests that the rescue event occurs later in the intracellular growth cycle than those events which lead to recombinants in a cross between normal infecting particles.

Other experiments are in progress to further characterize the recombination event which leads to rescue. CR experiments have been carried out using two unirradiated rII mutants and heavily irradiated wild-type phage. One of the rII parents is a point mutant, the other a mutant having a deletion involving both the rIIA and the rIIB cistrons. CR of the r+ allele of the irradiated wild type is observed in crosses to the unirradiated point mutant; in CR experiments using the deletion mutant as the unirradiated parent, no r+ rescues are observed. Bacteria were infected with a constant total multiplicity (18) of the two rII mutants and with a low multiplicity of heavily irradiated wild-type phage. The dependence of the frequency of r+ rescues upon the ratio of the two rII mutants in the infecting mixture was determined. The frequency of rescue was found to increase linearly with increasing multiplicity of the point mutant. This result suggests that only a single interaction between an irradiated and unirradiated genome is required for rescue.

166. INDUCED "MUTATIONS" IN SERRATIA PHAGE Kappa BY EXTRACELLULAR ULTRAVIOLET IRRADIATION

Investigator: Ulrich Winkler
Support: North Atlantic Treaty Organization

By irradiation of the free temperate phage Kappa with ultraviolet or X-rays one can induce heritable changes without irradiation of the indicator bacteria strain HY (Naturwissenschaften 46:150, 1959; Nature 186:330, 1960). The altered phage
have lost their capacity for lysogenization in part or completely. Attempts will be made to find whether the cause of the heritable change is 1) a real mutation in the phage, 2) an exchange of genetically homologous material between phage and host bacteria or 3) a recombination between genetic material of the irradiated phage and those of an unknown prophage (in the host bacteria) which is genetically different from Kappa. A further question to be studied is whether only heritable changes affecting the capacity for lysogenization can be induced or whether other changes, for example, those of the host range, can be induced.
VISITING LECTURERS

A number of visiting scientists gave general biology seminars during the year. The lecturers and their subjects were:

Dr. Albert Szent-Györgyi, Marine Biology Laboratory, Woods Hole, Massachusetts: "Submolecular Biology."

Dr. Giuseppe Attardi, University of Padua, Italy: "Analysis of Antibody Synthesis at the Cellular Level."

Dr. S. R. Pelc, University of California Medical Center, San Francisco, and M.R.C. Biophysics Research Unit, King's College, London, England: "Incorporation of Tritiated Thymidine and Reproduction of Chromosomes."

Dr. Waldo Cohn, Oak Ridge National Laboratory: "Pseudouridine: A New Kind of Nucleoside from Ribonucleic Acids."

Dr. W. L. Russell, Oak Ridge National Laboratory: "Genetic Effects of Radiation in Mice."

Dr. Leonard Nelson, Department of Physiology, Emory University and Department of Medicine, University of California at Los Angeles: "Chemical and Morphological Aspects of Sperm Mobility."

Dr. Charles J. Brokaw, Department of Zoology, University of Minnesota: "Studies of Isolated Flagella."

Prof. Toki-O Yamamoto, Biological Institute, Faculty of Science, Nagoya University, Nagoya, Japan: "Artificial Induction of Sex Reversal in Fishes."

Dr. David Smith, Botany Department, Oxford University, England: "The Biology and Physiology of Symbiotic Plants -- Lichens."

Dr. C. S. French, Department of Plant Biology, Carnegie Institution of Washington, Stanford: "Light, Pigments, and Photosynthesis."

Dr. R. W. Siegel, Zoology Department, University of California at Los Angeles: "The Genic Basis of Sexuality in Paramecium."

Dr. Dietrich Schneider, Zoology Department, University of California at Los Angeles: "Olfactory Perception in the Silkworm, Bombyx mori."

Dr. Emil Witschi, Sigma Xi National Lecturer, Department of Zoology, State University of Iowa: "Sex Reversal in Animals and Man."

Dr. Henry Harris, John Innes Horticultural Institution, Hertford, England: "Studies on RNA Synthesis in vivo."

Dr. C. E. Ford, M.R.C. Radiobiological Research Unit, Harwell, England: "Recent Developments in Human Cytogenetics."

Dr. Silvio Ranzi, Department of Zoology, University of Milan, Italy: "Protein Differentiation during Development."

Prof. Arthur Kornberg, Department of Biochemistry, Stanford University School of Medicine: "Recent Studies on the Enzymatic Synthesis of DNA."

Dr. Homer Jacobson, Chemistry Department, Brooklyn College, Brooklyn, New York: "Self-reproducing Systems; Living and Artificial."

Prof. Erwin Blümmel, Botanical Institute, University of Tübingen, Germany: "Photoperiodism and Endogenous Rhythms in Blooms and Butterflies."

Dr. William Backwith, Department of Psychiatry, University of California at Los Angeles Medical School: "An Introduction to Modern Comparative Ethology."

GRADUATES

Eleven students were awarded the B.S. or the Ph.D. degree in Biology in June, 1960. The names, degrees conferred, the titles of doctoral theses, and the plans of the recipients for future work are as follows:

Alan Paul Berg, B.S. To University of Oregon Graduate School.

John Howland Campbell, B.S. To Harvard University Graduate School for advanced work in Zoology.
Leroy Edward Hood, B.S. To The Johns Hopkins University School of Medicine.
Thomas Michael Jovin, B.S. To The Johns Hopkins University School of Medicine.
Sidney Ronald Roth, B.S. To University of California at Los Angeles Graduate School for work in physiological psychology.
Robert Campbell Thompson, B.S. To The Johns Hopkins University School of Medicine.
Jacob Valentine Maizel, Jr., Ph.D. Thesis: "Isolation and Partial Characterization of Avenacin, an Antibiotic-like Substance from Oats." He is doing research at the Laboratory of Cell Biology, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Maryland.
John Robert Simmons, Ph.D. Thesis: "Studies on Amino Acid Incorporation in the Larvae of Drosophila melanogaster." He is doing research at the Biochemistry Department, Stanford Medical School, as a U.S. Public Health Service postdoctoral fellow.
Edward Harvey Simon, Ph.D. Thesis: "I. Transfer of DNA from Parent to Progeny Cell. II. A Study of the Possible Role of DNA in Viral RNA Synthesis during the Multiplication of an Animal Virus." He is doing research at the Department of Biology, Purdue University.
William Allen Strohl, Ph.D. Thesis: "Studies on the Modification, by Various Agents, of the Heat Sensitivity of Poliovirus." He is now Instructor, Department of Microbiology, St. Louis University School of Medicine.
Howard M. Temin, Ph.D. Thesis: "The interaction of Rous sarcoma Virus and Cells in vitro." He is now Assistant Professor of Oncology, McArdle Memorial Laboratory, The Medical School, University of Wisconsin.

GOSNEY FELLOWSHIP

Dr. Harriett Ephrussi-Taylor, of the Sorbonne, spent a part of the year at the Institute as a Gosney Research Fellow, studying the transforming principle in pneumococcal deoxyribonucleic acid.

GRADUATE STUDENT SUPPORT

During the year a training grant from the United States Public Health Service was used to support the following graduate students in Biology: Mr. Dennis Barrett, Mr. Alan P. Roberts, Mr. William A. Strohl, Mr. George Guthrie, Mr. Richard F. Squires, Mr. Stanley G. Krane, Mr. Robert L. Heath, and Mr. D. James Morre.

A National Science Foundation grant for graduate research training was used during the year to support Mr. Paul H. Deal, Mr. Harris Bernstein, Mr. F. William Studier, and Mr. Colwyn Trevarthen.

A grant from the Nutrition Foundation was used during the year for the support of the following graduate students: Mr. Thomas A. Cole, Mr. Michael Fried, Mr. Robert L. Millette, and Mr. John R. Simmons.

The Arthur McCallum Fund provided support for Mr. Robert L. Heath, Mr. John R. Simmons, Mr. William A. Strohl, Mr. Millard Susman, Mr. Howard M. Temin, and Mr. Ivan Jean Weiler.

During the year the following graduate students held nationally awarded predoctoral fellowships:

National Institutes of Health, Public Health Service: Mr. George Guthrie,
Mr. Charles R. Hamilton, Mr. Boyd A. Hardesty, Mr. Jacob A. Maizel, Jr., Mr. Martin Mendelson, Miss Ann Roller, Mr. Edward H. Simon, and Mr. Millard Susman.

National Science Foundation: Mr. Dennis Barrett, Mr. John R. Menninger, Mr. Donald C. Shreffler, and Mr. Robert A. Weisberg.

The National Foundation provided fellowship support during the year for Morton H. Kirsch, M.D., who has begun graduate work for the Ph.D. degree.

HONORS AND AWARDS

G. W. Beadle received the American Cancer Society's national award for outstanding service; was given the Kimber Genetics Award by the National Academy of Sciences for "distinguished contributions to the science of genetics;" was elected a foreign member of The Royal Society; was appointed a member of the President's Science Advisory Committee; was invited to give the Arthur Dehon Little Memorial lecture at the Massachusetts Institute of Technology.

Renato Dulbecco and A. H. Sturtevant were appointed members of the American Cancer Society Subcommittee on Leukemia and Lymphomas.

A. J. Haagen-Smit was appointed by Governor Edmund Brown to the State Automobile Emission Control Board.

R. W. Sperry was elected a member of the National Academy of Sciences.

FINANCIAL SUPPORT

The financial support available for the work of the Division of Biology comes from many sources: from general institute endowment and from special endowment funds for broad areas of work; from grants from or contracts with individuals, companies, foundations and U.S. governmental agencies for specific projects; from unrestricted annual gifts; and from contributions to general funds provided by Industrial Associates and Institute Associates.

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Los Angeles County Air Pollution Control District | Smog research on plants
Mrs. Austin McManus for Desert Plant Research | Plant research in desert areas
Malcolm McNaghten      | Scholarships
National Foundation for Infantile Paralysis | Bacterial and animal virus research; fellowship supply grants
National Science Foundation | Studies in animal physiology, biochemistry, biophysics, genetics, plant biochemistry, plant physiology
Nutrition Foundation   | Research on biochemical genetics of Neurospora
Nutrition Foundation - McCallum Foundation | Predoctoral fellowships
Rockefeller Foundation | General biology support; studies of water relations and of chemical aspects of climatic responses of plants
Harlan Shoemaker Fund for Paralytics | Research on muscle reinnervation
U.S. Atomic Energy Commission | Research in radiation genetics, protein synthesis
U.S. Department of Agriculture | Plant physiology and corn cytogenetics research
U.S. Geologic Survey   | Plant physiology research
U.S. Navy, Office of Naval Research | Research on spinal reflexes in cords
U.S. Public Health Service | Studies in basic experimental biology, animal physiology, biochemistry, biophysics, embryology, genetics, plant physiology, psychobiology, virology, graduate research training
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