A Report for the Year 1960-1961
on the Research and other Activities
of the
Division of Biology
at the
California Institute of Technology
Pasadena, California
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

Annual reports conventionally note gains during the year; they rarely emphasize losses. This one can only begin with the mention of a major loss: the departure of our Chairman of the past 14 years, first to share his time as Dean of the Faculty, and in January to leave to become Chancellor of the University of Chicago. Our loss is profound, but so is our gratitude to George W. Beadle for all that he did for us. We wish him the quality of conspicuous success he gave us, in his new responsibility. Another loss is less regrettable only because it is the kind that must occur in the natural course of time: E. G. Anderson, one of the first to join the Divisional Faculty when it was founded by T. H. Morgan, reached full retirement age and was named Professor Emeritus.

Curriculum Revisions

There were, of course, many gains. The year saw the effective occupation of the new Gordon Alles Laboratory for Molecular Biology and the Campbell Plant Research Laboratory, the completion of which was described in the previous annual report. In undergraduate instruction, a sharp increase prevails in the number of students electing the Biology Option; in the present undergraduate years each successive class sets a new record number. Curriculum revision has progressed in a highly satisfactory manner; the new course in cell biology, a sophomore elective devoted to such subjects as cellular ultrastructure, electrical phenomena in cells, permeability and active transport into cells, feedback mechanisms in cellular differentiation, the dynamic consideration of cell division, and other chemical and physical approaches to cell biology, has proved to fit in well with the interests and training of Caltech undergraduates at that level. The consolidation and acceleration of subject matter within the area of plant biology into a single first-term junior course, taught for the first time this past year, is providing a successful answer to a problem that plagues all the sciences—the necessity of telescoping and integrating knowledge that is accreting at an increasing rate into the earlier training of the student, so that he can get on to more specialized frontiers. A similar development will be undertaken this coming year on the animal side of biology; that this new effort will combine the talents of the newest and youngest member of our staff, Dr. Charles Brokaw, who will be joining the faculty in September, with those of our senior scholar, Professor A. H. Sturtevant, who will be in his final year before full retirement, offers secure promise that the contribution of this new course will be of the highest significance and value. Biochemistry and biophysics have been integrated into a one-year junior intense course. The summer course in marine biology, at our Marine Laboratory in Corona del Mar, taught by Dr. Wheeler North, continued the standard of excellence set the previous summer by Dr. Brokaw, guest teacher.
Undergraduate Research Participation

Not the least contribution to the atmosphere of youth, vigor and enthusiasm in the Division was provided by the Summer Research Apprentices Program. Thirty-eight undergraduates, about half of them selected from a large number of applicants from many colleges and universities all over the country, and the other half Caltech undergraduates, spent periods of at least ten weeks working on research problems in laboratories under the direct supervision of members of the Biology Faculty. The progress toward the status of mature investigators displayed by many of these apprentices has been indeed remarkable.

Research Activities: The Brain

The research activities of students at the graduate level, the stimulating, energetic and diverse contributions of a large number of postdoctoral fellows here for a year or two from various backgrounds, and the investigational and integrating programs of our faculty are summarized in this report. Only two areas will be selected for brief mention here; these are drawn from near the opposite ends of the spectrum of organization among living things.

The control centers of the brain come in matched pairs, a complete set to each half of the brain. Normally, the right and left halves are in direct communication through a series of fibers that cross the midline and make connections between corresponding structures on the two sides. It has been known for some time that surgical section of the largest of these fiber tracts in human patients has remarkably little effect on any aspect of behavior. Animal studies at Caltech over the past several years have shown that cats and monkeys recover easily from such an operation and are virtually indistinguishable from their normal cage mates under practically all conditions of testing and training. They perceive, learn, and remember much as normal animals do. But if special training and testing conditions are set up, in which different sensory information flows to each of the divided hemispheres, each hemisphere proves to have its own independent perceptual, learning, memory and other mental processes. In normal animals, the midline fiber tracts create on each side a weak "carbon copy" of the engram stored on the other. But when the tracts have been sectioned it is as if each of the separated hemispheres is unaware of what has been experienced by the other, as if the animal had two separate brains. It is possible to train such an animal to give opposite responses to the same visual cue, depending on which eye he sees it with. When he sees both contradictory cues at the same time, there is little evidence of mental conflict between the two brains; one generally dominates. Contributions already made by this kind of study to an understanding of brain structure and function are of the greatest interest. The potentialities are even more impressive. Because the animal has effectively two brains, which may be simply separated without affecting his health, comfort, or general behavior, a "control" and "experimental" brain co-exist in the same organism. The system is optimally designed for all sorts of experiments, ranging from short-term studies on inanimate organization to studies of the long-term effects of early experience on adult behavior. As Roger Sperry said in concluding a talk on this subject, "Those of us working in brain research find ourselves today, as never before, surrounded by seemingly endless possibilities just waiting to be explored." His interests are, of course, strongly reinforced at Caltech by the long-established and productive programs of Dr. Van Harreveld, working in the field of central nervous system physiology of mammals, and Dr. Wiersma, whose group traces in painstaking detail the nervous connections in the peripheral nervous systems of invertebrates. Current plans for extensions of joint electrical engineering and biology interest into the field of computer simulation of cerebral mechanisms will provide further reinforcement. Perhaps in time extensions in another direction, from psychobiology into psychology, may also be undertaken.
Research Activities: Nucleic Acids

At the other end of the spectrum, in a way, lies the area of molecular biology, where the nature of molecules important to living systems, their forms, functions, and organization are under study. Kingpins in this molecular empire are the nucleic acids, which carry and transmit the coded information of heredity, and control the synthesis of other molecules, such as enzymes and hemoglobins, which direct the chemistry of organisms. This is an area in which Caltech has long been a leader, and the Annual Report gives evidence of the extensive effort being maintained in this connection. Current studies run the gamut, from the remarkable single-stranded deoxyribonucleic acid of the tiny phage $\phi X174$ being studied on several fronts mainly by members of Dr. Sinsheimer's group, to the "chromosome" of an ordinary bacteriophage which turns out, excitingly enough, to behave like a circle rather than a linear structure, and which directs remarkable processes of differentiation in the bacteriophage. These phages are far more than formless tiny blobs; they have a beautifully defined structure, a head, a tail, fibers running through the tail and exposed at its tip; a base-plate; they attach to and inject their nucleic acid into the bacterium they are infecting with a contraction leading to an action like that of a hypodermic syringe. These details of structure can only be visualized with the electron microscope; now, through the development of Dr. Hodge's suite of laboratories, the Biology Division is equipped to go into matters of ultrastructure in the cells of higher plants and animals as well as the viruses, with the highest degree of competence. And how do the nucleic acid molecules go about controlling the biochemical activities of cells? Here, too, the attack at Caltech is many-pronged, from extensive and detailed studies of the sequence of reactions in pea embryos, which turn out to be an admirable material for this kind of study, and the red blood cell forming tissues of animals and man, in which hemoglobin formation continues to be a major concern of our biochemists, to the control of enzymes affecting the biochemistry of the mold Neurospora, and the biochemistry and biophysics of viruses, extending, particularly in Dr. Dulbecco's group in animal virology, into the area of how viruses affect cells, and how the cells of a higher animal come to be different from each other during the course of development.

Between these two areas on the spectrum, from molecular biology and viruses to the complex organization and function of the animal brain, lies much of the realm of biology, part of it strongly represented at Caltech. To borrow again a part of Roger Sperry's phrase, we "find ourselves today, as never before, surrounded by seemingly endless possibilities just waiting to be explored."
STAFF OF INSTRUCTION AND RESEARCH
SUMMARY
DIVISION OF BIOLOGY

Ray D. Owen, Acting Chairman
succeeding G. W. Beadle

Professor Emeritus

George E. MacGinitie, M.A.

Professors

Ernest G. Anderson, Ph.D.
George W. Beadle, Ph.D., D.Sc., Nobel Laureate
James F. Bonner, Ph.D.
Henry Borsook, Ph.D., M.D.
Max Delbrück, Ph.D.
Renato Dulbecco, M.D.
Sterling Emerson, Ph.D.
Arie Jan Haagen-Smit, Ph.D.
Alan J. Hodge, Ph.D.
Norman H. Horowitz, Ph.D.
Anton Lang, Ph.D.
Edward B. Lewis, Ph.D.
Herschel K. Mitchell, Ph.D.
Ray D. Owen, Ph.D.
Robert L. Sinsheimer, Ph.D.
Roger W. Sperry, Ph.D.
Alfred H. Sturtevant, Ph.D., D.Sc.
Albert Tyler, Ph.D.
Anthonie van Harreveld, Ph.D., M.D.
Cornelis A. G. Wiersma, Ph.D.

Biography
Genetics
Biochemistry
Bio-Organic Chemistry
Hixon Professor of Psychobiology
Thomas Hunt Morgan Professor of Genetics
Embryology

Visiting Professors

David G. Catcheside, M.A., D.Sc.
Eduard Kellenberger, D.Sc.

Research Associates

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William S. Stewart, Ph.D.
Jean J. Weigle, Ph.D.
Visiting Associates

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John R. Laughnan, ² Ph. D.  
Paul A. Walker, ³ Ph. D.  

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² Guggenheim Fellow  
³ Randolph-Macon Woman's College

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John H. Fessler, Ph.D.  
Henry Heilmers, ¹ 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.  

Instructor

Charles M. Steinberg, Ph.D.  

Research Fellows

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Giuseppe Attardi, M.D.  
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Klaus Bayreuther, Ph.D.  
Benigna M. Blondel, ² Ph.D.  
Joseph Bossom, ¹ Ph.D.  
Sidney Brenner, D.Phil., M.B.B.Ch.  
Hans J. Burkhardt, Ph.D.  
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Donald Caplenor, ³ Ph.D.  
Edward A. Carusi, Ph.D.  
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Rowland H. Davis, ³ Ph.D.  
Bruce M. Eberhart, ⁶ Ph.D.  
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Marguerite Fling, Ph.D.  
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Hildegarde Lamfrom, Ph. D.
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Jerry B. Lingrel, Ph. D.
Bruno A. Lobo, 12 M. D.
James A. Lockhart, Ph. D.
John B. Loefler,13 Ph. D.
Peter H. Lowy, Doctorandum
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Marian Michniewicz,4 D. Sc.
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1. CENTRAL CONNECTIONS BETWEEN THE THORACIC AND ABDOMINAL CORDS OF THE CRAYFISH

Investigators: C. A. G. Wiersma, B. M. H. Bush
Support: National Science Foundation

In continuation of the studies on the anatomical-physiological connections of the crayfish central nervous system, the cord was investigated at the level between thorax and abdomen. Insight was thus obtained into the information that is exchanged between these different body parts, and in what form the exchange takes place. A total of 130 entities was established, which denotes an increase above the two previously investigated levels—the connections between the brain and the rest of the cord, and between the 3rd and 4th abdominal ganglia, the latter in the isolated abdomen.

About 50 of the total of 75 entities found in the abdominal cord were recognized with certainty. Of the 100 "commissural" units some 30 were found back at the level between thorax and abdomen.

Apart from the stretch receptor interneurons, few primary sensory fibers appeared, and these were mostly limited to ones entering the last thoracic or the first abdominal ganglion. Of some special interest was the finding that at least the most posterior N-cell (Wiersma & Pilgrim, 1960) sends a branch down the cord as do all stretch receptors.

The interneurons found could be clearly divided into two main classes. Those of the first order reacted clearly to input in the peripheral sensory fibers. Again there was a formation of bundles of those interneurons reacting to stimulation of single segments, or parts of such segments. None were found in this class coming from the head region, in contrast to the findings at the commissural level.

The largest number of interneurons found belonged to interneurons of this class, but they reacted to stimulation of sensory fields in two or more different segments. A number of these responded both to thoracic and abdominal fields, and some to fields on any part of the body.

Clear evidence was obtained for quite readily reacting interneurons, whose input nevertheless was secondary. Such interneurons react, in contrast to primary interneurons, as well to stimulation of any part of their sensory field after they had been cut as before, but only in either the anterior or the posterior lead, and not in both. This shows that they obtained their input through primary interneurons. They are therefore more difficult to study, since they will not react any longer in this fashion, when those primary interneurons are cut, as will occur during continued preparation.
In addition an unknown number have high thresholds, and it becomes difficult or impossible to gain certainty about their input channels. Also, in this type, central inhibition plays a more and more prominent part. Thus it was not rare to find one interneuron, responding at first readily to a certain stimulation, then failing completely on such stimulation, and later responding readily again. The method employed reaches in such cases the limit of its usefulness.

It was observed that it is especially the high threshold interneurons of this type which cause readily observable effects, such as leg movements or abdominal flexions or extensions, whereas those with lower thresholds do not do this.

Publications:


2. COMPUTER ANALYSIS OF INTERNEURONS WITH MORE THAN ONE INPUT

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

Computer analysis of crayfish interneurons with two or more inputs and two outputs is in progress. It has been shown that the two outputs display the same number of impulses, with only slight differences apparent in the spacing of the impulses. This leads to the conclusion that such interneurons have no way of transmitting information concerning the locality of the stimulation within their total sensory
field. The loss of "information" (number of impulses suppressed by collision) becomes stationary after a maximum has been reached, at which time about half the number of impulses have been suppressed. If two inputs are kept constant, and a third varied, the last is considerably suppressed until this maximum is reached. Under no conditions does the combination of two or more inputs tend to give rise to uniformly spaced impulses. On the contrary, typically there arise patterns in which shorter and longer intervals between impulses alternate in various time sequences. This leads to the speculation that the lack of proportionality between input and output frequencies at lower input rates may be compensated for physiologically by the pattern-sensitivity of the final analyzer (e.g. the motor neurons).

Mr. Dennis Benson, an undergraduate at the Institute, assisted in this investigation.

3. MEMBRANE POTENTIAL CHANGES ON ACTIVATION IN CRUSTACEAN MUSCLE FIBER

Intracellular investigations have increased our knowledge of the processes taking place in crustacean muscle fibers upon stimulation of neurons, but one of the problems still to be solved is the nature of the relationship between the membrane changes and contraction of the muscle. The claw opener muscle of the European hermit crab is innervated by a single motor axon, and has previously shown two distinct response sequences to differing stimuli applied to this axon. Further investigation of this preparation, using internal electrodes to determine the muscle membrane response accompanying the two reaction types, was undertaken.

The limb of the crab was arranged and dissected in such a way that the motor axon could be stimulated in a variable way, while at the same time the muscle response was recorded through glass capillary microelectrodes inserted into individual fibers, both stimulus and responses being fed into an oscilloscope and photographed. Stimulation by a train of constant low-frequency impulses produced a gradual increase in the depolarization of the cell membrane until a maximum level of depolarization was reached, this being maintained for any further duration of the stimulus at a voltage level halfway between the resting and the zero membrane potential levels. A slow contraction takes place under these circumstances. A short burst of high frequency pulses (300 to 400/sec.) gave rise to a quick attainment of this level, from which arose, depending on circumstances, either abortive or full grown spike potentials, reaching or overshooting the zero membrane voltage level. These were accompanied by twitch-like contractions of the muscle. After the spike the membrane was partially repolarized; continued stimulation resulted in the establishment and maintenance of the maximum maintained depolarization level as before. The critical factor for spiking seemed to be the rate at which the initial depolarization took place. It is likely that the initial depolarization represents activity in the chemically excitable junctional area of the membrane beneath the nerve ending, while the spike represents activity in the remainder of the membrane which is electrically excitable; and that the maximum maintained level of depolarization is therefore produced by the complete activation of the junctional area. It appears that this maintained depolarization is also responsible for the slow late rise in muscle contraction which can follow the initial twitch in this muscle.

This work was done in collaboration with A. C. Bobbert at the University of Leiden.
4. RESPONSE OF CRAYFISH STRETCH RECEPTORS TO DYNAMIC STIMULI

Investigator: Martin Mendelson
Support: National Institutes of Health, Public Health Service
Arthur McCallum Fund
General Funds

In the past some work has been done on the response of stretch receptors to movements, or in other words to changes in the degree of stretch. Lowenstein and Finlayson have used the abdominal stretch receptors of insects, Pringle and Wilson studied the position sensitive cells of the spines on the cockroach leg. Lippold and associates have examined the stretch receptor elements of mammalian muscle. These investigators found, in units that were supposed to respond to the position of a joint or to the stretch of a muscle, that at low frequencies of stretch-relaxation the units did indeed respond as expected, showing a higher frequency at maximal displacement and a decrease in output at lesser displacement or stretch. At higher stimulus repetition rates, using approximately sine shaped stimuli, they observed the output frequency maximum approaching the point in time at which the rate of displacement was maximal. It was decided to test this response on the abdominal stretch receptors of the crayfish, which seemed ideally suited for the purpose. In each abdominal segment there are two types of stretch receptor. One type responds to stepwise stretch with a stepwise increase in output. At each increase of stretch the receptor responds with an increase in frequency. The output of this type of receptor for any given degree of stretch may stay constant for as much as six hours. The second type responds to stepwise stretch with a short burst at each step but does not maintain its discharge if the stretch is maintained. Here, it seemed, was a receptor almost exclusively sensitive to displacement and one sensitive only to increases of displacement.

The experiments consisted of removing a receptor, mounting its ends in clamps (one of which could be driven back and forth by an electro-mechanical system) and then submitting it to cyclic stretch and relaxation. The stimulus waveform used was of triangular shape; that is the organ was stretched at constant velocity and relaxed at constant velocity. Cycling rate (velocity of stretch) and amplitude were independently variable allowing a separation of the effect of these two factors. Such a separation is much more difficult with a sine wave where velocity is a function of amplitude. With this improved technique it was observed that the output of the two types of receptors is almost entirely displacement sensitive. In no case was the frequency maximum seen to shift with constant amplitude and changing stretch rate, even over a very broad range of rates. The output maximum always coincided with the point of maximum displacement. It was noted though that the output was asymmetrical about the maximal displacement point. In the second type of receptor described above the asymmetry increased with increasing cycling rate to the point where the output ceased at or just after the reversal of direction. Recording from the interior of these cells during cyclic stimulation shows why. The inside of the cell is maintained by metabolic processes at a potential negative to the outside. Stimulation causes a decrease in the negativity which in turn elicits the impulses. During the period of increasing stretch the negativity smoothly decreases and impulses are discharged. At just subfiring levels of stretching the negativity was seen to decrease smoothly, but at the turning point of the movement there was a sharper return of negativity than expected. Such a sharp return, accentuated at higher speed and stretches could easily be responsible for the silencing of the cell at the turning point. This rebound is attributed to a process called adaptation which is stimulated by the excitatory process and antagonizes it. The stronger the excitatory process, the stronger the adaptation it elicits. Assuming that diminution of the adaptation follows diminution of excitation with a lag will account for the observed phenomenon. When the excitation has begun to diminish past the turning point of the stimulus, the adaptation may not have begun to diminish yet or may even be still increasing. Summing of these two processes would give the brief rebound.
5. PHYSIOLOGICAL OBSERVATIONS ON CRAB MOVEMENT RECEPTORS

Investigator: Martin Mendelson
Support: National Institutes of Health, Public Health Service
       Arthur McCallum Fund
       General Funds

The movement receptors of crustacea have been described and studied by Wiersma and Boettiger. These organs, which take the form of a cluster of nerve cells on an elastic band across the joint, have been found in the legs of numerous species. They are of particular interest to the physiologist because of some unique characteristics. The individual movement receptor cell responds to movement of the joint in one direction only, provided that the speed of the movement exceeds a certain threshold level. With a relatively small increase in the movement speed the impulse frequency rises to about 50 to 60 per second and further increase in the movement speed will increase the frequency. This is in contrast to most sensory receptors, which are capable of firing at frequencies of several hundred per second. In addition, at the saturation frequency the movement receptor fires very regularly with remarkably even spacing between impulses; and as the frequency decreases the interspike intervals are integral multiples of the saturation interval. This strongly suggests a timing mechanism that makes it impossible for impulses to occur except at certain times. Thus two questions arise: first, what makes a cell respond to movement of the joint in one direction only and since cells are present for each direction how is the distinction between the two directions of movement made? Second, what is the timing mechanism built into the individual cells? A partial answer is now available for the first and some suggestive data for the second.

The band mentioned above is situated so that it runs from the tendon of a muscle in one segment of the leg to the shell of the next segment. When the joint moves in one direction the band is stretched and the angle between it and the tendon is reduced so that the two are parallel. When the joint moves in the other direction the band is relaxed and the angle between band and tendon increases, stretching some short fibrous connections in the angle. It was suggested that the movement which stretches the tendon stimulates some of the cells and then the movement in the other direction stimulates cells attached to the short fibers across the angle. Yet removal of the organ from the leg and subsequent stimulation by stretching and relaxing of the band has shown that certain cells will respond to the stretching and others will respond to relaxation of the band in the absence of any pull upon the short fibers. It is proposed that there is a relative motion between parts of the band and the cells attached thereto, and that this is the real stimulus. Electron microscope pictures by M. Whitear at Cambridge have shown two types of cell endings in the band and it is supposed that one type responds to relative motion in one direction. The other, which faces the opposite way, responds to motion in the other direction.

With regard to the timing mechanism, attempts to record from inside the cells with special micropipette electrodes have led to repeated failure. This in spite of the fact that other mechanoreceptors have been successfully recorded under more rigorous conditions. Instead, it was found that the application of the drug nicotine to
the movement receptors provokes a discharge of impulses. Recording from single nerve fibers has shown that the frequency of discharge elicited by nicotine may be as high as 250 per second whereas the same cell could not be made to respond to movement stimulation with a frequency greater than 55 per second. Obviously, the cells are capable of firing at high rates and it is apparent that the two types of stimulation work through different pathways within the cell. It appears that the impulse firing mechanism of the cell is more accessible to the nicotine than to the mechanical stimulation. This could result from greater spatial separation between the mechanical stimulation point and the impulse initiating point than is the case between the latter and the nicotine stimulation point. Alternatively, the degree of mechanical coupling between band cell may be low such that no more than a certain amount of mechanical force can be transmitted to the cell through this coupling. Of further interest is the observation that the nicotine discharge, when its frequency falls to low levels, does not show any of the rhythmic attributes of the mechanically evoked output. This implies that the timing system is a part of the mechanism which transduces mechanical stress into electrical signals. Another attempt is now in progress to record intracellular events from these cells.

Reference:


6. THE CONTINUED ANALYSIS OF FACTOR I IN MAMMALIAN BRAIN

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

Electrophoresis of active cow brain extracts was carried out. Although this led to a separation into more and less active fractions of Factor I, a compound that inhibits discharge of crayfish stretch receptors, this method alone did not lead to great purification. Sublimation under controlled conditions produced an extract of greater purity. Preliminary efforts were made to identify the active fraction by infrared spectroscopy. Some indications were obtained that one of the active substances may be related to, but not identical with, neuraminic acid.

7. METRAZOL AND GLUTAMATE METABOLISM IN THE BRAIN

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

Experiments have been carried out to determine whether metrazol, a convulsant, inhibits the metabolism or movement uptake of L-glutamic acid in the cerebral cortex of anesthetized rabbits or slices of rat brain. In vivo, radioactive L-glutamate applied to the surface of the cerebral hemispheres is metabolized rapidly within 1 to 3 minutes, primarily to form glutamine. The presence of metrazol as 47 to 50 mg per kg body weight does not inhibit this measurably. Cortical slices of rat brain incubated with radioactive glutamate produce as much radioactive product when metrazol is present as in its absence. The concentrations of metrazol used have been 50 mg/L and 625 mg/L.

The accumulation of L-glutamic acid by cortical slices of rat brain was unimpeded by metrazol at 682 mg/L.

The production of ammonia by brain slices in the absence of added glucose was unaffected by 250 mg/L of metrazol.
The enzyme, glutamine synthetase from beef brain, was studied for any effects on its rate of activity with metrazol concentrations of 42.8 mg/L to 2.3 g/L. No effects were demonstrated.

The latest experiments involved incubation of cortical slices of rat brain in Krebs Ringers with 55mM potassium chloride rather than the usual 5mM. This higher concentration of potassium ions accelerates aerobic glycolysis as well as the turnover of glutamic acid. This higher rate of metabolism is considered by some neurochemists to more nearly approach the functional state of nervous tissue. Metrazol at the 50 mg/L level still did not significantly alter the overall amount of radioactive product formed from the glutamic acid.

8. IMPEDANCE CHANGES AND ELECTROLYTE DISTRIBUTION IN THE SUBMAXILLARY GLAND

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

It has been long known that the electrical impedance of the submaxillary salivary gland of the rabbit increases markedly during the first minutes of secretion. This change has been explained in various ways in the past. However, recent concepts of tissue impedance, based on the investigations of Fricke and Cole on the impedance of cell suspensions, suggest that such an increase in tissue impedance is caused by a loss of electrolytes from the extracellular compartment. A (low frequency) current measuring impedance is carried mainly by the extracellular ions, the intracellular electrolytes being surrounded by a high resistance cell membrane.

The impedance was measured during secretion induced by an injection of pilocarpine. When the impedance had reached a maximum (often doubling in value) the gland was removed for the determination of the concentrations of chloride, sodium and potassium ions. The potassium was removed from the gland in the saliva (and in the blood) and was replaced by sodium. The chloride concentration of the gland decreased. In other experiments the gland at maximum impedance was removed quickly, frozen in isopentane, cooled to its fusion point, and subjected to substitution fixation at -25°C in alcohol containing silver nitrate. Histological preparations made from this material were treated to show the chloride as a brown silver subhalide. As compared with control glands the submaxillary at maximum impedance showed a marked loss of chloride from the intertubular spaces, which is in agreement with the overall loss of chloride observed. This loss of electrolytes from an extracellular space explains fully the impedance rise during secretion.

9. EFFECTS OF DEHYDRATION ON BRAIN ELECTROLYTES AND CORTICAL IMPEDANCE

Investigators: A. Van Harreveld, N. K. Hooper, J. T. Cusick
Support: National Institutes of Health, Public Health Service, National Science Foundation

A considerable dehydration of the brain of the rabbit can be established by the slow infusion of a hypertonic glucose solution. An analysis of the dehydrated brain showed that it loses about 25% of its water and a considerable percentage of its chloride and sodium. Practically no potassium was lost from the brain.

The cortical impedance rises during the dehydration (35%). A parallel between losses of chloride and sodium, which are in general extracellular ions, and an
Increase in tissue impedance was thus found, as in the submaxillary gland (see above). This observation is of interest in view of the paucity of intercellular space as revealed by electronmicroscopy of the nervous system. The question can be posed as to the location of the chloride and sodium ions which apparently carry the measuring current in the brain as well as in the gland. Two solutions of this problem present themselves. Either there is more extracellular space in the living tissue than is suggested by the narrow spaces in electronmicrographs, or some or all of the glia contains sodium and chloride and has cell membranes which are readily ion permeable. In that case glia would have the value of an extracellular space in the nervous system.

In press:


10. REVERSIBILITY OF ASPHYXIAL CHANGES IN THE APICAL DENDRITES OF THE RABBIT'S CEREBRAL CORTEX

Investigators: A. Van Harreveld, S. Tachibana
Support: National Institutes of Health, Public Health Service
National Science Foundation

Asphyxiation of the cortex causes, after a latency of about 3 minutes, a sudden increase in the cortical impedance. This is caused by a transport of water and electrolytes into the apical dendrites. As an expression of this transport an increase in the chloride content of the apical dendrites has been observed. It was found that this transport of water and electrolytes is quickly reversible (within 15 minutes) after asphyxiation up to 30 minutes. After asphyxiations lasting 45 to 75 minutes the original situation was not always restored after 15 minutes. In some preparations the apical dendrites had not returned to their original diameter. In other instances the diameter of the dendrites was within normal range but they contained definitely more chloride than normally. This may indicate that in these experiments sodium had been removed accompanied by another anion (perhaps bicarbonate). There was a satisfactory correspondence between the recovery of the apical dendrites and a reduction of the cortical impedance.

These findings emphasize the considerable resistivity to lack of oxygen on the part of the processes necessary for the maintenance of the ionic distribution and for the reactions involved in impulse conduction. This resistivity, which is well known to exist in the peripheral nervous system, is contrasted with the great sensitivity of the process necessary for the maintenance of living nerve cells. Asphyxiation of 20 minutes of the spinal cord usually causes considerable cell death. In the cortex the cells may still be more sensitive.

11. SUBSTITUTION FIXATION OF NERVOUS TISSUE

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

Substitution fixation of nervous tissue at temperatures of -25°C in alcohol containing silver nitrate has been used to study the location of chloride in tissue. Although useful for the location of chloride with the light microscope these preparations show extensive ice formation, which prevent their examination with the electron
microscope. To ascertain the location of chloride either in intercellular spaces or in glia a technique has been worked out by which a very small tissue sample (1 to 2 mm$^3$) can be punched out of the cortex and blown immediately in isopentane cooled to its fusion point. The speed with which these operations are carried out will prevent any extensive redistribution of electrolytes and water over extra- and intracellular space, which on the basis of impedance measurements may occur within minutes after disturbance of the cortex. Furthermore, a technique has been worked out by which the substitution fixation can be performed at much lower temperature (about -90°C). It is hoped that at this temperature the ice formation will not seriously hamper electron microscopic examination of the tissue. Light microscopy has failed to show the massive ice formation observed at -25°C.

Publications:


12. ELECTROPHYSIOLOGY OF ASPHYXIATED CORDS

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

Reflex activity was investigated in cats whose spinal cords had been asphyxiated from 30 to 90 minutes and who had recovered for periods of a few hours to four weeks. This series of experiments which was begun in 1959 is now finished and the results are being analyzed. The main results have been reported in Biology 1960. Sufficient material has been collected to give information about the variability among such preparations.

Publication:


13. THE INNERVATION OF THE CRICOTHYROID MUSCLE OF THE RABBIT

Investigators: Harlan Shoemaker Foundation
Support: National Science Foundation
General Funds

The cricothyroid muscle is innervated by two motor nerves. It has recently been a point of discussion whether or not an appreciable percentage of the muscle fibers is innervated by both motor nerves. A solution of this question was attempted
by recording from individual muscle fibers with intracellular electrodes. Only 6% of the muscle fibers reacted with spike potentials on stimulation of both motor nerves. Not more than 2.5% of gold stained and macerated muscle fibers showed double motor nerve endings. The method used did not make it possible to decide whether the two end plates on a single muscle fiber receive their innervation from a single or from two motor nerve fibers. The spike potentials observed in 6% of the fibers on stimulating the motor nerves can be explained in other ways than by double innervation of the muscle fiber. The conclusion was that few fibers of the cricothyroid muscle and possibly none were doubly innervated.

14. EFFECTS OF CEREBRAL ASPHYXIATION ON CONDITIONED REFLEXES

Investigators: A. Van Harreveld, S. Tachibana
Support: National Science Foundation
General Funds

The series of experiments on the effects of cerebral asphyxiation on conditioned reflexes in the rabbit was continued and is now finished. In addition to the method of asphyxiation by increasing the intracranial pressure above the blood pressure, another method was used in which the large vessels arising from the arc of the aorta were clamped. This allows experiments in which oxygenation of the brain is alternated with periods of asphyxiation. Several such schedules were used. The results have been much as described in Biology 1960. The conditioned reflexes could usually be elicited again in the surviving animals after days or weeks. However, in a percentage of the preparations the reflex could not be elicited for more than four weeks. Such animals could be conditioned again in a short time.
15. AMINO ACID INCORPORATION INTO PROTEIN BY SUBUNITS OF RABBIT RETICULOCYTE MICROSOMES

Investigators: Ellen Glowacki, Hildegard Lamfrom
Support: National Institutes of Health, Public Health Service

The state of aggregation of microsomes can be ascertained by analytical ultracentrifugation. The extent of aggregation of microsomes, including those from rabbit reticulocytes, depends on the magnesium concentration and the ionic strength of the medium. The usual incubation medium in which rabbit reticulocyte microsomes incorporate amino acids into hemoglobin contains $1.5 \times 10^{-3}$M magnesium, $5 \times 10^{-2}$M potassium chloride and $1 \times 10^{-9}$M tris pH 8.0; in it microsomes exist predominantly as 70s particles. In solutions of higher magnesium ion concentration microsomes yield microsomal aggregates of 100s to 140s. In solutions without magnesium ion or with low magnesium ion concentrations the microsomes reversibly dissociate into smaller nucleoprotein subunits. There is good evidence that one 70s microsomal particle dissociates into one 30s and one 50s particle.

Various methods are available for the removal of magnesium from reticulocyte microsomal particles, in order to achieve dissociation.

1. Dialysis against a medium without magnesium ion.
2. Passing reticulocyte microsomes through a sephadex column
3. The addition of a chelating agent, e.g., pyrophosphate.

With the first two methods only partial dissociation of microsomes into subunits could be obtained and some 70s particles always remained. With the third method complete dissociation of reticulocyte microsomes into 30s and 50s subunits was possible. Moreover, by varying the relative concentrations of microsome and pyrophosphate any desired extent of dissociation into subunits was feasible.
2. Are subunits of microsomes active in protein synthesis?

With a method available for completely dissociating microsomes into subunits it became possible to determine whether or not microsomes, after dissociation, could still function in protein synthesis.

To test this idea the following experiment was carried out: Microsomes were dissociated with pyrophosphate, the pyrophosphate was removed with pyrophosphatase and the resulting orthophosphate removed with sephadex. The incubation for amino acid incorporation was done in the usual medium containing magnesium ion, in which the microsomes reaggregated to 70s particles.

It was observed that once microsomes had been completely dissociated into subunits they lost the capacity to incorporate amino acids into protein and none of the usual supernatant factors were capable of restoring this property.

Microsomal preparations which contained as few as 5% of undissociated microsomes after the magnesium ion removal step retained unimpaired ability for protein synthesis, incorporating as much C14 leucine into protein as control preparations (containing 95% 70s particles).

These observations indicate that in a usual undissociated microsome preparation only a small fraction, approximately 5%, of the microsomes present may be actively participating in protein synthesis. The results are also consistent with the idea that the 70s particles which are "active" are more resistant to dissociation.

16. THE BIOLOGICAL SYNTHESIS OF PROTEIN

Investigators: H. Borsook, Jerry B. Lingrel
Support: National Institutes of Health, Public Health Service
U.S. Atomic Energy Commission

a) Cytoplasmic constituents with a role in protein synthesis

Reticulocytes do not have an intact nucleus; yet they synthesize a specific hemoglobin. The experimental results obtained indicate that the specificity depends on the combined action of the ribosomes and some as yet unidentified component of the extra-particular cytoplasm other than amino acid activating enzymes and S-RNA. This conclusion has been drawn from experiments in which ribosomes of one species and supernatant solution of another (e.g. rabbit and sheep respectively) were used to synthesize hemoglobin in vitro. Rabbit and sheep hemoglobins, which are separable by column chromatography, were used as carriers and the location of the radioactivity of the labeled amino acid indicated which kind of hemoglobin had been synthesized.

DNAase, unlike RNAase which inhibits completely, does not inhibit the synthesis of hemoglobin in this cell-free system. This finding appears to exclude any function here of the residual DNA in reticulocytes.

b) Reconstitution of lysed reticulocytes

After erythrocytes have been lysed by a hypotonic solution, restoration of isotonicity with salt restores the integrity of the membrane with respect to its ability to function as an osmometer and to retain hemoglobin. Attempts with reticulocytes have failed to achieve any significant degree of reconstitution. Variations in pH, temperature and salt composition were examined exhaustively in the three stages of the process: lysis, reconstitution, and washing of the "reconstituted" cells. Once the adsorption of the generally dissolved hemoglobin was taken into account there was no evidence of any significant reconstitution. The insoluble material of the lysate has a great affinity for dissolved hemoglobin and the adsorbed hemoglobin was not removed by repeated washing with large volumes of isotonic saline.
It may be that erythrocytes can be reconstituted after lysis because their membrane is elastic; erythrocytes swell before lysis and reversal of the swelling restores the integrity of the membrane. In the maturation of reticulocytes to erythrocytes the volume is constricted to about half; the inability to reverse the lysis of reticulocytes may be associated with inability to stretch the reticulocyte membrane further during lysis by hypotonic solution.

An attempt was made to incorporate RNA (from reticulocytes) into normal erythrocytes by adding it to the lysate and then reconstituting the membrane. No RNA was included in the reconstituted erythrocytes.

Reticulocytes which had been lysed, then submitted to the reconstitution procedure and washed with isotonic saline, were tested for their ability to synthesize protein. The cell-free systems in current use are very slow; the rate is about 0.1% of that of the intact cells, and it stops in about one-half hour, whereas it goes on for hours in intact cells. The lysed and "reconstituted" reticulocytes were several times more active than the lysed and non-'reconstituted' preparation; but as a means of obtaining a more active cell-free preparation, this improvement is not useful enough.

c) Intermediates in protein synthesis

The widely accepted hypothesis of the pathway of protein synthesis is that amino acids are activated at their COOH groups by enzymes specific for each amino acid using adenosine triphosphate, and the activated amino acid is transferred to an ester linkage on S-RNA. The activated amino acids are then transferred to ribosomes, which are the template on which the specific array of amino acids is assembled, the peptide bonds made and the complete protein removed. It is taken, on the basis of a little evidence, which is not conclusive, that the array is formed by extension, amino acid by amino acid, from the N-terminal end of the protein. The evidence is very strong (in spite of certain exceptions) for the pathway up to the linkage of the activated amino acid to S-RNA. The evidence for the path beyond that point is fragmentary and there are some serious conflicts.

It is to this area that some present work is directed. Measurements are being made of the number of residues of labeled amino acid incorporated per ribosome particle under various conditions of retardation of synthesis. If all the ribosomes were active and each were concerned with the making of 1 of the 4 peptides of hemoglobin, one would expect to find the ribosomes half filled in the steady state and to contain 9 to 10 leucine residues. The steady state is attained in much less than five minutes and the number of leucine residues found corresponded to 13.5% of the ribosomes being active if each ribosome made 1 of the 4 peptides of hemoglobin. If all 4 peptides are made on each active ribosome then only about 3.3% are active.

The maximum incorporation of the labeled amino acid into the ribosomes occurs at 37.5°C in less than five minutes. From then on, for at least an hour, there is no change so long as the specific activity of the labeled free amino acid within the cells remains unchanged. When labeled is replaced by unlabeled amino acid the ribosomes quickly lose their labeling. It is clear, then, that the amino acid labeling of the ribosomes is in the nature of a dynamic steady state which responds quickly to changes in the amino acid content of the medium. The turnover number of the ribosomes can be calculated from the ratio obtained by dividing the amount of labeled amino acid in the hemoglobin by the steady state value of the ribosome. On the hypothesis that the peptides of globin are made on the ribosomes the ratio gives the number of times each ribosome has been filled and emptied in the stated time interval. The time divided by the ratio gives the turnover number. In reticulocytes at 37.5°C this number is close to 0.25 minutes. On the basis that each ribosome makes only 1 of the 4 peptides of hemoglobin then 4 ribosomes are involved in the making of a hemoglobin molecule and the time required is 0.25 minutes. If alternatively an active ribosome
makes all 4 peptides of hemoglobin then it accomplishes this in 0.25 minutes. The turnover number is based only on the experimental data, i.e. it contains no assumptions regarding mechanism and its magnitude is independent of that of the specific activity of the labeled amino acid. A turnover number of 0.25 minutes connotes a much faster rate of hemoglobin synthesis in reticulocytes than any hitherto reported. It corresponds to a little less than 0.1 seconds per peptide bond (not taking into account the time required to synthesize 1 heme molecule per peptide). In order to obtain these values it was necessary to determine the specific activity of the free (pool) leucine within the cell.

A deficiency of iron retards protein synthesis greatly; in this condition the labeling of the ribosome decreases to about 40% of the value at full activity. This was an unexpected finding. Iron is a component of the heme of hemoglobin and it was to be expected that a deficiency of heme, because of a lack of iron, might retard release of the completed globin peptides from the ribosomes. This is probably the case; but the finding of a reduction to 40% of the labeling of the ribosomes indicates that iron is required also for "loading" of the ribosomes.

One can calculate from the amino acid sequence of globin an "expected" degree of incomplete labeling with leucine as a consequence of failure of incorporation of any other amino acid. On this basis it was expected that tryptamine, which blocks activation and hence incorporation of tryptophan, would reduce the labeling of the ribosomes by leucine to 20% of the full value. This was found to be the case. Such studies are being extended. The information obtained is, it is felt, necessary to a choice of experimental conditions for the isolation of the compounds (peptides probably) in which the labeled amino acids are incorporated on ribosomes.

One needs to know the nature of the bond by which intermediates in protein synthesis are attached to ribosomes in order to proceed rationally in the devising of a method for the isolation of such intermediates. It has been found that they are attached to the protein of the ribosomes so firmly that most (if not all) are not released by treatment which disintegrates the RNA. The determination of the nature of the bond is of central importance in any hypothesis of protein synthesis which implicates ribosomes.

17. PROTEIN SYNTHESIS IN A MIXED SYSTEM: COLI S-RNA AND RABBIT RETICULOCEYTE MICROSONES

Investigators: Hildegard Lamfrom, Richard Squires
Support: National Institutes of Health, Public Health Service

In the currently accepted scheme of protein synthesis four experimentally identifiable steps are visualized:

1. The coupling of a specific amino acid to a specific RNA (S-RNA)
2. The transfer of the amino acid from the S-RNA to the microsome
3. The condensation of amino acids into peptides on the microsome
4. The release of the completed protein from the microsome into the soluble phase.

An experiment was done to determine whether coli S-RNA, when charged with amino acids, could act as a donor of amino acids to reticulocyte microsomes, and if so whether the protein which was made was hemoglobin. It was found that coli S-RNA could transfer amino acids to reticulocyte microsomes with the same
efficiency as reticulocyte S-RNA. However, while with the reticulocyte S-RNA the incorporation of amino acids was primarily into the rabbit major hemoglobin component, with coli S-RNA as the donor the amino acid incorporation was mainly into protein other than the rabbit major hemoglobin.

One can attempt to interpret these observations in the light of Crick's adapter hypothesis proposed for step 2 above.

This hypothesis postulates that a particular amino acid would be inserted in the appropriate place on the microsomal template due to base pairing between a region of its specific S-RNA and a portion of the microsomal template. Only if the S-RNA and the microsomal template had the same code would the amino acids be placed properly in the peptide sequence.

In a cell-free system where both S-RNA and microsomes are chosen from the same cell, as for example from the rabbit reticulocyte, it is not unexpected that the codes should match and the correct protein is synthesized. It is not even too surprising that the S-RNA can be substituted from another tissue of the same animal, such as from the rabbit liver, and still the codes seem to be the same, since hemoglobin is synthesized.

However, the RNA code in unrelated organisms need not be the same. The code is apparently different in coli and rabbit reticulocytes. While some kind of base pairing must have been possible as evidenced by the transfer of amino acids from coli S-RNA to reticulocyte microsomes, the amino acids were probably not placed in the correct position on the template due to the difference in code, and the resulting sequence of amino acids in the newly synthesized protein was not that of rabbit hemoglobin.

Reference:


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

Investigators: Hildegard Lamfrom, Douglas K. Stewart, Leland Hartwell, Miriam Wright, Alexander Miller
Support: National Institutes of Health, Public Health Service

In reticulocyte cell-free systems containing three cellular components--microsomes, pH 5 fraction and supernatant--radioactive amino acids are incorporated into a soluble protein fraction. This labeled protein has chromatographic properties corresponding to the hemoglobin of the cell from which the microsomes and supernatant are obtained. Previous results indicated that a supernatant fraction obtained from reticulocytes was absolutely required for the release of soluble proteins. However, experiments in which rabbit and sheep reticulocyte components were combined clearly indicated that in those experiments in which a heterologous supernatant fraction was used, a protein was formed which did not correspond to the hemoglobin of the cells from which the microsomes were obtained. Indeed, this protein could not be distinguished from the hemoglobin corresponding to the heterologous supernatant.

As an extension of these experiments, cellular components were prepared from reticulocytes of rabbits and ducks and incubated in various combinations with C14 amino acids under appropriate conditions. The newly formed protein was separated from the microsomes and subjected to starch electrophoresis in the presence of both duck and rabbit carrier hemoglobin. When either duck or rabbit microsomes
were incubated with supernatant from the heterologous species, an appreciable amount of protein was labeled which did not correspond in electrophoretic behavior to the microsomal hemoglobin. Furthermore, this effect was intensified by the addition of pH 5 fraction corresponding to the supernatant.

The nature of the new protein formed in such mixed systems, the bulk of which does not correspond to the major hemoglobin component of either species, is now under investigation. Two possible explanations for this which are being considered are: a) Duck-rabbit hybrid hemoglobins are being formed under the conditions of incubation; b) A protein of altered sequence is formed under the influence of heterologous supernatant.

Reference:

19. NATURALLY OCCURRING PEPTIDES AND OTHER AMINO ACID DERIVATIVES

Investigator: H. K. Mitchell
Support: National Science Foundation
National Institutes of Health, Public Health Service

Previous work has shown that Drosophila contains high concentrations of peptides and other amino acid derivatives and some more recent observations have shown that similar substances occur in rabbit brain tissue and in Neurospora but in much lower concentrations. The continued program is concerned with the problems of isolation, identification, biosynthesis and metabolism of these substances especially in relation to their role in protein synthesis and protein metabolism.

Techniques of extraction avoiding changes (enzymatic and otherwise) have been refined as have techniques for fractionation. Several successive ion exchange systems have been found to be necessary for purification of components of the complex mixture in Drosophila.

As a basis for further metabolic studies of peptides and proteins in Drosophila an extensive study was made of changes in alkaline phosphatase and total proteins at all stages of development during the life cycle of Drosophila. Cyclical changes in enzyme activity and protein stability have been observed corresponding to hatching, molting and pupation.

Discussions summarizing the work with Drosophila were held at the Free Amino Acid Conference at the City of Hope in May, 1961. Two papers will be published in the Conference collection by Elsevier Press, 1961.

20. TYROSINE-O-PHOSPHATE, A NEW NATURALLY OCCURRING PHOSPHATE ESTER

Investigator: H. K. Mitchell
Support: National Science Foundation
National Institutes of Health, Public Health Service

Most of the known phosphate esters and anhydrides have been found to have fundamental biological functions, and early observations on the existence of tyrosine-O-phosphate in Drosophila have received further attention.
Isolation of tyrosine-O-phosphate by means of ion exchange chromatography on Dowex-50 and Dowex-1 followed by crystallization from aqueous ethanol has been achieved and the product compared with the synthetic compound obtained from direct phosphorylation of tyrosine by heating with phosphorus pentoxide and phosphoric acid. The products are identical by several physical criteria. The U.V. absorption spectrum of tyrosine-O-phosphate is similar in shape to that of tyrosine but its maximum is at 10 mμ shorter wave length and its extinction coefficient is about 60% that of tyrosine.

In wild-type Drosophila both tyrosine and the phosphate ester exist in the free state while the mutant lethal translucida contains only the ester in third instar larvae. Wild type appears to contain also bound forms of the ester. Tyrosine-O-phosphate has not been found in rabbit brain or Neurospora but it probably exists in mosquitoes.

Publication:


21. HYDROXAMIC ACID-FORMING COMPOUNDS IN DROSOPHILA

Investigator: Tom Cole
Support: Nutrition Foundation
General Funds

The question of peptide intermediates in protein synthesis is now being seriously entertained in several laboratories. A significant number of nucleotide-peptides have been reported, but the type of linkage and the purity of the compounds are not well defined.

In Drosophila pupae and eggs there are compounds which are soluble in cold 50% ethanol and will form hydroxamic acids upon treatment with hydroxylamine. They can be separated on a Dowex-1 column. The techniques of column chromatography and continuous-flow electrophoresis are being used for further purification. Efforts are taken to keep the temperature below 40°C and at a mild acid pH. These are the best conditions for preserving the model compound, which is a phosphoanhydride linkage between a peptide and a nucleotide.

22. ERYTHROPOIETIC FACTOR

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

The term erthropoietin is becoming more and more the accepted name for the circulating erythropoietic factor or factors. This substance appears to regulate the level of red cells and hemoglobin in blood. When injected into normal animals of many species, it produces a polycythemia, with increased total red cell mass, hematocrit, red cell count and hemoglobin. The extra red cells cannot be distinguished from other normal red cells. None of the known antianemic agents can produce such increases in normal animals.

Erythropoietin can be found in the plasma of anemic animals, rabbits, rats, sheep, etc. It can be found in hypoxic animals, in the plasma of anemic humans, and in the urine of severely anemic animals. Active materials from all sources produce similar effects when injected in test animals. For convenience, assays are usually made with rats or mice, but other species respond.
With the collaboration of Dr. Elizabeth Russell of the Jackson Memorial Laboratory, tests have been run in closely related normal and genetically anemic mice. The anemic mice fail to respond to doses of erythropoietin many times greater than doses which are effective in their normal littermates. But anemic mice do respond to lowered atmospheric pressure with a typical increase of the red cells and hemoglobin content.

Red cell producing tissue from normal mice can be grafted on the anemic littermates, and this grafted tissue, in the anemic hosts, responds to erythropoietin. The experiments raise many questions. For instance, erythropoietin may not be the only stimulus to increased erythropoiesis, or erythropoietin and oxygen each may operate on different stages of erythropoiesis, if not through different mechanisms. Further exploration of these results may throw light on these questions.

Through the Childrens Hospital of Los Angeles with the help of Dr. Denman Hammond, the program of isolating erythropoietin from the urine of anemic patients is continuing.

Erythropoietin can be isolated from anemic rabbit plasma in good reproducible yield and specific activity. A 500 to 1,000 fold concentration of the plasma protein is obtained by ethanol and ion exchange column fractionations.

At this stage of purification it is a homogeneous appearing acid glycoprotein. The similarity in yield and physical properties of inactive isolates from normal plasma makes it likely that the active moiety is a small portion of the glycoprotein.

The material is heat stable in aqueous solution. Activity is destroyed by proteolytic enzymes or neuraminidase, indicating that an intact glycoprotein is necessary for activity.

It remains to be seen whether the active component is glycoprotein or whether glycoprotein serves as stabilizer of a more labile material.

The active material is extractable into the aqueous phase of a two-layer phenol water system. This observation has led us to the use of small amounts of phenol in column buffers, precipitants and dialyzing solutions.

Considerable and erratic losses of activity in fractionation of urines was found. The use of as little as 0.1% phenol now enables recovery of over 50% of the activity present in urine before fractionation.

Publication:


23. TRIPOLYPHOSPHATASE IN NEUROSPORA

Investigators: Alexander Miller, Frances Hardin
Support: National Institutes of Health, Public Health Service

It has been previously shown that the concentration of inorganic polyphosphate in Neurospora can be caused to vary widely as a function of the incubation medium. Furthermore, evidence has been obtained which suggests that the polyphosphate, even
under conditions of maximum accumulation, is present in the free form in the cyto-
plasm. The preliminary observation that polyphosphate is localized in cell walls has 
been analyzed in detail and shown to be an artifact resulting from adsorption of poly-
phosphate on the external surface of the cell wall.

This therefore led to consideration of mechanisms whereby enzymes leading 
to the synthesis or degradation of polyphosphates were influenced by growth conditions. 
The possibility that enzymes hydrolyzing polyphosphates decreased, leading to ac-
cumulation, was investigated.

A soluble polyphosphatase was found in Neurospora which hydrolyzed tri-
opolyphosphate but not pyrophosphate or longer chain polyphosphates. However, this 
enzyme remained constant in specific activity under a variety of conditions where the 
intracellular polyphosphate concentration was greatly different. Yet to be analyzed 
are enzymes degrading long-chain polyphosphates and those involved in synthesis of 
polyphosphate; this work is being pursued by F. M. Harold, National Jewish Hospital, 
Denver, Colorado.

References:

A. Miller, F. M. Harold. Biology 1958, No. 8, p. 16.
F. M. Harold. Unpublished observations.

24. FROG URICASE IN EMBRYOGENY

Investigator: Dennis Barrett
Support: National Institutes of Health, Public Health Service

Studies of the changes in concentration of the enzyme uricase in frog de-
velopment, which were begun last year on the European grass frog, have been con-
tinued this year on the bullfrog. The aim is to discover the mechanisms involved 
in the regulation of enzyme concentration.

Two suggested mechanisms have been ruled out. Since uricase first appears 
in demonstrable quantity at the stage when the larva first ingests food, induction by 
some foodstuff was suspected. The pattern of uricase development, however, was 
found quite parallel with normal, in completely starved animals. Secondly, on sus-
picion of a link with hormonal control uricase was assayed in a series of animals in-
duced by thyroxin to metamorphose prematurely. Instead of speeding the rise of 
uricase concentration to normal metamorphosis level, the treatment caused a de-
crease in uricase.

Hence the embryonic pattern of uricase development is directly bound 
neither to an exogenous inducer, to food availability, nor to morphologic age.

25. ENZYMOLOGY OF FROG URICASE

Investigator: Dennis Barrett
Support: National Institutes of Health, Public Health Service

The enzyme has been prepared from frog liver with a 2,500-fold purification 
over the liver homogenate, or 240-fold over the acetone particle stage. The frog
enzyme has been found to be very similar to that isolated from swine by Mahler, and many of the steps in the swine isolation procedure have been useful.

The enzyme has been found in particles which sediment just above the mitochondria. Thus a preliminary centrifugal fractionation to free the uricase particles from the bulk of mitochondria has been a valuable addition to the procedure.

The impure enzyme shows two interesting properties: it is slowly inactivated during the catalytic process, though it is stable in the absence of substrate; and it is activated by heat, gaining 50% activity in one hour at 60°C. Both effects disappear after the last few purification steps.

A new assay for uricase is based on capturing and counting $\text{C}^{14}_2$, released from uric acid-6-$\text{C}^{14}$, as the enzyme decarboxylates at the 6 position. If the method proves reliable, it should be 100 to 1,000 times more sensitive than the present spectrophotometric assay, and should fill in the picture of uricase concentration in the stages before its activity is now detectable.

A dialyzable, rather chemically stable, non-competitive inhibitor of in vitro uricase activity has been discovered in unfertilized eggs, and it is being compared with similar activities in the liver, kidney and pancreas. The developmental significance of the inhibitor in vivo and in embryogeny will be studied.

26. EFFECTS ON A BACILLUS OF CONTINUED MAINTENANCE IN THE LOGARITHMIC PHASE OF GROWTH

Investigators: R. L. Heath, H. K. Mitchell
Support: National Institutes of Health, Public Health Service
General Funds

In connection with studies on endospore formation in B. megatherium, it was attempted to obtain a continuously maintained spore-free vegetative cell population by means of the Chemostat. The Chemostat is an apparatus for automatically maintaining a growing culture at a constant cell population and in a homogeneous physiological state in a given volume of medium by constantly feeding fresh growth medium and drawing off excess cell suspension. When an essentially spore-free cell suspension of B. megatherium was placed in the chemostat and maintained at $5 \times 10^7$ cells per ml on minimal medium (sucrose-inorganic salts), the heat-resistant spore count increased gradually until after 3 days the spore count was 20% of the total count. When the total population was maintained at $1 \times 10^7$ or less per ml, the spore count never exceeded 1% of the total count. When a complex medium was used, even at $2 \times 10^8$ cells per ml, the spore count increased only from $10^1$ to $10^4$ per ml during the first two days and remained constant thereafter. The nature of the changes occurring in minimal medium at high cell population may give some insight into the initiation of the sporulation process.

The effects of repeated transfer of part of a growing culture of B. megatherium to a flask of fresh growth medium were also studied. Using minimal medium, with a maximum cell population of $10^8$ per ml and a 10-fold dilution at each transfer, cultures showed a marked decrease or complete cessation of growth after 5 to 7 transfers, accompanied by extensive cell death; after several hours, the cultures resumed growth and grew uninterruptedly thereafter. There is no such variation in growth rate in complex medium. The results may reflect a hitherto unreported nutritional requirement of B. megatherium exhibited only after prolonged growth in a deficient medium.
27. THE ACTIVATION OF SULFHYDRYL GROUPS BY VITAMIN B_{12} COENZYMES

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

The aging of E. coli mutant 113-3 cells resulted in a general decrease in enzyme activity. The addition of vitamin B_{12} to these cells increased enzyme activity. These changes were correlated with changes in reactivity of the total protein sulfhydryl groups of the cell. Two coenzyme forms of the vitamin were isolated which increased the activity of cell-free preparations of glucose-6-phosphate dehydrogenase, but this system was too crude for a study of the corresponding changes in the activity of the sulfhydryl groups of the enzyme.

A study was undertaken of the effect of aging on the sulfhydryl groups of pure proteins. It was found that the reactivity of the sulfhydryl group of crystalline human serum albumin in solution became less reactive over a period of months. Addition of the coenzyme form of the vitamin increased the reactivity of the sulfhydryl group. This is the simplest system in which an effect of a B_{12} coenzyme has been demonstrated and is evidence for one mode of action of vitamin B_{12}. The same reaction has been demonstrated in aged whole human serum and suggests the possibility of an assay for coenzyme B_{12} deficiency in pathological conditions.

28. EFFECTS OF ERYTHROPOIESIS STIMULATING FACTOR (ESF) AT DIFFERENT TIME INTERVALS AFTER SINGLE INJECTION

Investigator: G. Hodgson
Support: National Academy of Sciences
American Cancer Society

The effects of ESF on uptake of C^{14}-labeled amino acids and Fe^{59} into hemoglobin and on reticulocyte levels of normal mice were studied as a function of time after single intravenous injection. The first change observed was a marked increase of C^{14} amino acid uptake into hemoglobin at 24 hours, followed by increased consumption by erythroid tissue at 48 hours, and an increased reticulocyte count in the peripheral blood at 72 hours.

Publication:


29. STUDIES ON RIBOSOMAL RIBONUCLEIC ACID FROM RETICULOCYTES

Investigator: T. A. Sundararajan

A new method for the isolation of ribosomal RNA from rabbit reticulocytes was developed. The method was based on the use of low concentration (0.1%) sodium dodecyl sulfate for the dissociation of the ribosome and aggregating the protein in the presence of 1M sodium chloride. Following the removal of the protein by low
NATURE OF THE LONG CHAIN ALCOHOL IN CHLOROPHYLL

Investigators: A. W. Noltes, James B. Boyd
Support: National Institutes of Health, Public Health Service
National Academy of Sciences

Isoprene type compounds are of general occurrence in plants and animals and many have important physiological functions. The molecule of the green leaf pigment chlorophyll contains a diterpene type alcohol. Present day analytical techniques would for the first time seem capable of differentiating among phytol, its isomers or other closely related long chain alcohols. It was therefore of interest to determine whether phytol is the only long chain fatty alcohol occurring in chlorophyll or others serve the same function.

Up to now chlorophylls from three different types of plants have been investigated. They include Cystoseira osmundacea, a marine alga collected at Corona del Mar; Salicornua ambiguа ("pickleweed"), obtained at Badwater, Death Valley; and Euphorbia canadensis from Huntington Gardens, Pasadena.

The plant material was rapidly chopped up, pigments extracted and immediately chromatographed on powdered sugar columns according to known methods. Chlorophylls were isolated and subjected to mild alkaline hydrolysis, which afforded the corresponding long chain alcohols. Thin layer chromatography on silicic acid plates and gas chromatography were used as analytical methods. Silicone gum SE-30 and chromosorb W at 225° and diethylene glycol polyester on base treated celite at 205° afforded good separations of phytol and isophytol, whereas on several other media dehydration occurred. So far no indications have been obtained as to the presence of long chain alcohols different from phytol in any of the above plant materials.
RESEARCH UPON BACTERIOPHAGE ØX-174

Investigators: Robert L. Sinsheimer, Carolyn Nagler, Shirley Guthrie
Support: National Institutes of Health, Public Health Service
Commonwealth Fund

Research has continued upon the fate of the DNA of bacteriophage ØX-174 during infection of susceptible cells. With the use of radioactive (P³²), density-labeled (N¹⁵, or O + N¹⁵) parental bacteriophage it has been shown that the parental DNA is converted to an altered form, called a "replicative form" (RF). This form was initially characterized by the fact that it bands at a lighter density than ØX DNA in a cesium chloride density gradient.

The RF of ØX DNA is infective in the protoplast system. The amount of RF within the infected cell increases manifol during infection. At no time during infection is there evidence of a pool of free single-stranded DNA. Single-stranded DNA is apparently built into the progeny particles as rapidly as it is formed.

If the infection is carried out in the presence of chloramphenicol, no progeny phage are formed. No pool of single-strand DNA can be found. RF is formed from the parental DNA and again increases manyfold, albeit more slowly than in normal cells. Evidently extensive protein synthesis is not necessary for formation or multiplication of RF.

Upon heating, the RF increases in buoyant density (analogous to the increase in density upon denaturation of double-strand DNA); some single-strand parental DNA is released. RF is also 8 to 10 times as resistant as single-strand ØX DNA to ultraviolet inactivation (by germicidal lamp); this resistance can be melted out by heating.

THE PROTEIN SUBUNIT OF BACTERIOPHAGE ØX-174

Investigator: Edward A. Carusi
Support: National Institutes of Health, Public Health Service

It is currently believed that the genetic material DNA functions by specifying either directly or indirectly the linear sequence of amino acids within proteins. One
approach to testing this belief is to demonstrate a correlation between the arrangement of nucleotides within a particular species of DNA and the arrangement of amino acids in a protein which the DNA specifies.

Because of its relative simplicity, bacteriophage $\phi X-174$ appears to be well-suited to this purpose, since each virus particle contains one molecule of single-stranded, relatively low molecular weight DNA, and a "coat" consisting of a large number of reasonably low molecular weight protein subunits.

When subjected to suitable, mild conditions, $\phi X$ particles disintegrate, liberating free DNA and protein into solution. These can be separated from each other by selective precipitation of the protein. The purified protein was subjected to physical and chemical investigation by the methods of sedimentation equilibrium and amino acid analysis. Because of a marked tendency of the protein subunits to aggregate in the usual aqueous solvents, it was necessary to carry out physical measurements in a three-component system containing a dispersing agent in addition to protein and buffer salts. Despite the difficulties of interpreting three-component systems, it could be demonstrated that the protein coat consists of physically identical subunits having molecular weights of 26,000. There are thus approximately 180 subunits arranged on the surface of each virus particle. The spatial arrangement of these subunits is most probably that of an icosahedron, based on geometric considerations and evidence from electron microscopy.

The amino acid composition of $\phi X$ protein has been determined using standard column procedures. The molecular weight calculated from the constituent 213 amino acids is in excellent agreement with the weight obtained by physical methods. Since the number of amino acids is reasonably small, sequence determination of $\phi X$ protein seems feasible.

37. NUCLEOTIDE SEQUENCES IN DNA FROM $\phi X-174$

Investigator: J. B. Hall
Support: National Institutes of Health, Public Health Service

Genetic information is presently believed to be carried by molecules of deoxyribonucleic acid. It is thought that the DNA carries this information in the form of a code consisting of certain sequences of nucleotides. The nature of this code is largely unknown.

The DNA of the bacteriophage $\phi X-174$ is an exceptionally good subject for the study of this coding problem, since it apparently consists of a single molecular species which encodes the information for the production of this relatively simple virus. The problem has been further simplified by the degradation of the DNA to apurinic acid, which leaves only the sequences of pyrimidine nucleotides intact. These can then be separated by chromatography on a DEAE column by a procedure which appears to fractionate them into groups according to the length of the oligonucleotides. The longer oligonucleotides, those containing 10 or 11 nucleotide subunits or more, are of particular interest.

Present work is directed towards obtaining $\phi X$ DNA highly labeled with $^{14}C$ in the pyrimidines. This is necessary because of the small amount of bacteriophage DNA available.
38. STUDIES OF THE DEOXYRIBONUCLEIC ACID FROM THE BACTERIOPHAGE φX-174

Investigator: Alice Burton
Support: National Institutes of Health, Public Health Service

From the bacteriophage φX-174, deoxyribonucleic acid has been prepared which is single-stranded, rather than having the more usual double-stranded form. However, during its replication, the phage DNA passes through a stage in which its physical properties are markedly different from those of the single-stranded form. The isolation and purification of this replicative factor is in progress. When sufficient material is available, the chemistry of this factor will be investigated.

39. THE RELATIONSHIP BETWEEN CHEMICAL STRUCTURE AND BIOLOGICAL ACTIVITY IN THE BACTERIOPHAGE φX-174 DEOXYRIBONUCLEIC ACID

Investigator: Walter Fiers
Support: Rockefeller Foundation
National Institutes of Health, Public Health Service

The availability of almost homogeneous φX-174 DNA and of a method for evaluating its infectivity offers a convenient possibility to study the relationship between chemical structure and biological activity.

In principle, a limited and controllable degradation of a DNA chain can be obtained by means of exopolynucleotidases such as the Escherichia coli phosphodiesterase. This enzyme attacks only the 3'-hydroxyl end of single stranded DNA chains and results in the step by step liberation of 5'-deoxyribonucleotides. The acid solubility of the latter offers a basis for assaying the enzymic activity.

It was found that about 20% of a φX DNA preparation can be readily hydrolyzed by the E. coli diesterase. This, however, is due to the complete degradation of some of the chains, while the bulk remains intact. These results may be explained either on the basis of an undissociable enzyme-substrate complex or on the assumption that about 80% of the DNA chains bear a block on the 3'-hydroxyl end which protects them from the enzymic attack. The latter explanation proved to be correct.

The nature of the block remains to be elucidated.

40. DEAMINATION STUDIES WITH NUCLEOTIDES AND NUCLEIC ACIDS

Investigator: Homer Jacobson
Support: John Simon Guggenheim Memorial Foundation
National Institutes of Health, Public Health Service
General Funds

Studies of rates of deamination (by nitrous acid) of the bases in nucleic acids give a chemical "target size" and allow biological evaluation of the effects of a clean-cut chemical reaction of known speed, when compared with the rates of the corresponding biological events, i.e. inactivation and mutation. Moreover, detailed knowledge of the separate values of the purine and pyrimidine base deamination rates allows some judgment as to which of the base deaminations are responsible for a given mutation, group of mutations, or inactivation.
Control system for providing a constant concentration and pH of the unstable HNO₂, assay methods for measuring deamination extent by two-dimensional paper chromatography of hydrolyzed radioactive nucleic acids with isotopic nucleotide diluents, and isolation methods for the nucleotides of φX-174 bacteriophage whole virus after deamination, have been worked out. Rates of reaction for the pure deoxy-nucleotides of adenine cytosine and guanine, and for all three deaminable bases in whole φX-174 virus and in free viral DNA have been measured.

The reaction rate for deoxycytidine-5'-phosphate (and, presumably the rate for deamination of any such compound) was found proportional neither to the concentrations of H⁺ nor of NO₂⁻ in such systems. At high (H⁺), and also at low (NO₂⁻), there is a less-than-proportional increase and more-than-proportional decrease in the rate, respectively. This is understandably due to partial tieup of the deaminating HNO₂ into increasing proportions of inactive NO, NO₂, and HNO₃, under these conditions. Consequently, caution must be exercised when comparing deamination rates reported in various laboratories under varying conditions. The NO₂⁻ concentration effect has been confirmed with the inactivation of φX-174 whole virus, which is similarly non-linear in (NO₂⁻) at constant pH.

Reaction rates of free nucleotides have been found to be about tenfold as great as when bound into φX-174 virus; free φX-174 DNA, at this ionic strength, deaminates about twice as fast as DNA in virus. Adenylic acid is the slowest-deaminating nucleotide in the virus, although the fastest in the free state. Comparison with loss of biological activity shows that, both for free and viral DNA, there cannot be much more than a single deamination per particle inactivated, i.e. most deaminations of φX-174 are lethal. Data scatter do not allow a lower limit for the number of deaminations per lethal hit per particle (although it cannot be less than 1.0), but make more than 1.5 hits improbable. Thus at least two-thirds of the genome chain in this organism is "essential" in the sense that replacement of any of these bases by their deaminated equivalents is lethal.

### 41. SOME STUDIES ON THE INTRACELLULAR GROWTH OF φX-174

**Investigator:** David T. Denhardt  
**Support:**  
National Science Foundation  
National Institutes of Health, Public Health Service  
General Funds

In order to study precisely the growth characteristics of the phage φX-174 in the infected bacterium E. coli C it is necessary to have the development of the virus synchronized in all the infected bacteria in the culture. This insures that all of the infected complexes will be at the same stage at the same time. The first technique used was to add the phage to the bacteria in the presence of cyanide (KCN, 0.003M). This permits adsorption, but not production of mature phage. Dilution into fresh broth allows development to occur synchronously in the infected complexes. The criterion for synchronization was a rapid increase in the phage titer, ranging over 4 factors of 10, and reaching the final burst size within a period of 5 minutes. There was an eclipse phase (when no whole phage can be found inside the cell) of about 8 minutes; whole phage appeared in the infected cell by the 9th minute after dilution away from cyanide. It was found, both by banding the DNA in a cesium chloride density gradient and by a characteristic decrease in the sensitivity of the complex to ultraviolet light, that some development could occur in the presence of cyanide after 10 to 15 minutes; the single strand of phage DNA assumed a less dense, more ultraviolet (UV) resistant form called RF (replicating factor) and thought to be double-stranded DNA.
With the goal of preventing formation of RF, synchronization was attempted using starved bacteria. The cells were aerated for one hour in buffer, then phage was added. By 10 minutes 99% of the phage had disappeared and gone into eclipse; similar behavior was observed when cyanide was used to synchronize. Absence of the characteristic band in the density gradient and failure of the UV sensitivity to decrease led to the conclusion that RF was not formed after 20 minutes incubation of the infected complex in buffer. Addition of broth permitted growth to begin: the UV sensitivity decreased by a factor of about two, but remained single-hit, during the first 2 minutes; and whole phage began to appear inside the cell after 10 minutes. It thus appears that it takes about 1 minute to form RF at 37°C.

The UV studies were done in Luria-Latarjet type experiments where the UV sensitivity of the infected complex is studied at various stages of its development. Between 4 and 8 minutes after growth starts the number of "targets" in the cell that must be "hit" to destroy the cell's ability to produce whole phage increases from 1 to about 20. Multiplicity reactivation could not be demonstrated, probably because of damage to the cell. Damage to the bacterial cell's capacity (ability to produce phage) is suspected both because the initial slope of the multi-hit survival curves is steeper than expected from target theory and because the difference in UV sensitivity between single-stranded phage DNA and RF in vitro is much greater than the difference observed here. The limiting slope of the multi-hit survival curves indicates that at least one of the targets is RF.

42. STUDIES ON φX DNA DURING REPLICATION

Investigator: Anne Haywood
Support: American Cancer Society

The question of whether a pool of φX DNA is formed during infection of a bacterium arises in considering the mechanism of φX replication. Since as yet it is impossible to separate a "replicative factor" (RF) by chemical methods, a protoplast assay was used to define the amount of DNA present in a cell lysate. This assay measures only the free φX DNA present and will not measure the DNA of whole phage unless the protein coat is removed by phenol. One portion of a cell lysate was treated with phenol, and the other portion was brought to a density of 1.70 with cesium chloride and centrifuged to separate the free DNA from the whole phage. Both these cell lysates were assayed for φX DNA at every other minute during the course of infection.

When cells are infected with φX at a multiplicity of 0.1, the assay of phenol-treated DNA stays approximately constant (1 to 2 per infective center) until 15 minutes after infection. At that time, which coincides with the initiation of production of intracellular phage, there is a sharp rise in the assay of phenol treated DNA. The centrifuged DNA, initially very low, increases rapidly in amount until between 7 and 9 minutes where it catches up with the phenol-treated. During the next 4 minutes the amount of centrifuged DNA about equals the amount of the phenol-treated DNA and increases only slowly. After 11 to 13 minutes, it again increases more rapidly in amount.

When a multiplicity of 5 is used, the pattern is approximately the same except that at 7 minutes there are 5 to 10 DNA particles per infective center.

Some evidence suggests that RF infects protoplasts with a different efficiency than does the mature phage DNA, which is used as the standard for the assay.
43. CONTINUED INVESTIGATIONS ON THE PROTOPLAST-SUBVIRAL PARTICLE SYSTEM

Investigators: George Guthrie, Thomas Creighton
Support: National Institutes of Health, Public Health Service
American Cancer Society

The basic method for the assay of subviral particles, reported in Biology 1960, has since been published. These data demonstrated that DNA molecules as well as subviral particles from the bacteriophage \( \Phi X-174 \) are biologically active against protoplasts. However, in order to use the protoplast system effectively in investigating the replication of \( \Phi X \) DNA, it has been necessary to characterize more completely the optimum conditions to be used in these experiments.

Investigations have been carried out to try to establish the optimum conditions for the particular experiments of interest. Such factors as salt concentration during infection as well as the type of salt have proved important. Similar studies will continue as long as the uses of the system demand it.

In addition to this a critical investigation of the multiplication of DNA in the infected protoplasts themselves has been initiated. This work is in its earliest stages with the primary concern being the development of new techniques for the recovery and assay of the developing intra-protoplast viral nucleic acid.

Reference:

44. SEQUENCE STUDIES ON TMV RNA

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

The isolated RNA moiety of tobacco mosaic virus is a monostranded macromolecule made up of approximately 1,600 adenylic-, 1,700 guanylic-, 1,100 cytidylic-, and 1,600 uridylic-acid residues. The unknown sequence of these 6,000 nucleotides determines the genetic information carried by this viral RNA. Experiments are underway to gain some information on this sequence. This is being attempted by splitting the TMV RNA with a RNase highly specific for the secondary phosphodiester linkages of the 3'-guanylic-acid residues, followed by isolation of the oligonucleotides thus obtained according to their size and nucleotide sequence.

45. STUDIES ON THE IODINATION OF TOBACCO MOSAIC VIRUS NUCLEIC ACID

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

TMV RNA absorbs iodine in the presence of sodium bicarbonate, and this absorption results in inactivation of its biological activity. Incubation of TMV RNA with iodine-sodium bicarbonate mixture for one hour at 35°C is sufficient for 95% to 98% inactivation. Without the presence of sodium bicarbonate, there is no absorption of iodine by TMV RNA, and correspondingly no inactivation. The mere presence of iodine even slows down the spontaneous degradation of TMV RNA in solution. The fact has been demonstrated not only by the infectivity experiment, but also by studying the ultracentrifuge patterns under schlieren optics. Sodium bicarbonate alone at
the concentration of 0.05M, or even higher at 0.1M, does not inactivate the infectivity of TMV RNA. Inactivation of TMV RNA by iodine can be obtained in the presence of sodium bicarbonate at a concentration not less than 0.01M.

The absorption of iodine by purines and pyrimidines and their nucleotides and nucleosides was studied in the presence of sodium bicarbonate. In the case of uridylic acid and uridine, the absorption of iodine was manifested by a gradual loss of maximal ultraviolet absorption spectrum. However, complete loss of ultraviolet absorption spectrum was not obtained at the concentration of sodium bicarbonate generally used for studies with TMV RNA, 0.05M to 0.1M. At 0.1M sodium bicarbonate, the reduction in absorption is about 50% for 5' uridylic acid, 42% for uridine, and 31% for 2' and 3' uridylic acids. When uracil is incubated in the same manner with iodine and sodium bicarbonate, there is a gradual increase in maximal absorption at 282 m\(\mu\) accompanied by a gradual loss of absorption at 259 m\(\mu\), indicating the formation of iodouracil.

There were no evidences of any change of absorption spectra when other nucleotides and nucleosides were tried in a similar manner. In the case of cytosine, however, there was a shift of maximal absorption and a gradual decrease in maximal absorption.

Preliminary determination on the rate of iodine absorption by TMV RNA was made by measuring the decrease in the absorption due to iodine at 350 m\(\mu\). The result indicated a rapid absorption of iodine by TMV RNA within a 1-hour period at 35\(^\circ\)C, and a leveling-off afterwards. The total amount of iodine absorbed in 6 hours of incubation was approximately 1 mole of iodine per 2 moles of nucleotides of TMV RNA. This would indicate either that each pyrimidine fraction absorbed 1 molecule of iodine per nucleotide, or that uridylic acid absorbed 2 molecules of iodine.

46. PROPERTIES OF DNA FROM THYMINELESS BACTERIA

Investigator: Philip C. Hanawalt
Support: American Cancer Society
National Institutes of Health, Public Health Service

Thymineless bacteria are of interest because they lose viability in a characteristic manner when the specific DNA precursor, thymine, is withheld. Studies with the triple mutant, E. coli 15T-A-U-(thymine, arginine, and uracil requirement) have indicated that protein and/or RNA synthesis is necessary to initiate but not to sustain DNA replication. A culture of this strain in which DNA replication has continued to completion (+T-A-U) does not lose viability when thymine is subsequently removed. Also, such a culture exhibits a striking resistance to inactivation by ultraviolet light, as compared to a normal, exponentially-growing culture.

A detailed study is being made of the physical state of the DNA from bacterial cultures in each of three physiological states defined as follows:

1. A normal culture is balanced, exponential growth (+T+A+U)

2. A culture in which thymineless growth has reduced viability to 1% survival. (2 hours -T+A+U)

3. A culture in which DNA replication has run to completion during protein synthesis and RNA synthesis inhibition. (90 minutes +T -A-U)

The bacteria are opened by lysozyme-versene and depronol, and the nucleic acid is deproteinized with cold phenol; or, to minimize deproteinization, the bacteria
are opened by grinding with alumino in the presence of magnesium ions. No differences in buoyant density in cesium chloride have been found for DNA isolated by either of the above methods from cultures in the three defined states. It is concluded that there is less than a 1% component of DNA-associated protein which is stable in the cesium chloride solution, even under ionic conditions in which the ribosomes are stable. C\textsuperscript{14}-thymine labeled DNA forms a single Gaussian band in the cesium chloride density gradient under the above conditions, indicating density homogeneity.

Present experiments using tritiated-uridine to label RNA are designed to determine the presence and extent of bound RNA in the DNA fraction in the three physiological states. A workable method of assay has been developed which involves the collecting of nucleic acid samples on a microfilter which selectively retains high molecular weight DNA (native or denatured) but passes free RNA. The dried filter is placed directly in the liquid scintillator to give a counting efficiency of about 8% for tritium.

References:


47. INFECTION OF PROTOPLASTS FROM SYNCHRONOUS CULTURES

Investigators: James E. Davis, Lesley H. Menninger
Support: National Science Foundation
National Institutes of Health, Public Health Service

The infection of protoplasts of E. coli with purified DNA from \ensuremath{\Phi}X-174 is not a very efficient process; only about 0.1% of the protoplasts may be infected by DNA. The "competence peaks" observed in the transformation of synchronous cultures of Pneumococcus suggested that a similar improvement in infectibility might be found in protoplasts made from synchronous cultures. Attempts were made to prepare protoplasts from synchronous cultures of E. coli at various stages in the division cycle to see if any differences in infectibility would result.

Good synchronization was obtained by several cycles of mild temperature shock treatments (28 minutes at 25°C followed by 7 minutes at 37°C). Division of 50 to 75% of the cells occurred during the 7 minute period at 37°C during the third and fourth cycles of the treatment.

No significant effects on protoplast infectibility were noted as a function of stage in the division cycle. Further attempts to obtain synchronous cultures by filtration methods were not very successful, and the project has been discontinued.

Reference:

48. LARGE ULTRAVIOLET MONOCHROMATOR

Investigators: H. E. Johns, M. Delbrück
Support: National Foundation
American Cancer Society
Commonwealth Fund
General Funds

Detailed studies of the action of ultraviolet light on biological and chemical systems require a light source which is very intense as well as highly monochromatic. To meet this requirement, a large water prism monochromator has been constructed. Mirrors, rather than lenses, are used throughout in order to avoid chromatic effects inherent in lens systems. The prism consists of a quartz shell filled with water. The monochromator is designed to operate from the visible region down to 2,000 Å with a dispersion of 0.2 mm per Å at 2,300 Å.

49. QUANTUM YIELD FOR THE PRODUCTION OF THYMINE FROM THYMINE DIMERS

Investigators: H. E. Johns, S. Rapaport, M. Delbrück
Support: National Foundation
National Science Foundation
General Funds

Upon ultraviolet irradiation of a frozen solution of thymine, a dimer product is formed. In the frozen state, the thymine molecules are spatially oriented in such a fashion that dimer production is favored. In liquid solution, however, the reverse reaction is favored. The quantum efficiency for the production of thymine from the dimer in liquid solution was studied, using the quartz monochromator. The monochromator was calibrated, using malachite green leucocyanide as a chemical dosimeter. Thymine is assayed spectrophotometrically.

The quantum yield is close to 0.6 from 220 mµ to 289 mµ. The same dimerization reaction was also studied in the thymine dinucleotides TpT, pTpT, and cyclic pTpT, at 275 mµ. The quantum yields for the "dimerization" reaction (i.e., the linking of the two thymines across their carbon atoms 5 and 6) is $3 \times 10^{-3}$ for TpT, $9 \times 10^{-3}$ for pTpT, and $10^{-3}$ for cyclic pTpT. For the reverse reaction the quantum yield at 235 mµ is 0.6 for pTpT.

50. STUDIES ON THE DENATURATION AND RENATURATION OF COLLAGEN MACROMOLECULES

Investigators: Alan J. Hodge, J. H. Fessler, Nancy Martin
Support: National Institutes of Health, Public Health Service

The macromolecules of soluble collagens from various sources show characteristic helix-coil transitions on heating, the transition temperature depending on the particular collagen. The denatured collagen (parent gelatin) is then susceptible to attack by trypsin. Experiments are underway to ascertain by following the optical rotation as a function of temperature and the number of bonds susceptible to tryptic attack at any given temperature (using a pH-stat) whether certain regions of the collagen macromolecule are more easily denatured than others. It is hoped that the process may also be followed by electron microscopic examination of ordered aggregates of segment long spacing (SLS) type, in which the macromolecules are packed side-by-side with all like features in register, i.e. in homoregister.
On thermal denaturation, the three chains of the collagen macromolecule dissociate into two components as seen in the ultracentrifuge. (1) the α component, consisting of a single chain, and (2) the β component, comprising two chains linked together by one or more cross-links of unknown nature. In a small fraction of the macromolecules, the three chains are cross-linked together and behave as a single kinetic unit (the γ component) on denaturation. It has previously been shown that renaturation of intact collagen molecules apparently involves only the latter fraction, which can be fractionated out of the mixture by precipitation as SLS aggregates after cooling. The viscosity and optical rotation recovery rates indicate a strong facilitation or reformation of the polyproline-like helical structure in those molecules having the three polypeptide chains cross-linked, and suggest that reassociation of the α and β components is at best a much slower process and may not occur in a meaningful fashion. The rates of recovery of viscosity and optical rotation are being studied in both systems as a function of temperature. The results may have implications for the renaturation of other multi-strand systems such as DNA, and also for collagen synthesis.

In addition to the physical studies, amino acid analyses will be carried out on the α, β, and γ components to determine whether any significant differences in composition exist. Fingerprinting studies may reveal the nature of the cross-links involved in the β and γ components.

51. STUDIES ON THE HYDRATION CHANGES IN COLLAGEN MACROMOLECULES DURING FIBROGENESIS AND DENATURATION

Investigators: J. H. Fessler, Alan J. Hodge
Support: National Institutes of Health, Public Health Service

When a neutral solution of mammalian collagen is warmed to near body temperature typical striated collagen fibrils are formed, which may be dissolved again on cooling, and definite equilibria can be established. This phenomenon can be interpreted as a change with temperature of the affinities between the collagen macromolecules themselves and with the solvent. In view of recent evidence suggesting that ordered water may play an important role in stabilizing the characteristic helical structure of the polypeptide chains in the collagen molecule and since the packing arrangement of the molecules in the fibrils formed on warming results in a juxtaposition of nonpolar regions, it was felt that this phenomenon might result from a reduction in the hydration of the molecules with increased temperatures, thus allowing the formation of "hydrophobic bonds."

It has recently been shown that the effective hydration of DNA macromolecules can be measured from their buoyant density as determined by density gradient ultracentrifugation, and this technique is being applied to study the buoyant density of the collagen system as a function of temperature. Preliminary results indicate that the buoyant density of the fibrils formed on warming is higher than that of the molecules in solution, a result in agreement with the postulated change in hydration.

The effect of thermal denaturation on the buoyant density of collagen in solution has also been studied. When collagen is denatured to gelatin, the component polypeptide strands are wholly or partly separated and assume a random coil configuration. If structurally bound water is involved in the stabilization of the helical structure of the polypeptide chains, then denaturation should result in a considerable decrease in hydration, with a concomitant increase in the buoyant density. Preliminary results indicate that the buoyant density of gelatin is higher than that of both the collagen macromolecules and the fibrils formed on warming.
52. SIDE-CHAIN DISTRIBUTION IN THE COLLAGEN MACROMOLECULE

Investigator: Alan J. Hodge 
Support: National Institutes of Health, Public Health Service

It has previously been shown by electron microscope studies of the band distribution in crystallites of segment long spacing (SLS) type after staining with phosphotungstic acid (PTA) and with uranyl nitrate that the polar side-chains, both basic and acidic, are located in well-defined clusters or loci separated by relatively long regions, which because of their lack of staining are presumed to consist mostly (or entirely) of nonpolar residues. In the SLS crystalline form, the collagen macromolecules are arranged in parallel fashion, with all like features in register, so that in principle it is possible to determine the distribution of any residue capable of reacting specifically with an "electron stain." The band pattern observed in the electron microscope is thus a "fingerprint" of the macromolecule in terms of any group for which the stain is specific.

In view of the success of the negative contrast technique in elucidating the topography and subunit structure of a number of viruses, and since the polar side-chains are bulky relative to most nonpolar ones, this method was used in an attempt to determine the distribution of the bulky polar side-chains in SLS crystallites. Results so far, using neutralized PTA as the negative contrast agent, show a band-for-band correspondence with the images obtained for SLS crystallites after positive staining with PTA and cationic uranium. It is hoped that appropriate densitometric measurements of the band intensities in these three types of images may yield a more quantitative picture of the distribution of the basic and acidic residues within the staining loci. It may also be possible to determine an approximate electron density distribution for the collagen macromolecule, a result which has not been forthcoming from X-ray diffraction data since the only sufficiently oriented specimens available consist of fibrils in which the collagen macromolecules are arranged in a longitudinally staggered array.

Reference:

53. FRACTIONATION OF NEUTRAL SALT-SOLUBLE COLLAGEN

Investigator: J. H. Fessler 
Support: National Institutes of Health, Public Health Service

Solutions of collagen can be obtained from rapidly growing connective tissue by extraction with buffers at neutral pH, and this "neutral-salt-soluble collagen" is probably material at an early stage of fibrogenesis. Warming causes fiber formation in vitro and the purified material can thereby be separated into three fractions: the fibers of one fraction dissolve again on cooling, those of another do not, and a third fraction only forms fibers at a higher ionic strength. The problem is: what are the differences between these fractions and how are the differences related to the fiber forming properties?

The fractions have been isolated. Their amino acid compositions are being measured. Their ion binding behavior, buoyant densities and solubilities are being studied, and also the variation of these with temperature.
54. ULTRATHIN SECTIONING OF VITRIFIED TISSUE FOR ELECTRON MICROSCOPY

Investigator: David Norman
Support: American Diabetes Association
National Institutes of Health, Public Health Service

A technique for the examination of biological material in the electron microscope which bypasses the need for chemical fixation and supportive medium infiltration has been studied. Using liquid nitrogen as the coolant and liquid propane as the immediate surround, it is possible to transform the water of living cells from liquid to solid state without the apparent formation of crystalline ice. This observation is based on biological criteria rather than physical methods for distinguishing between vitreous and crystalline ice. It is well known that certain kinds of living cells are very sensitive to the usual methods of freezing and will rupture or die as a result of such treatment. In these studies, the continued viability of such cells following immersion at temperatures approximating that of liquid nitrogen strongly suggests the existence of an amorphous or vitreous water state during the low temperature period of the freezing procedure. Using a continuous liquid nitrogen flow system it is possible to maintain the temperature of the specimen to be sectioned at something lower than -150°C and thereby obtain sections with the cell water present.

Attempts to encapsulate such sections with various plastic polymers in order to retain the water during observation in the electron microscope have uniformly failed. These attempts were unsuccessful because of the low temperature which must be maintained during the encapsulation procedure. There remains the possibility of overcoming this obstacle by the use of a previously constructed micro-chamber into which the frozen section can be transferred in the presence of the coolant.

55. STUDIES ON FERRITIN AND HEMOSIDERIN

Investigators: Arne Shoden, Alan J. Hodge
Support: National Institutes of Health, Public Health Service

Ferritin and hemosiderin are two iron compounds found in various tissues, primarily liver, spleen and bone marrow. It is generally believed that they function as a reserve of iron which can be drawn upon when needed in acute blood loss. Ferritin is a water soluble iron protein containing up to 20% iron in the form of six spheres of hydrous ferric oxide located at the apices of a regular octahedron. These iron hydroxide micelles are contained in an envelope of protein (apo-ferritin) which has a molecular weight of 460,000 and a diameter of 105 Å.

Hemosiderin is a granular, insoluble material containing iron, as much as 36%. It was generally believed that hemosiderin formation was a result of rapid and excessive red cell destruction. Present work has shown, however, that hemosiderin has two distinct cellular locations in the liver, Kupffer and parenchymal cells, and that hemosiderin in the parenchymal cells may result from degradation of ferritin, while Kupffer cell hemosiderin may result from various forms of iron engulfed by these cells.

The transition from ferritin to parenchymal hemosiderin is being followed with the help of the electron microscope and also by determination of changes in the amino acid content. Another aspect of the present study involves the possible existence of subunits and also the changes in the amounts of apo-ferritin found in the liver cells as a result of stimulation of apo-ferritin production by injection of small amounts of iron, as well as by heavy iron loading which tend to saturate apo-ferritin with iron.
56. **LIGHTWEIGHT SPACE-FILLING MACROMOLECULAR MODELS**

Investigators: John Petruska, Alan J. Hodge  
Support: National Institutes of Health, Public Health Service

The accurate space-filling atomic models designed by Pauling and Corey are conventionally made as hardwood or elastomer-plastic units with metal fasteners. These units, while possessing many excellent features, are not entirely suitable for building research models of the macromolecular structures found in nucleic acids and proteins. The models turn out to be too heavy and rigid to be manipulated to the degree desired and also too expensive when large numbers of atoms are involved.

In an attempt to overcome these disadvantages a technique for making the atomic models of a durable form plastic has been developed. The technique, in which a quick-setting foaming plastic is poured into easily prepared silicone-rubber molds and allowed to set, is simple and inexpensive enough to be widely used. Atomic models may be cast in single-atom or many-atom units, which can be connected together by a variety of means depending on the bond properties desired.

In one mode of use particularly valuable for protein structure investigations, amino acid residues are connected together by two pin-and-socket connectors assuring planarity of the peptide bond. Side chains, except in the case of pyrrolidines, are connected to the a carbon by a single pin and socket allowing free rotation. By stringing together NH, CH and CO units in the sequence -HN-C=CO-HN-C=CO- and plugging four such strands together at the peptide connectors, any desired number of residues can be placed in a rigid a-helical configuration in which the side chains can be substituted at will.

57. **THE FOLDING OF POLYPEPTIDE CHAINS IN GLOBULAR PROTEINS**

Investigators: John Petruska, Alan J. Hodge  
Support: National Institutes of Health, Public Health Service

Now that the amino acid sequences have been determined in a number of globular proteins, it is of considerable interest to know how the polypeptide chains are folded in these proteins, as the folding seems to play a crucial role in determining the specific activity of the protein in the biological system.

Hopefully, there are some rules to be found governing the relationship between sequence and folding. In search of such rules, a study was made of the folding characteristics of the polypeptide chains in myoglobin, hemoglobin, ribonuclease and TMV protein, whose sequences are completely or nearly completely determined. To simulate the polypeptide chains space-filling models were used built of light-weight (foam plastic) Pauling-Corey type atoms, possessing rotational freedom about single bonds. To arrive at the folding there are employed experimental findings about the structure of some of these proteins and other simpler (fibrous) proteins from X-ray crystallographic analysis and the physical-chemical behavior of the proteins in solution under various conditions.

A detailed correlation has already been made between the sequences in myoglobin and hemoglobin and the folding deduced by X-ray crystallographers. On the basis of this correlation and some known facts regarding the enzymatic activity and its alteration by reagent attack at specific amino-acid residues, a model of ribonuclease has been built showing how the 124-residue polypeptide chain is likely to be folded. In this model the chain is in the form of a coil, a-helical in some places,
that bends back on itself five times. The near-180° bends A, B, C, D and E occur around residues 24, 42, 69, 93 and 114 from the N-terminal end, respectively. Bends B, D and E involve proline residues; while A and C involve concentrations of residues with hydrogen-bonding side chains (serine, threonine, asparagine and glutamine). In the regions B-C and D-E the chain is mostly in a tightly wound, α-helical form (presumably right-handed) whereas in the other regions it is mostly more loosely wound, the looser winding being the result of interactions involving side chains. The active site is considered to involve residues in the regions A-B, B-C and D-E, which lie close together in the model. Studies aimed at refining the model and elucidating the enzyme mechanisms are now under way.

58. INTERPRETATION OF THE SPECTRAL CHANGES ON CHEMICAL SUBSTITUTION IN AROMATIC HYDROCARBONS

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

A quantum-mechanical perturbation theory has been developed for describing how the electronic transitions in aromatic hydrocarbons change in intensity and frequency (or wavelength) as a function of the nature, the number and arrangement of substituents. With this theory it has been possible to derive general formulas for benzene and all other (hypothetical) aromatic cyclic polyenes of type \( C_{4N+2}H_{4N+2} \) (where \( N \) is an integer) and for their cross-linked derivatives of type \( C_{4N+2}H_{2N+4} \) which include naphthalene, azulene, anthracene, phenanthrene, etc. By applying these formulas to observed spectral data certain perturbation parameters have been evaluated for a number of different substituents.

Having obtained the values of these parameters one can do a number of things. One can insert them into the theoretical formulas and predict the spectral changes for various possible arrangements of the substituents. One can also try to interpret them and relate them to other changes accompanying substitution, e.g. changes in ionization potential and chemical reactivity.

An examination of the perturbation parameters in terms of molecular orbital theory has revealed that one of the two parameters (\( \nu \)) evaluated from the frequency shifts is almost a pure measure of the "inductive" effect of substitution, i.e. the change in the electrostatic field acting on the \( \pi \)-orbitals of the carbon framework. The other parameter (\( \zeta \)) also contains a contribution from the "inductive" effect, but for most substituents is principally a measure of the "conjugative" effect, i.e. the interaction of the \( \pi \)-orbitals of the substituent with the \( \pi \)-orbitals of the carbon framework. It is observed that the transition moment parameter (\( q \)) evaluated from the intensity changes in the first two transitions of the aromatic hydrocarbons is almost proportional to \( \nu \) and therefore principally arises from the "inductive" effect. Another observation, that the parameters \( q \) and \( \nu \) correlate quite well with the difference (\( \Delta \sigma \)) between the Hammett's sigma values for meta and para substitution in benzene, indicates that \( \Delta \sigma \) also arises principally from the "inductive" effect.

More comparative and theoretical studies along these lines are under way to gain a deeper understanding of the electronic changes associated with chemical substitution.
References:


II. "Application of Perturbation Theory to Substituted-Benzene Spectra." Ibid. 34:1120.

EMBRYOLOGY

STAFF:
Professor: Albert Tyler
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59. A STUDY ON THE POSSIBILITY OF DISPERMIC ANDROGENESIS IN X-IRRADIATED RAT EGGS

Investigator: Lajos Piko
Support: Lalor Foundation

Androgenesis (development of eggs with only paternal genes) in eggs previously exposed to X-rays is a well-known phenomenon in Amphibia and many other lower animals. Viable androgenetic adults have been produced so far in the wasp Habrobracon and in the silk-worm; in the latter diploidy is achieved through the fusion of two sperm nuclei.

For examination of the possibility of induction of this phenomenon in the rat, where dispermy is not infrequent, the effects of different levels of X-irradiation on fertilization and cleavage were followed. Unmated estrous females were irradiated at 600, 1,200, 2,500, 6,300 and 13,400 r towards the end of estrus when they contained tubal eggs in metaphase II. At 2,500 r, and higher, the anterior half of the body was shielded. The females were mated immediately after irradiation and sacrificed within three days.

A total of 195 eggs, coming from 19 females, was examined by phase microscopy in the living state, and after aceto-carmine staining. Sperm penetration was normal although with the higher doses the proportion of fertilized eggs decreased (to 60% at 13,400 r). Emission of 2nd polar body took place and pronuclear development proceeded nearly normally in all groups. Cleavage was delayed, especially after the higher doses. But the main observable anomaly was nuclear fragmentation at the first and later cleavages. Six dispermic eggs (with 2 sperm tails in the cytoplasm) were observed. Three of these, obtained in the 6,300 and 13,400 r group, were in the 2-cell stage and their nuclei appeared nearly normal. Studies on the later development of such eggs are in progress.

60. OBSERVATIONS ON THE EFFECTS OF HYPERTERMHY ON FERTILIZATION AND CLEAVAGE IN THE RAT

Investigator: Lajos Piko
Support: Lalor Foundation

Hyperthermy (elevation of the body temperature) before and at the time of sperm penetration was reported to increase considerably the chances of polyspermic fertilization in the rat (up to 34% polyspermy in an extreme group); other anomalies, such as cytoplasmic and nuclear fragmentation, occurred at the same time.

Thirty-three females were subjected to hyperthermy during about two hours following delayed mating (i.e., mating towards the end of estrus). The body temperature was raised to 40.5-42.0 °C (normal level about 38.5 °C), either by exposing the animals to infrared irradiation or by putting them into a warm (40.0 to 41.0 °C) waterbath. The animals were later sacrificed and the eggs examined for cytological details by phase microscopy.
In 300 fertilized eggs the incidence of dispermy was practically unchanged (4.7% dispermic eggs as compared to 4.1% in the control group). Cytoplasmic and nuclear fragmentation were observed occasionally. In addition, the higher temperatures frequently caused abnormal spindle formation (multipolar spindles) at first cleavage and suppressed cleavage completely in many eggs. The nature of this inhibitory effect needs further clarification.

These data show that an elevation of the body temperature (in the range of moderate to high fever) leads to abnormalities of fertilization and early development.

Reference:


61. INTRA-TUBAL INJECTIONS FOR AN EXPERIMENTAL STUDY OF FERTILIZATION IN THE RAT

Investigator: Lajos Piko
Support: Lalor Foundation

For studies, in mammals, of effects of changes in the immediate environment of the eggs at the time of fertilization, a method has been developed whereby small amounts of fluid are injected directly into the Fallopian tube by means of a micro-syringe.

The operation is performed usually after ovulation, but before sperm penetration takes place, the Fallopian tube and the ovary being exteriorized through a small dorso-lateral incision.

This method has been used so far to study the effects of a change in the ionic content of the tube. It is to be extended to the study of more specific substances, such as antisera against eggs and sperms, certain enzyme inhibitors. One of the effects noted following use of a chelating agent, EDTA, is the occurrence within the egg of additional sperm that fail to transform into pronuclei.

62. IN VITRO OBSERVATION OF CLEAVAGE OF DISPERMIC RAT EGGS

Investigator: Lajos Piko
Support: Lalor Foundation

Earlier observations indicated that dispermic rat eggs cleave normally and result in triploid embryos. For further exploration of the cleavage behavior of dispermic eggs attempts were made to culture rat eggs in vitro after examination by phase microscopy.

Fertilized eggs were collected from 22 female rats of the "Sherman" strain which had been mated towards the end of estrus (about two hours after the average time of ovulation). Six of the females were subjected to a moderate hyperthermia (body temperature raised to 40-41 °C during about two hours after mating) in order to increase the frequency of polyspermy. In cleaved eggs the evidence of dispermy was based on the presence of two sperm tails within the egg cytoplasm.

A total of 128 eggs, 8 of them dispermic, in the 1-, 2- and 4-cell stage was cultured at 38°C in a variety of media (mostly in a phosphate-bicarbonate-buffered balanced salt solution containing 1 mg/ml glucose and 1% human serum albumin and
gassed with 5% CO₂ in air). Many eggs, including some dispermic ones, made one further regular division (1st, 2nd, or 3rd cleavage) in vitro, particularly if put into culture when cleavage in vivo was approaching. None underwent more than one cleavage in vitro.

An additional 33 eggs at the 16 to 32 cell stage (recovered four days after mating) were cultured in the presence of 0.5 gamma/ml colchicine. The eggs were then treated by a hypotonic saline, fixed, stained by aceto-carmine, and squashed. Sixteen eggs furnished analyzable mitoses. Of these, 15 were diploid (2n = 42); in a dispermic egg however (with the remnants of two sperm mid-pieces in the egg) the two countable metaphase groups were triploid.

These data accord with earlier in vivo observations that dispermic rat eggs cleave like monospermic eggs, but are triploid.

References:


63. ENZYME INDUCTION IN SEA URCHIN EGGS AND EMBRYOS

Investigator: Paul C. Denny
Support: National Institutes of Health, Public Health Service General Funds

The acid phosphatase activity of the sea urchins Strongylocentrotus purpuratus and Lytechinus pictus is steady in the mature egg and early embryo. On the other hand alkaline phosphatase activity is very low or non-existent in the mature egg and early zygote. Some time around ten hours after fertilization alkaline phosphatase activity becomes measurable and increases until it reaches a steady amount. Work is in progress to induce alkaline phosphatase activity prior to the time when it normally becomes measurable.

64. DEVELOPMENTAL IMMUNOGENETIC ANALYSIS OF CANCER

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

In last year's report a brief outline was given of a comprehensive theory of cancer proposed by this investigator. The theory has been since published, as noted below, with considerable documentation of pertinent evidence from many published investigations relating to the subject. A supplement to this account has also been prepared recently and presented at the International Symposium on Biological Interactions in Normal and Neoplastic Growth at the Henry Ford Hospital, Detroit, May 18-20, 1961. The summary of this supplementary account follows:

"A relatively simple theory of cancer has been constructed on the basis of developmental immunogenetic considerations, involving especially analogies with transplantation disease. The latter, in the form that involves transplantation of cells from pure-line parent to F₁ hybrid is taken as the experimental counterpart of the tumor-host relationship. The initiating event in cancer is pinpointed as the loss or
inactivation of a gene or genes at one or more of the histocompatibility (H) loci of the animal. At heterozygous loci only one allele would need to be lost or inactivated, while at homozygous loci (and in inbred animals) loss or inactivation of both alleles at the locus would be required. Differences of opinion and results with regard to chromosomal aberrations, and to the form of the cancer incidence-dose of carcinogen relation, appear to be resolved on the basis of the theory.

"Proliferation is attributable to the operation of the same kind of factors, namely stimulation by foreign H-antigen, that operate in the graft-versus-host reaction and which reflect the proliferative behavior of immunologically competent cells, in general, when exposed to foreign antigen. This is consistent with evidence showing no intrinsic differences between cancer cells and the corresponding normal cells with respect to proliferative capacity, and with evidence that there need be no difference from normal cells in structure and function, although in any particular tumor and at various stages there may be differences in the relative proportions of cells in the proliferative and the differentiated states.

"Production of humoral and cell-fixed antibodies, by some or all cells of the tumor, against host cells is considered to be primarily responsible for the essential pathological features of cancer. Leads to ameliorating and therapeutic measures are briefly discussed with regard to agents that interfere with secondary effects of antigen-antibody reactions, agents that suppress antibody formation, auto- and hetero-immunization procedures, restoration of lost H-gene by appropriate DNA or formation within the cell of lost H-antigen by appropriate RNA, administration of genetically aggressive immunologically competent cells, and destruction of the neoplastic cells by allergic damage by shocking doses of the antigen against which they have become specifically sensitized."

Publications:


65. IMMUNOLOGIC APPROACHES TO PROBLEMS OF HUMAN FERTILITY

Investigators: Albert Tyler, Delores Williams
Support: National Institutes of Health, Public Health Service

Extensive reviews have recently been written, as noted below, of immunological investigations relating to problems of reproduction in animals. The reviews analyze the major published investigations and tabulate most of the literature concerning immunological factors in animal reproduction. The topics include: attempts at active, or passive, immunization of females with seminal materials; treatment of spermatozoa in vitro with antibodies; exploration of possible selective action of antibodies with respect to sex and other genetic characteristics; suppression of spermatogenesis by immunization with extracts of testes or of spermatozoa along with adjuvants; occurrence of sperm-agglutinins in sterile men; immunization with placental and fetal material; antibodies against various hormones that affect the reproductive system; possible blood group incompatibilities; and natural heteroagglutinins. The direct interference with fertilization and development by means of specific antibodies is illustrated principally with experiments performed with eggs and sperm of
invertebrate animals and antisera prepared against purified extracts of these materials. Also, an account is presented of the naturally-occurring antigen-antibody-like system of eggs and sperm, namely the fertilizins and antifertilizins (see below) as the specific receptor substances by which the gametes unite, and experiments are described that show how the extracted receptors, themselves, may be employed to block fertilization, without evident injury to the gametes, simply by being added in excess to the medium in which sperm and eggs are mixed.

One of the problems currently under investigation concerns the occurrence of sperm-agglutinins in sterile men. This is being studied in direct collaboration with Dr. Edward T. Tyler and Dr. Nicholas Nakabayashi of the University of California at Los Angeles, and in more remote collaboration with Dr. Sheldon J. Segal of the Population Council, Inc., the Rockefeller Institute, New York, Dr. Philipp Rümke of the Central Laboratory of the Netherlands Red Cross Blood Transfusion Service, Amsterdam, and Dr. Shanta S. Rao of the Indian Cancer Research Center, Tata Memorial Hospital, Bombay. It has been found that about 3 to 5% of sterile men possess in their serum rather potent agglutinins for sperm of unselected donors. Also under investigation is the possibility that additional cases of sterility may be related to the presence of cell-fixed antibodies rather than antibodies of the circulating type. Other investigations deal with the antigenic structure of human spermatozoa, as studied by methods of gel-diffusion, immuno-electrophoresis, and fluorescent antibody staining.

Publications:


66. CULTURE OF MAMMALIAN EGGS AND CUMULUS CELLS

Investigators: Albert Tyler, Delores Williams
Support: National Institutes of Health, Public Health Service

Along with attempts to culture early division stages of mouse eggs, cultures have been established of the cumulus (granulosa) cells obtained from freshly ovulated eggs. Fertilized eggs, starting with the 1- or 2-cell stage, generally fail to continue development in culture in balanced salt solutions with added nutrients and serum. This has been the experience of many investigators working with rodent eggs. The frequency of development is higher when portions of the Fallopian tube along with estrogen are included in the medium. When started after the 4-cell stage the eggs develop to later blastocysts quite regularly in simple balanced salt solution.

The cumulus cells grow well in Eagle's medium with 10% horse and 10% human serum, as irregularly elongate flattened cells attached to glass, and without producing hyaluronate.
67. FERTILIZINS AND ANTIFERTILIZINS

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

Chemical and biological properties of the fertilizins and antifertilizins, the specific receptor substances of the eggs and sperm respectively, have been discussed in previous reports and publications. In the present report a brief account is given of a theory of fertilization in which the specific mutual adherence of the gametes and the engulfment of the sperm by the egg are interpreted in terms of fertilizin-antifertilizin interaction and specific pinocytosis. This theory which was previously presented (Tyler, 1959, Experimental Cell Research, supplement, 7:183-199) can account for most of the main observational and experimental features of fertilization. A complete catalog of these features cannot be presented here. However, a few may be mentioned to indicate the nature of the problems.

1. Fertilization is tissue- and species-specific.
2. The spermatozoa of most animals become immotile when they attach to the surface of the egg.
3. There are no openings in the surface (plasma membrane) of the egg large enough to admit the spermatozoon.
4. There is no opening left after the spermatozoon has entered.
5. In most species of animals only one spermatozoon is admitted, if the egg is in healthy condition and if the concentration of sperm is not extremely high. This block to polyspermy is established rapidly.
6. Upon fertilization there is an opening-up of granule-containing vesicles located in the cortex of the egg below the vitelline membrane.
7. There is an opening-up of an acrosomal vesicle on the head of the sperm upon contact with the egg.
8. The vitelline membrane (zona pellucida in mammals) lifts off the surface of the egg proper to become the fertilization membrane in most species of animals.
9. Removal of the fertilization membrane permits re-fertilization of the egg for a considerable period after the initial fertilization. Such de-membranated fertilized eggs readily accept many additional sperm (Tyler, Monroy and Metz, 1956).

To account for these and other features of fertilization a theory has been devised. The primary points of the theory are that specific adherence of egg and sperm occurs by virtue of binding by their respective receptor substances, fertilizin and antifertilizin, and that "penetration" of the sperm into the egg is rather a pinocytotic engulfment of the sperm by the egg. The process is designated pinocytotic to emphasize that it is a wrap-around process that is postulated.

These points can account for items 1 to 4 of the features of fertilization listed above. In order to account for items 5 to 9 it is necessary to assume that the plasma membrane bears the egg-receptors (fertilizin) and that this membrane protrudes through pores in the vitelline membrane. (Evidence for this is now accumulating from studies by electron microscopy in various laboratories and many different species of animals.) Also, the gelatinous coat, in species that possess this structure,
represents an attenuated extension of the essential substance (fertilizin) of the plasma membrane. Effective contact between sperm and egg is thus made by virtue of the antifertilizin of the sperm's surface and the fertilizin of the plasma membrane. Fertilizin molecules in the gelatinous coat may help to the extent to which they are anchored to the plasma membrane and contract upon interaction. However, any fertilizin molecule in the surrounding solution would tend to "neutralize" the sperm.

Immediately following contact with the first sperm to reach it in favorable position (and unreacted condition) the plasma membrane wraps itself around the sperm by progressive reaction of fertilizin and antifertilizin. At the same time the plasma membrane elsewhere retracts through the pores in the vitelline membrane, the retraction progressing rapidly as a wave of contraction from the region of the fertilizing sperm. The stresses set up on the surface of the sperm by its reaction with the fertilizin of the plasma membrane are considered to induce an opening up of its acrosomal vesicle, liberating lytic agents which soften the vitelline membrane so as to facilitate passage of the "plasma-membrane-wrapped" sperm through it. Contraction of the plasma membrane results in an opening up of its cortical vesicles (the walls of which are continuous with the plasma membrane), the liberated material serving to "tan" the elevating vitelline membrane, to act osmotically to help elevate the latter, and to participate in the gradual formation of a hyaline layer that slowly forms over the retracted plasma membrane.

It is suggested that, in addition to the specific adherence and engulfment phases of fertilization, the theory may also account for activation of the egg in terms of the intussuscepted portion of egg's plasma membrane brought in with the sperm.

Publications:


68. ANTI-DEVELOPMENTAL ACTION OF ANTISERA AGAINST FERTILIZINS AND AGAINST OTHER EGG-EXTRACTS OF SEA URCHINS

Investigators: Albert Tyler, Alberta Seaton, Jacques Signoret
Support: National Institutes of Health, Public Health Service
North American Treaty Organization

It has been shown previously that development can be blocked in fertilized eggs of sea urchins by treatment with antisera prepared against fertilizin or against saline extracts of jelly-less eggs (cf. A. Tyler, Exper. Cell Res., Suppl. 7:183-199, 1959). The antisera against fertilizin were generally found to be the more highly effective for such purpose; those against sperm and against other tissues of adult sea urchins were ineffective. Strong antisera can block cleavage within one-quarter of a division cycle.

Current studies with fluorescein-labeled antibodies against the fertilizins of the sea urchins Lytechinus pictus and Strongylocentrotus purpuratus show the presence of fertilizin-like antigens at the surface of the fertilized as well as unfertilized egg. The fertilization membrane does not show marked fluorescence. Regression of the surface fluorescence indicates decrease in these antigen during development.

Antisera prepared against sea urchin egg extracts exhibit many (eight or more) precipitation bands when interacting with the homologous antigen. Removal or decrease in amount of one or two of these bands can be effected by absorption of the
antisera with homologous sperm, without appreciable decrease in the development-blocking action. Absorption with fertilizin-coated sperm removes, or diminishes, another band and also removes, or diminishes, the anti-developmental action of the antisera.

The evidence indicates that anti-developmental action, whether by antisera against egg extract or by antisera against fertilizin, is due to antibodies against fertilizin-determinants. It supports further the view that fertilizin-like material is present in the plasma membrane (not the fertilization membrane or vitelline membrane) of the fertilized egg. The evidence shows the ineffectiveness, as cytotoxic agents, of antibodies directed against internal constituents of the cell, since by appropriate absorption such antisera may show many precipitation lines on agar-diffusion plates and yet be ineffective in blocking development, whereas the antisera against fertilizin are highly effective in this respect.
GENETICS

PART A. -- DROSOPHILA

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69. A NEW METHOD OF PREPARING DROSOPHILA CHROMOSOMES FOR CYTOLOGICAL ANALYSIS

Investigator: E. B. Lewis
Support: U.S. Atomic Energy Commission
National Science Foundation

A new method of treating and staining somatic chromosomes of the fruit fly Drosophila has been worked out. It is a modification of the colchicine-hypotonic saline method widely used in studying the chromosomes of vertebrates. One of the uses to which the new method is being put is to study certain kinds of chromosome breakage which occur after X-ray treatment of the fly. There is remarkable resemblance (at the metaphase stage) in size and shape between some of the autosomes of human beings and the major autosomes of Drosophila, when both are prepared by the colchicine (or colcemide)-hypotonic saline method. The new method has already proven particularly valuable in identifying ring chromosomes.

70. X-RAY STUDIES AT THE BITHORAX PSEUDOALLELIC SERIES

Investigator: E. B. Lewis
Support: U.S. Atomic Energy Commission
National Science Foundation

The bithorax cluster of genes in the third chromosome of Drosophila melanogaster is concerned with the growth and differentiation of certain thoracic and abdominal segments of the fly. For a number of reasons, the cluster is well suited to the study of radiation-induced mutation. There is a high mutation rate at this cluster when males are X-rayed. A wide spectrum of alleles is known spontaneously and each of such alleles, even those belonging to the same locus, is qualitatively distinguishable with ease from the others. Previous studies have shown that mutations induced by X-raying males have the following properties: almost all of them are associated with gross chromosomal rearrangements, and they act almost invariably as complete inactivations of the gene in question. It therefore is of interest to know whether X-rays are capable of inducing the same spectrum of alleles as is found spontaneously. This, moreover, is a general question of great importance to our understanding of gene mutation in all organisms. This question is being investigated by X-raying wild-type females and looking for new mutations involving the bithorax cluster. In this way gross chromosomal rearrangements are largely avoided owing to the rarity of their induction when females are X-rayed. Cytological analyses of the giant salivary gland chromosomes have been completed on a series of seven mutations induced in this manner. Four mutations have proved to be small deficiencies rather than point mutations. Three have proven to have no apparent rearrangement associated with them; of these two are, however, identical with the spontaneous mutant
known as Ultrabithorax, while one is a new type of recessive mutant. The mutation rate to viable recessive mutant alleles at the bithorax locus is extremely low, in spite of the relatively high frequency at which lethal mutations of the rearrangement type are induced and in spite of the variety of spontaneous bithorax mutants at hand. The mutants produced in these X-ray studies are being used as "tools" to study genetic fine structure, position effect and other gene-action phenomena.

71. X-RAY INDUCED REVERSE MUTATION OF THE IVORY MUTANT

Investigator: E. B. Lewis
Support: U.S. Atomic Energy Commission
National Science Foundation

The eye color gene known as ivory in Drosophila melanogaster mutates with a relatively high back mutation frequency following X-ray treatment of females; namely, at a rate of $3 \times 10^{-8}$/rad. A study has been made to determine whether the reversions might be due to non-allelic suppressors. These studies have in every case proved negative indicating that if suppressors are involved they are probably intragenic suppressors.

In order to have a still more sensitive system for analysis, a linear repetition of four or five sections of the region of the chromosome carrying this gene has been synthesized and investigated for its X-ray induced mutability rate. Irradiation of this "repeat" in the female germ line has, however, given a much lower back mutation rate than would be expected if the rate were simply the summation of the rates for the four or five ivory mutants contained in the repeat. Yet, somatically the back mutation rate of the repeat does seem to be proportional to the number of doses of ivory present. Thus a contradiction arises which resembles that already found when germinal and somatic reversion rates of ivory are studied in males and females. Thus, irradiation of ivory males has failed to increase significantly the reverse mutation rate, yet somatic reversion of the gene appears to be as readily induced in males as in females (other factors being equal).

The cause of the difference between germinal reversions in males and females remains obscure and indicates that there is some fundamental qualitative difference in mutability between oocytes and oogonia on the one hand and between spermatids and sperm on the other.

72. A STRAIN OF DROSOPHILA SELECTED FOR CANALIZATION OF THE PHENOTYPE OF A SCUTE (sc) MUTANT

Investigator: B. L. Sheldon
Support: Commonwealth Scientific and Industrial Research Organization, Australia
General Funds

Scute mutants generally reduce the number of bristles on various parts of the body of Drosophila. Mutant individuals are also extremely variable in phenotype as environmental conditions (e.g. temperature) vary, in contrast to the almost invariable ("canalized") wild type. A strain of scute flies had been selected successfully for low variability of number of scutellar bristles in a particular environment at 25°C. Subsequently it also proved to be quite insensitive to large changes in the temperature environment from that of the selection program. Transfer of this strain to this laboratory involved a change to a different culture medium. The selected strain was again insensitive to this environmental change by comparison with a control, highly-variable scute strain, confirming that selection for low variability in a
relatively uniform environment has produced a genotype that is buffered against marked variations in its environment. Some 12 generations of complete relaxation of selection have not altered the degree of canalization achieved by previous selection.

The selected strain has been further analyzed so far in three ways: (1) a 7-point linkage study of its X chromosome, (2) outcross to the control, a highly variable scute strain, (3) crosses to a tester with dominant markers on the II and III chromosomes, for assay of the importance of II and III chromosome recessives in the low-variance genotype. The results show fairly conclusively that the X chromosome is not involved in this phenomenon and that it is largely due to recessives on chromosome III.

Reference:

73. A POSSIBLE EFFECT OF SUPERNUMERARY SPERM IN DROSOPHILA

Investigators: B. L. Sheldon, L. Piko
Support: Commonwealth Scientific and Industrial Research Organization, Australia
Lalor Foundation

In Drosophila melanogaster several supernumerary sperm penetrate the egg at the time of fertilization. The erupt (er) Suppressor-erupt (Su-er) system of Glass (Science 126:683-689, 1957) has been used to determine whether Su-er supernumerary sperm have any suppression effect on the expression of erupt. Observations were made at the same time and in uniform culture conditions on two parental stocks

\[
\begin{align*}
\text{b Su-er}^+ \text{bw} &; \text{ster} &; \text{b Sup-er tu bw} &; \text{st er su-tu} \\
\text{b Su-er}^+ \text{bw} &; \text{st er } &; \text{b Sup-er tu bw} &; \text{st er su-tu}
\end{align*}
\]

(kindly supplied by Dr. Glass), the reciprocal F\(_1\)'s between them and the two backcrosses of F\(_1\) males to the Su-er\(^+\) parental strain females. While the analysis of the data is not complete, the results, both from the F\(_1\)'s and the backcrosses, show that Su-er\(^+\); er and Su-er\(^+\); er zygotes have a higher frequency of normal eyes and a lower frequency of extreme expression of erupt, when the supernumerary sperm are wholly or partly of the Su-er genotype. This suppression effect is interpreted as being due to the release of some Su-er gene product into the egg cytoplasm from the Su-er supernumerary sperm.

74. PHENOTYPIC AND GENETIC VARIABILITY OF SCUTELLAR BRISTLES IN WILD DROSOPHILA MELANOGASTER

Investigator: B. L. Sheldon
Support: Commonwealth Scientific and Industrial Research Organization, Australia
General Funds

The number and pattern of scutellar bristles in wild-type strains of D. melanogaster are highly invariable ("canalized"). It has long been recognized, however, that phenotypic invariability does not necessarily imply an absence or a lack of genetic variability. An extensive study has been undertaken to determine firstly,
the range of variation in the wild-type phenotype, including its response to different temperatures, and secondly, the way in which the different types of variants respond to selection. These data together with further genetic, developmental and biochemical analysis of the selected strains should give useful information on the properties of the base population genotype which bring about the canalization of the phenotype.

Four types of variants occur with a high enough frequency (say 1/200 or greater), at least under temperature stress, to allow accurate statistical description of the population variability and attempts at establishing selection lines on a broad genetic base. The variants are (1) one or more of the normal 4 bristles missing, (2) presence of extra bristles, (3) one or more of the normal 4 bristles greatly reduced in length and diameter, (4) presence of one or two very short and fine bristles at the center of the extreme, posterior margin of the scutellum. A good response to selection has been obtained for (2), (3) and (4), but not for (1). In the latter case some 20,000 flies were looked at to obtain 10 females and 45 males with one or more bristles missing. Some of these males (presumably sons of a single heterozygous female) turned out to be carriers of a particular new sex-linked recessive which removed bristles at specific sites and unfortunately tended to obscure other variation of type (1) in the selection line. Apart from this mutant, selection was not successful in establishing a strain with a higher frequency of flies with missing bristles. Further attempts will have to be made to accomplish this, as it is clear that the developmental buffering and its genetic control at this end of the phenotypic scale are more rigorous and finely-adjusted, probably more specific in terms of loci involved and their interactions, than at the other end of the scale leading to extra bristles. Detailed interpretation of the other selection lines and some correlated changes of the type (2), (3) and (4) variants in them must await a full statistical analysis.

75. GENES MODIFYING BRISTLE NUMBERS IN DROSOPHILA

Investigator: A. H. Sturtevant
Support: General Funds

The pattern of bristle arrangement in Drosophila is surprisingly stable. It appears that the named bristles have been present for at least 100,000,000 generations, so that natural selection has had ample time to fix a favorable pattern. However, there are a number of mutant genes that do alter this pattern, and the altered patterns are quite sensitive to differences in modifying genes. As reported last year, an attempt has been made to use this variability in a study of the specificity of the action of these modifying genes. The general result of the analysis, so far as yet carried out, is that each bristle is controlled by a set of genes that is to a large extent independent of those that control other bristles. There are genes that affect several bristles, but there is little evidence for a common pattern of such multiple effects—one gene will affect bristles a and b, another a and c, another b and c, and still others only one (or all three) of them.

76. A NEW TYPE OF NON-DISJUNCTION IN DROSOPHILA

Investigator: A. H. Sturtevant
Support: General Funds

A new mutant gene, a recessive near the left end of the X chromosome, leads to the production of from 1 to 30% of eggs with no X's. The result is that a female homozygous for this gene gives sons that receive their X's from their father. The variability in the frequency with which this occurs has so far not been explained; the only clue is that the frequency given by a female is negatively correlated with the frequency of crossing over, in the left end of the X, among her eggs that do carry
X's. There is also an increased frequency of eggs that do not receive a maternal fourth chromosome.

The further analysis has proved difficult, but is still being worked at. The case may be of interest, when it is better understood, because of the current active study of cases of non-disjunction in man.

77. SERIAL Duplications IN DROSOPHILA CHROMOSOMES

Investigator: John R. Laughnan
Support: National Science Foundation
Guggenheim Foundation
General Funds

Recently, in collaboration with Mrs. H. M. Peterson, the studies on serial duplication in maize have been extended to the Bar locus in Drosophila melanogaster. Since an adjacent serial duplication of the 16A section of the salivary X chromosome is involved in Bar, our model predicts that, in addition to the normal and double-bar crossover derivatives from Bar reported by Sturtevant, the duplication should also give rise to normal and double-bar occurrences that are nonrecombinants for marker loci. In addition to the expected recombinant exceptions, we have obtained a number of nonrecombinant exceptional derivatives. Cytological analyses of strains of the latter were initiated in Dr. Lewis' laboratory and continued at Cornell University. They indicate that the nonrecombinant derivatives are of three types: (a) spontaneous aberrations in the neighborhood of the Bar region, including five deficiencies, one inversion and one duplication, (b) genetic changes (4 cases identified) without identifiable cytological changes in the 16A region or nearby, and (c) a loss or gain of one 16A section correlated with the genetic change from Bar to normal or to double-bar (14 cases identified). These latter cases, which are without associated effects on viability and on crossing over in the marked segment, occur with a frequency of about one per 10,000 gametes tested. Their occurrence is consistent with the hypothesis of intrachromosomal recombination, and indicates that the phenomenon must be taken into account in considering the basis for nonconventional patterns of recombination in higher forms as well as in the microorganisms.
When an animal or person has rejected a tissue transplant from another individual, a second transplant will give a "second-set," accelerated rejection response if the two successive donors share one or more similar or identical antigens that are absent in the recipient. The probability that this will occur in any given instance is a function of the kind and amount of genetic variability in the population, and of the relationship among the recipient and the two successive donors. The algebra of Mendelian populations was explored in this regard; it became evident that the frequencies of "second-set" responses could provide measures of genetic variability different from and considerably more efficient than experimental designs developed for this purpose in the past. The method is particularly advantageous for studies of variation in natural and human populations, where little is known and much information is needed in the field of histocompatibility genetics. It also became evident that, in populations segregating for histocompatibility differences, the usual design of "immunological tolerance" experiments would be expected to give an impression of lesser specificity in tolerance than in immunization, as a function not of the inherent specificity of the mechanisms but of the genetic bases of tolerance and incompatibility.

A test of part of these predictions was performed by making skin grafts to 137 recipients from random donors in a segregating backcross mouse population, removing these first-set grafts at 8 days, and performing second grafts from random donors. Incompatibility for the strong H-2 locus in the 9th linkage group was avoided in these combinations. The frequency of "second-set" responses gives an estimate of 13 or 14 independently segregating loci affecting these responses, in addition to the known H-2. These results are in good agreement with estimates from less efficient designs, and indicate that the method may indeed be broadly useful.

Parabiotic intoxication is a disease usually observed when a hybrid mouse from a cross between two pure strains differing at the H-2 locus has been joined in
parabiosis with a mouse of one of the parental lines. This disease is usually fatal to the hybrid mouse. This animal exhibits a wasted, hunched appearance and severe anemia. Paradoxically, the pure strain mouse appears healthy and plethoric. Recently, Eichwald and his associates have presented evidence to support the hypothesis of Hall and Hall that the symptoms of intoxication are due to a shift of red cell mass from the anemic mouse to its hyperemic partner. These investigators expressed doubt that parabiotic intoxication is due to a direct effect of a graft versus host reaction. The purpose of the present investigations is to present new evidence for or against the concept of a graft versus host reaction in parent-F1 parabiotic intoxication.

The system most commonly employed in these investigations was the AKR/J mouse parabiosed to a (AKR/J x DBA/2J)F1 mouse. The mean survival time of the hybrid in this combination is 13.9 days, S.E. ± 0.74, with a range of 10 to 24 days. Another system used less extensively was the A/J and (A/J x C57BL/J)F1 combination. The mean survival time of the hybrid in this combination is 9.6 days, S.E. ± 0.48, with a range of 7 to 15 days.

Many of Eichwald's clinical and laboratory findings on this disease were confirmed. In addition, studies with chromium-51 and iron-59 labeled red blood cells have revealed that (a) the anemia and polycythemia are real and not due to simple hemodilution and hemocoagulation, respectively; (b) there is a true shift in red cell mass; (c) there is neither preferential selection nor preferential destruction of hybrid cells over the pure strain cells during the first 11 days of parabiosis; and (d) preliminary data suggest that there is an unequal cross-transfusion during the shifting of the red cell mass.

The problem of the graft versus host reaction in parent to F1 parabiosis was approached by employing lethally irradiated parental strain and hybrid strain mice as recipients for spleen cells obtained from intoxicated hybrid mice. The results of these experiments indicate that immunologically competent parental cells colonize the hybrid's spleen during parabiosis. In another series of experiments, parental strain mice were sub-lethally X-irradiated prior to parabiosis with their F1 hybrids. Parabiotic intoxication was abrogated by X-irradiation. These findings indicate that the parent to F1 parabiotic intoxication is the result of an immunological reaction by the parental strain mouse against the hybrid. This entity is therefore analogous to homologous disease.

References:


80. BLOOD GROUPS IN RHESUS

Investigators: Ray D. Owen, Margaret MacGillivray Sondra Clearwaters

Support: U. S. Atomic Energy Commission General Funds

Several specificities of the r.b.c. of Rhesus monkeys were identified by means of rabbit-anti-rhesus r.b.c. sera subjected to absorption analysis and tested with antiglobulin technique in which goat-anti-rabbit globulin serum provided the developing reagent. The main objective in this study was to provide markers for studies of erythropoietic transplants in irradiated monkeys; the reagents have proved
useful for this purpose. They are strong and specific, but there is no full assurance that they are "unit" in either a serological or a genetic sense. Reagents are tentatively identified as anti-A, -B, -C, -D and -E, respectively. In tests on 269 monkeys, positive reactions occurred with the following frequencies: A: 34.9%; B: 98.1%; C: 48.7%; D: 18.2%. Tests on a sample of 84 additional monkeys from another population gave significantly lower frequencies of A and C. The anti-E reagent was included only in the latter tests; E-positive reactions occurred with a frequency of 35.7%. The population distributions indicate that A, C, and D are independent; all B-negative animals are however A-positive and the association is highly significant statistically, suggesting allelism of A and B. The proportions of A, AB, and B animals are consistent with this hypothesis. There is a statistically significant tendency \((0.01 < p < 0.05)\) for C and E to be present or absent together. Lytic and saline-agglutination tests of absorbed rabbit- and chicken-anti-Rhesus r.b.c. sera gave only weak, variable, unrepeatable or negative results, and normal and isoimmune Rhesus sera were negative in tests with panels of Rhesus r.b.c. This work was done in collaboration with Donald R. Anderson, School of Aviation Medicine, Brooks Air Force Base, Texas.

Publications:


81. THE EFFECTS OF IRRADIATION ON THE STATE OF IMMUNOLOGICAL SUPPRESSION (TOLERANCE)

Investigators: W. H. Stone, Zeola Collins, Marilyn Moffat, R. D. Owen


An animal whose immunologic response has been partially or totally suppressed as a result of pre-treatment with antigen is said to exhibit immunological suppression or tolerance toward the antigen. The object of these experiments was to determine whether an animal suppressed for a given antigen would 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 injected with sheep cells at various periods after irradiation. Serum samples were obtained and tested for their antibody content as an indication of the effect the irradiation had on the state of suppression.

The results indicated that the state of suppression had been lost after irradiation. One explanation might be that the extensive proliferation and differentiation of cells and the increased loss of antigen that follows irradiation accounts for the loss of suppression. If the continued persistence of antigen is necessary for the maintenance of suppression, this explanation would fit the data.
82. BLOOD GROUPS IN THE RAT

Investigator: R. D. Owen
Support: U.S. Atomic Energy Commission
          General Funds

The serum of an albino rat of unknown ancestry was found to have normal antibody against red cells of all other rats tested; this rat provided the basis for a line (since lost) uniform for possession of the antibody. The difference A (for the antigen) vs. a (for the antibody) was controlled by a simple allelic alternative, the allele controlling the presence of the antigen being dominant. The symbol was chosen because it was assumed that this might be the alternative encountered by Burhoe. Four other unit specificities were defined in saline agglutination tests of rabbit-anti-rat red blood cell sera subjected to absorption analysis; these were assigned symbols C, D, E and F. The C and D reactivities are controlled by a pair of codominant alleles; in tests on several thousand rats, no additional allele has been identified at this locus. Alleles for E and F are dominant to those for their respective absences; they are genetically independent of each other and of the C-D and A-a loci, so that four independent loci were marked. Reagents for the C-D alternative are exceptionally strong and specific; only these two have been maintained to the present, and they proved useful for early studies of red blood cell chimerism following parabiosis, embryonic injection, and bone marrow injection after X-irradiation. In isoimmunization only anti-E appeared; on these uncertain grounds anti-E may be the same as Burhoe's earlier anti-M and isoimmune reagents later identified by others.

An apparent "dosage-effect" is noted at the C-D locus, and some developmental and possible organ-distributional aspects of these cellular specificities have been studied.

Publication:


83. GENETICALLY DETERMINED MOUSE SERUM PROTEIN VARIANTS

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

Genetically determined serum protein variants are known in humans and a number of other mammalian species. These investigations were conducted to detect serum protein variant systems in mice and to define their genetic control, using starch gel electrophoresis, immunodiffusion, and immunoelectrophoresis. Studies have been carried out to define the nongenetic variations occurring in mouse serum proteins during development, pregnancy and stress. Several quantitative and qualitative changes occur during postnatal development and during pregnancy, and certain characteristic changes occur under conditions of stress.

Two serum variant systems are detected electrophoretically. One involves the major serum beta-globulin, shown by Fe59 autoradiography on starch gels to be transferrin. The variant type is found only in the CBA line of 14 inbred lines thus far tested, and is detectable by an increased electrophoretic mobility. It has been shown by extensive genetic studies to be controlled by a single locus in the second linkage group of the mouse, about 14.5 map units from the dilution locus. Two
codominant alleles control the difference, and each allele determines the specificity of at least three electrophoretically distinct transferrin components. Under stress and at certain stages in postnatal development, shifts occur in the relative proportions of these components. The physiological significance of these changes is not known.

A second electrophoretic difference occurs among inbred lines in the presence or absence of a component at the leading edge of the principal prealbumin zone on starch gel electrophoresis. This difference is controlled by a pair of alleles without apparent dominance at a single autosomal locus.

A serological variant system in mouse serum involves a quantitative difference between inbred lines detected by immunodiffusion. Genetic studies have established that the difference is determined by a single locus and that this locus is closely linked with, or identical to, the Histocompatibility-2 locus in the ninth linkage group, a locus concerned with both transplantation reactions and red blood cell types. At least two alleles have been clearly defined and there is some evidence for a third. A consistent association between one serum type and one allele at the H-2 locus suggests direct control by the H-2 locus, though this may be contradicted by a few possible recombinants. Immunoelectrophoretic analyses suggest that the variant component is probably an alpha-globulin of high molecular weight. Studies to characterize the difference antigenically indicate that the principal difference is quantitative. There is no evidence for qualitative differences, but they are not excluded. The serum component is not detectably related antigenically to H-2 erythrocyte antigens and is not a product of erythrocyte degradation.

Publications:


84. SERUM PROTEIN TYPES OF HOMOLOGOUSLY TREATED IRRADIATED MOUSE CHIMERAS

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

In mice treated with a lethal does of X-irradiation and injected with blood-forming tissues from rats (heterologous treatment), or mice of other inbred lines (homologous treatment), it is found that transplants of the blood-forming tissues become established and the red blood cells and certain other hematopoietic tissues are of the specific type of the donor. Several studies have been conducted by various workers to determine whether the proteins in the serum of heterologous chimeras are also of donor type. These studies have indicated that little if any donor type serum protein is present, except possibly gamma-globulins.

It had not previously been possible to conduct this kind of study in homologously treated animals because no differences were known in mouse serum proteins.
which could be used as markers. Recent definition of three serum protein variant systems, however, provided the necessary markers. Chimeras were prepared from strain combinations which would permit distinction between donor and host type transferrins, prealbumins and serologically variant alpha-globulins. The sera of these chimeras were examined weekly up to 12 weeks after treatment. Donor type proteins appear briefly from 2 to 6 weeks in a few individuals, but animals surviving for three months or more after treatment have no detectable donor serum proteins, even though erythrocytes are entirely of donor type, as determined by a hemoglobin marker. These results suggest that the cells producing these three serum components are less sensitive to or more protected from the effects of X-irradiation than are certain of the other blood-forming cells.

85. THE ROLE OF INFECTION IN HOMOLOGOUS DISEASE

Investigator: Alan P. Roberts
Support: National Institutes of Health, Public Health Service
U.S. Atomic Energy Commission

Homologous disease occurs in mice treated after lethal doses of X-irradiation by a transplant of hematopoietic tissue from mice of a different strain. It appears following initial recovery from the effects of irradiation as a secondary syndrome often leading to death. This is now usually interpreted as some form of immunological reaction between the host and the grafted tissue. While such an immunological reaction is almost certainly the basis of the syndrome it may have no directly observable connection with those symptoms which may be seen in the mornbund host. Altered reactivity of the normal host resistance mechanisms may be sufficient to account for most of the homologous disease syndrome.

The strain combination used for this study is Balb/c/Jax bone marrow transplanted into (C57Bl/6/Jax X A/Jax)F1, (BAF1), forming a relatively compatible transplant. In a number of groups of such chimeras which have been prepared, homologous disease has occurred to only a slight degree and long term survival has approached 100%. These chimeras show the presence of the transplant, on the basis of hemoglobin solubility determinations which indicate the presence of donor type hemoglobin that differs from the host type. In other groups of chimeras homologous disease is more severe, often leading to 100% mortality. This marked variation cannot be the result of differences in conditions of irradiation or preparation and injection of bone marrow suspensions. The most likely explanation would seem to be different endemic, potentially pathogenic organisms present in the colony which cannot be adequately controlled with the housing conditions available, and without using germ-free animals. This possibility has been tested by isolation of bacteria from various organs of animals showing homologous disease. Positive cultures of a wide variety of bacteria have been obtained from most animals with a homologous transplant. Positive cultures are also obtained from animals with an isologous (from the same inbred strain) transplant, with lower frequency. It has not been possible to reproduce or to intensify homologous disease by injection of any of these cultures into homologous, isologous or normal animals or animals receiving X-irradiation only. This may indicate the activity of a bacterial synergism of two or more species in producing the disease normally or it may indicate the presence of more fastidious pathogens than could be obtained by the culture methods used. It may still be significant that homologous animals have bacteria present internally more often than do normal or isologous animals.

That homologous chimeras are less capable of handling invading bacteria than are isologous chimeras or normal animals has been shown by injecting a very mildly pathogenic culture of Salmonella paratyphi B (Sal. schottmulleri) at the time of
irradiation. After 2 to 3 weeks it was no longer possible to retrieve the same organism from isologously treated animals, either from feces or from internal organs. The bacterium was still present in homologous chimeras as long as 6 weeks after treatment. It could be found with increasing frequency in moribund animals and was constantly being excreted in the feces. The increase in numbers of salmonellae found in moribund animals does not necessarily indicate that it is causally associated with homologous disease. In an attempt to define more clearly the variations in resistance the clearance of bacteria from the circulation will be studied by the injection of P32 labeled organisms.

86. STUDIES OF MOUSE URINARY PROTEINS

Investigator: Alan P. Roberts
Support: National Institutes of Health, Public Health Service
U.S. Atomic Energy Commission

It has been known for some years that the normal mouse excretes a large amount of protein in the urine, the protein concentration being much higher in urine from males than from females. Studies are being undertaken to determine the origin and type of this protein.

The possibility that the urinary proteins might contain substances concerned with transplantation compatibility has been checked by determining their ability to alter the reaction against skin grafts. Urinary proteins precipitated by ammonium sulfate were used to immunize mice of another strain. Skin grafts from the same animals that supplied the urine were then put on the immunized animals. In all cases the grafts were rejected in the same manner as grafts on normal controls, suggesting the lack of transplantation antigen specificity in the urine. The possibility that antigens may have been damaged by the ammonium sulfate precipitation has been considered and this work will therefore be repeated with whole urine used for immunization.

By electrophoresis on starch gel the urinary protein is seen to move much more rapidly than serum proteins. This technique has been used to show a separation of the urinary proteins into at least six separate bands. The electrophoretic pattern shows the presence of a band given by urines from C57Bl mice, which is not shown by urines of six other inbred strains tested. Preliminary testing of segregating progeny, F2's and backcrosses, from the cross C57Bl/6 X CBA, suggests that this difference is probably under genetic control. A partially inbred line of Flexed mice has been shown to be segregating for this difference.

Rabbit antisera prepared against whole mouse urine form a precipitate with urine and also show strong precipitation with mouse serum. Preliminary tests, as yet inadequately resolved, of double diffusion in agar gels by the Ouchterlony technique show a number of precipitation lines with the homologous urine but give apparently single lines with urines from other inbred strains. A number of bands also appear in tests with homologous mouse serum, but at present these bands diffuse and the extent of cross reaction cannot be determined. It is planned to extend this study by the use of immunoelectrophoresis in an attempt to determine identity of the urinary proteins with serum proteins and possibly also with respect to components of organ extracts.
87. ISOIMMUNE ANEMIA IN MICE

Investigators: Margaret MacGillivray, S. Tokuda
Support: U.S. Atomic Energy Commission
National Institutes of Health, Public Health Service

AKR mice have been observed to develop anemia and positive Coombs tests following repeated injections of their own red blood cells. The incidence of these abnormalities is especially high when trypsinized red blood cells combined with Freund's adjuvant are injected.

The purpose of these experiments was to study this entity in an attempt to clarify the causative factors.

AKR and B6AF₁ mice were used. The experimental group was given weekly intraperitoneal injections of trypsinized isologous red blood cells in Freund's adjuvant. The control group was given Freund's adjuvant and saline. Hematocrit, Coombs, and Gorer-Mikulska tests were done at weekly intervals after the fourth injection.

Anemia developed more rapidly and was more severe in the experimental group than in the control group. Fifty percent of the experimental group showed weakly positive Coombs or Gorer-Mikulska tests in the first ten weeks. Eighteen percent of the control group showed similarly weakly positive Gorer-Mikulska reactions. This probably followed auto-immunization resulting from intraperitoneal bleeding after the injections. Red blood cell survival times, using Cr51, indicate a diminished survival of red blood cells in the experimental group but not in the control group. (Experimental group 50% survival = 12 days. Controls and normals 50% survival = 15 days.)

Iron uptakes using Fe59 were only slightly elevated in the experimental group indicating the absence of rapid or severe hemolysis.

Preliminary attempts to transfer the anemia passively using spleen and lymph node suspensions have not been successful.

The anemia resulting from isologous red blood cell injections in mice is probably due to immune destruction of red blood cells as indicated by their shortened survival times and positive immunologic tests.

Further work includes studying the effect of X-irradiation on this entity and repeated attempts at passive transfer.

Reference:

88. HOMOTRANSPLANTATION OF TESTICULAR TISSUE IN THE RAT, FOLLOWING TOTAL-BODY IRRADIATION AND BONE MARROW TRANSPLANTATION

Investigator: Marian Blake
Support: National Science Foundation
U.S. Atomic Energy Commission

Twenty-six rats were irradiated, then injected with bone marrow from donors differing at two phenotypic marker loci, a red-cell antigen controlling locus and a coat-color locus. Testicular cell suspensions were prepared from the corresponding
donors, and injected into one of the two testes of the treated recipients. The cir-
culating red cells of the treated animals were followed for the detection of an erythro-
poietic transplant, and histological examination of the testicular tissue was undertaken,
to determine the extent of reorganization within the host testes which had been in-
jected with donor germ cells. Unsuccessful efforts were also made to obtain progeny
from the treated rats. In all of the tissue sections examined, there were no signifi-
cant differences between the uninjected testes of the treated animals and those of
radiation controls. However, the histological pictures of the left (injected) and the
right (uninjected) testes of the treated animals were markedly dissimilar. Many
"free" cells appeared at all stages of spermatogenesis, both within the tubules and
interstitially, in the injected testes. The observations suggest that introduced cells
do survive and proliferate, but there is as yet no evidence that they function under the
conditions of these experiments.

89. THE FAILURE TO FIND BLOOD GROUP FACTORS ON HUMAN BLOOD
CELLS RESULTING FROM GENIC INTERACTION

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

Most blood groups are controlled by dominant genes, so a blood group is not
expected on the red cells of an individual unless also present on the cells of one or
both parents. Exceptions to this in birds, rabbits and possibly cattle suggest that
some kind of genic interaction may produce "new" blood groups in heterozygotes
(called "hybrid substances"). The object of these experiments was to determine if
this phenomenon occurred in humans. Rabbits were immunized with blood from chil-
dren and the antisera absorbed with pooled cells from the parents. The absorbed
sera were then tested with the cells of the child to detect any "hybrid" specificities.
With respect to any one blood group locus, only absorptions with cells from homozy-
gous parents would be expected to leave antibodies against the cells of heterozygous
children. So far, eight of eleven families have been studied, but with negative re-
sults. However, there were only four families with children who were possibly
heterozygous at either one or two of the blood group loci ABO, MN or Rh. The pos-
sibility that a "hybrid substance" occurs on human blood cells is by no means excluded
by these experiments. The experiments are being continued with additional families
of the appropriate genotypes.

Acknowledgment is made of the generous contributions of materials for this
study by Dr. Phillip Sturgeon, American Red Cross, Los Angeles, and by Dr. Roy Fisk,
Hyland Laboratories, Los Angeles.

Reference:

W. J. Miller. The hybrid-substance of the erythrocytes of the
hybrids between Columba livia and Streptopelia risoria.
Genetics, 41:700, 1956.

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

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

One limitation in studying human blood groups is the scarce supply of certain
typing fluids (reagents). Even reagents that are readily available are usually from
different individuals and often show variation in specificity and strength. Therefore,
a source of large quantities of strong and specific reagents from a single individual would be useful, especially for genetic studies. Cattle are readily available and are a good source of large amounts of sera. These experiments were designed to determine if cattle could be used to produce immune sera against human cells from which large quantities of typing fluids could be prepared.

Antisera in 18 cattle were prepared against the cells of six human donors. Each of three cows immunized with human M cells produced sera from which potent, specific anti-M reagents have been prepared. These reagents were tested with more than 100 cells of known type and have reacted strongly and specifically with cells of types M and MN. These cattle M reagents seem to be superior to the rabbit M reagents used routinely in human blood grouping. Preliminary analyses of these cattle anti-human sera indicate that at least three "new" specificities of human cells have been detected. An attempt is being made to produce reagents reactive with these new specificities.

Acknowledgment is made of generous contribution of materials for this study by Dr. Roy Fisk of the Hyland Laboratories, Los Angeles.

Reference:


91. MOLECULAR HYBRIDIZATION OF SHEEP HEMOGLOBINS

Investigators: D. C. Shreffler, J. R. Vinograd*
Support: National Science Foundation
National Institutes of Health, Public Health Service

It has been found in studies of the abnormal human hemoglobins that treatment at low or high pH causes dissociation of the hemoglobin molecule into subunits. If a hemoglobin of one type is labeled radioactively and mixed with a hemoglobin of a second type at low pH an exchange of a common subunit occurs between the two types; labeled subunits are transferred to the previously unlabeled hemoglobin to form a "hybrid" molecule. These studies have been useful in defining the structure of the human hemoglobin molecule and the genetic control of its specificity. The defective subunit of an abnormal hemoglobin can be determined by its hybridization behavior with other hemoglobins.

This technique has been applied to the two electrophoretically different sheep hemoglobins. The dissociation behavior of the two types has been studied and shown to be similar to that of human hemoglobin. Using radioactive and ferriheme labels on either of the two sheep hemoglobin types, it has been shown that some exchange of subunits occurs, but less than would be predicted with complete dissociation and random reassociation. This incomplete exchange indicates that either dissociation of the molecule is symmetrical, leading to incompatible half molecules from the two types, or that both kinds of subunits differ in the two types and are only partially cross-compatible. The results cannot distinguish these alternatives. The former is more attractive genetically, but the latter is possible with certain assumptions. The principal electrophoretic difference between the two types occurs in one of the two kinds of subunits.

* Division of Chemistry and Chemical Engineering, California Institute of Technology
92. SEROLOGY OF TETRAHYMENA

Investigator: John B. Loefer
Support: U.S. Atomic Energy Commission
U.S. Navy, Office of Naval Research
General Funds

Studies on serology of 78 strains of Tetrahymena pyriformis, variety 1, have continued along lines reviewed in Biology, 1960. Observations on seven "wild" and 71 inbred strains cultured in an Aerobacter-Cerophyl medium at 25°C indicate that all the strains may be classified into six distinct serological categories. These, insofar as studied, appear to be associated with a single genetic locus, designated as the H locus by Nanney and Dubert. Results so far obtained from breeding analyses indicate four distinctive alleles, HA, HC, HD and HE to be established in different inbred strains. It has not been determined whether HB and HF are at the H locus. Induced or I serotypes have been also invoked by culture of HA, HD and HB serotypes at 25°C in the presence of dilute homologous antisera. Observations are continuing on serotypes expressed by the same strains at 15°C and 35°C. More information regarding the characterization and distribution of these serotypes is given in the papers cited below.

Another study, originally begun as a separate phase of the serology problem and conducted jointly with associates at U.C.L.A., has led to several summary reports on amino acids of protozoa. Chromatographic patterns of free and protein amino acids were determined for Tetrahymena limacis and seven strains of T. pyriformis (F, Gf-J, HS, L1, L3, PR and WH52). Most comparisons were from axenic stationary phase material cultured at 25°C, although some work was also done with several strains grown at 10°C and 35°C. A few variations were observed among the free amino acid patterns of the strains studied, although it was not possible to correlate specific serotypes with these variations. A tabulated summary showing all reports to date for amino acids reported from protozoa is included in the publication first cited.

Publications:


References:


PART C. --NEUROSPORA

STAFF:
Professors: S. H. Emerson, N. H. Horowitz
Visiting Professor: D. G. Catcheside
Research Fellows: R. H. Davis, Bruce M. Eberhart, Marguerite Fling, P. C. Huang, Alexander Miller, Mary B. Mitchell, Yair Parag, Alfred S. Sussman, Paul A. Walker, Yasushi Watanabe
Graduate Students: Harris Bernstein, John C. Urey
Student Assistants: Stephen F. Heineman, Wendall Y. G. Ing, Janet L. Paschal
Research Assistants: Mary R. Emerson, Helen Macleod
Research Aides: Judith D. Elliott, Frances K. Hardin

93. GENETIC STUDIES WITH SLIME

Investigators: S. Emerson, M. R. Emerson
Support: National Science Foundation
Rockefeller Foundation

Further genetic tests of the plasmodioid variant of Neurospora (reported in Biology 1960) suggest that three mutant genes are responsible for the plasmodial growth habit. These are os (osmotic), which was present in the irradiated parent, a gene temporarily designated fz which is responsible for a rather fuzzy, usually buff-colored, phenotype, and one temporarily designated sp, which causes ascospores to germinate spontaneously (i.e., without heat activation) and produces a rather scummy, surface-restricted growth. Ascospores carrying these three genes, os fz sp, germinate without heat activation over a period of from one to five days in any of three forms: (1) as a plasmodial flow resembling the original "slime" growing very slowly, often dying before becoming established, and frequently changing to a hyphal-type growth; (2) as broad flattened hyphae which usually die, but may continue as in (1); and (3) as extremely fine, densely branched, hyphal growth which almost always (perhaps always) dies after forming a colony one or two millimeters in diameter. Type (1) may remain typically plasmodial if the original tube, especially in the agar has become dry, but almost always "reverts" to hyphal growth on subculturing. Such isolates (os fz sp) have occasionally been obtained in crosses of the original slime, (1207-1A)UV, to wild type, in which viability has been too poor for genetic analysis. Backcrosses of such slime-like isolates to wild type, however, have often given nearly complete germination of ascospores, and it is on the progeny of such crosses that the genetic interpretation has been based.

Whereas the original slime always produces plasmodial growth on an agar surface, the slime-like isolates from crosses to wild type generally produce hyphae, either directly upon ascospore germination or as a reversion from an original plasmodial growth. With a number of the slime-like isolates, however, it has been possible to establish, by vegetative selection, plasmodial sub-lines which have not produced hyphal growth even after a number of sub-cultures. The basis of such vegetative segregation has not been established.

Finally, among the genetic studies to date there appears to be a relatively high frequency of asci with various types of aberrant segregation.
94. CYTOLOGICAL STUDIES WITH SLIME

Investigators: S. Emerson, Judith Elliott
Support: National Science Foundation

When grown on agar, plasmodioid strains of Neurospora produce colonies whose margins are apparently completely devoid of cell walls. In the older parts of such colonies, multinucleate spherical cells develop, often with cell walls of considerable thickness. In liquid culture, hyphae of a sort are produced along with numerous spherical cells, both of which may have cell walls or apparently lack them.

Growing margins of extreme thinness are produced when slime colonies are grown between an agar surface and a cover glass. In such preparations, somatic nuclear divisions may be followed with considerable ease by phase contrast microscopy. The absence of cell walls in such marginal flows eliminates the diffraction halos characteristically produced by cells with cell walls. The growth is often sufficiently thin that no cytoplasmic structures are present above or below the nuclei, and, judged by their diameters, the nuclei, must often be considerably flattened. In such preparations it can be seen that the nuclear membrane persists throughout division. Elongation and fission of the nucleolus is preceded by division of less contrasting material which is presumed to be the chromatin material. In this laboratory the resolution of the presumptive chromatin is insufficient to permit definite statements regarding mitotic stages, though the recognition of the early stages is verified by the completion of nuclear division within the next two or three minutes.

Cultures of slime have been distributed to some cytologists interested in mitosis in fungi. This laboratory will gladly send cultures to all investigators wishing to study this material or to use it in class demonstration.

95. STUDIES ON INTERALLELIC COMPLEMENTATION

Investigator: D. G. Catcheside
Support: General Funds

When a series of biochemical mutants of Neurospora crassa are tested together in pairs to determine whether they complement in heterocaryons, it is found that they fall into groups distinguished by the fact that mutants from different groups always complement one another. Thus complementation occurs regularly between non-allelic mutants. However, within a group of allelic mutants either no complementation occurs between any pair or else complementation occurs between a few pairs. The latter phenomenon is thought to depend upon interaction between similar, differently altered protein units constituting an enzyme. The prediction is that alleles showing complementation determine enzymes, or proteins, which are polymers, while those not showing complementation determine enzymes which are monomers. It is therefore important to find cases where both complementation and enzyme constitution may be studied together, and especially to find cases of high probability that they are noncomplementary.

Two of the four genes concerned with steps in tryptophan biosynthesis have not so far shown any interallelic complementation. Rather few mutants have previously been available for these two (try-2 and try-4), so searches have been made for more of them. So far, tests of the few new ones with the old ones have provided no instance of complementation, but still the series are too small to establish absence of complementation with any certainty.
It is possible to show the relation between complementing allelic genes by a two-dimensional matrix or, more interestingly, by a map in one dimension in which each mutant is represented as inactivating a longer or shorter part of a line conceived as denoting the normal function.

More than half of the groups of alleles sufficiently investigated show inter-allelic complementation, but in no case has there been extensive enough study to show whether the ideal map is linear or requires more dimensions. A presumed case (Catcheside, 1960) of a two-dimensional map of the histidine-1 function has proved false. The erroneous diagnosis arose because one of the mutants reverts and this gave the appearance of complementation where none really occurred. Proof was obtained by preparing heterocaryons, showing presumed complementation, from components labeled with various combinations of other markers.

The additional tryptophan-1 and tryptophan-3 mutants encountered in the searches mentioned above are also being studied in relation to the sets of mutants previously available. The complementation map for try-1 proves to be more complicated than previously reported, now showing four segments instead of two. Ability to grow on anthranilic acid is associated with one end of the map and accumulation of this compound with the other end of the map, but details are not yet well enough known to make a complete correlation possible.

Representatives of the td (tryptophan desmolase=tryptophan-3) mutants, studied by Bonner and Yanofsky, have now been introduced, by repeated backcrossing to Emerson wild types, into the genetic background of these mutants, so that they are now heterocaryon compatible with them. An extensive test shows a relationship which can be represented by a one-dimensional complementation map in which all mutants are represented by a single continuous region of defect, with one possible exception, at present under further study. Eight segments (I to VIII) are distinguishable in the map, but further study may reduce this number by one or two. The mutant (A78) able to carry out the indole to tryptophan part of the reaction is defective in segment VIII. Those (td2, td71, A 69 and A 74) able to carry out the indole glycerol phosphate to the indole part of the reaction are defective in segment VII and may also be in one or more adjacent segments, namely IV to VI, though not in segment VIII. Yet other mutants, apparently unable to carry out either part of the reaction, are like some of these in their complementation maps. The correlation between complementation function and enzyme function is evidently fairly complex.

References:


96. GENETIC AND COMPLEMENTATION STUDIES WITH SEVERAL ALBINO MUTANTS OF NEUROSPORA

Investigator: P. C. Huang
Support: National Institutes of Health, Public Health Service

It is noted that most of the albino (al) mutants in N. crassa so far reported in the literature are located in linkage group I. While Hungate (1945, Stanford thesis) subgrouped her seven mutants into two, namely al-1 and al-2, Perkins (1959, Genetics
77

44:1185-1208) provided evidence for allelism or close linkage among nine previously unassigned albino mutants in relation to both al-2 and aur (aurescent). The non-allelism between al-2 and aur, which is allelic to al-1, was established earlier by Hungate. The present study is aimed to test whether the albino mutants indeed form a cluster of loci and to examine how much the genetic map agrees with or deviates from the results based upon complementation.

Two technicalities have hampered the recombination and complementation analyses of the albino mutants in Neurospora. First, unlike prototrophic mutants, albinos and their recombinants cannot be scored by the enrichment technique; this limits the genetic resolving power. Second, the synthetic pathway of the carotenoids, which are the bases for complementarity between albinos, is not yet fully agreed upon, making the supplementation type of techniques not feasible. The first point has been moderated by applying a modified Newmeyer's technique, i.e. to plate the ascospores directly onto a 1% sorbose-0.1% sucrose medium. The germinated ascospores each develop into a restricted colony and can be readily identified for albino versus wild pigmentation after seven days of incubation. A population of 100 to 150 random ascospores can be examined on the same plate instead of using separate tubes. The second point is being approached by analyzing chemically the various carotenoids present in each mutant and their complementation products. In one compatible pair analyzed, alCA and alSA, the complementary products produced are qualitatively the same as those carotenoids normally present in the wild-type strains (Krzeminski and Quackenbush, Arch. Biochem. Biophys. 88:287-293, 1960). These include, in the epiphasic fraction, spirilloxanthin, lycopene, neurosporene, zeta-like carotene, gamma carotene, beta carotene, phytofluene, and phytoene. Only the latter two polyenes are found to be present in the albino mutants.

The albino mutants used in this study were mainly obtained from Professor Dr. D. D. Perkins, Stanford University.

Publication:


97. DUPLICATE PHENOTYPES AMONG NEIGHBORING ASCI

Investigator: Mary B. Mitchell
Support: National Institutes of Health, Public Health Service

The asci of Neurospora crassa are regularly formed in closely associated pairs which appear to develop, or, in some cases, to cease developing and degenerate, concurrently (Genetics 45:507-517, 1960). The members of such a pair are attached at their bases and are so strikingly similar in morphological characteristics as to suggest that the distribution of genetic factors is the same in each. This could hardly be so if, as it has been customary to assume, genetic segregation takes place at meiosis which occurs independently in each ascus after it is established as an individual. It was therefore of interest to know whether patterns of segregation or ordinary genetic markers occur in pairs.

This situation is less readily observable than that of twin morphologies among developing asci. Scoring of commonly used markers requires isolation and culture of spores of mature asci which are fragile and easily ruptured or dissociated from one another. Also, only the grosser details of morphology which would serve to identify twins are visible under conditions suitable for free-hand dissection of asci. However, consecutively dissected duplicates (duplicate with respect to pheno-
types of segregants and their arrangement in the ascus) were found more often when an effort was made to dissect neighboring asci consecutively than when positions of the asci relative to one another were ignored.

More recently increased familiarity with the structure of the ascus cluster has led to greater success in predicting that a given pair of asci will show duplicate phenotypes or, more often, that a small group of asci, associated in a characteristic way, will contain duplicates. For example, one such group of 15 asci included 5 paired phenotypes and another group of 7, 3. Recurrences of infrequent phenotypes within small, closely associated groups are also observed.

If, as the observations are thought to indicate, asci regularly occur as phenotypically identical twins, then it would appear either that meiosis does not take place in the ascus after it becomes a separate entity, or that the ascus phenotypes are not determined at meiosis. To escape from this difficulty it might be suggested that, following meiosis, the whole ascus divides longitudinally. However, such divisions have not been observed and there are other reasons as well for doubting that the answer can be so simple. For example, there is evidence of further clustering of similar but not entirely identical phenotypes, suggesting close genetic relationships between neighboring twins. The basis of the clustering is not sufficiently well understood to merit discussion at present but it seems likely that this situation and that of twin phenotypes will be explainable in the same terms.

98. ISOLATION AND PROPERTIES OF NEUROSPORA TYROSINASE

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

It was reported last year that tyrosinase S has been isolated in electrophoretically and ultracentrifugally homogeneous form. This has now been accomplished for tyrosinase L, as well, and both tyrosinases have been crystallized.

The molecular weight of tyrosinase S has been revised downward from last year's estimate of 50,000-100,000. Sedimentation-diffusion measurements indicate a molecular weight of 34,500. Total amino acid analysis, combined with fingerprint data, gives the value 32,000-33,000. Sedimentation equilibrium measurements show a distribution of molecular weights from 35,000 to over 100,000. The enzyme apparently associates in solution. (Prof. Sinsheimer and Dr. Carusi provided the ultracentrifugal analyses). The molecular weight of tyrosinase L is of the same order of magnitude as that of tyrosinase S.

Total amino acid compositions of the two enzymes are similar. No cystine or cysteine has been detected in them. Fingerprint comparisons are in progress.

99. EXPERIMENTS ON THE ANTIPODAL SPECIFICITY OF TYROSINASE INDUCTION IN NEUROSPORA

Investigators: Helen Macleod, Yasushi Watanabe, M. Fling, N. H. Horowitz
Support: National Science Foundation

Tyrosinase activity is induced in Neurospora by the naturally occurring aromatic amino acids tyrosine, dopa, phenylalanine, and tryptophan. In every case,
however, the D-stereoisomer is much more effective as an inducer than the L-stereoisomer. It was shown in last year's report that the antipodal specificity is abolished in the case of the fluorophenylalanines, where both antipodes are effective inducers. The fluorophenylalanines are also growth inhibitors, a property which is shared by the D-aromatic amino acids. This suggested that the ability to inhibit growth is an essential property of tyrosinase inducers and led to the testing of a number of amino acid and purine analogs for induction. It was found that ethionine, 2,6-diaminopurine, and 8-azaguanine are both growth inhibitors and tyrosinase inducers. Canavanine, thienylalanine, and sulfanilamide are growth inhibitors, but do not induce. A number of other analogs, including 5-methyltryptophan are weak growth inhibitors and are also weak or ineffectively as inducers. It thus appears that all inducers inhibit growth, but not all growth inhibitors induce. These results also demonstrate that an aromatic ring is not necessarily necessary for induction, nor is the D configuration required. Experiments with the oxD1 mutation (D-amino acid oxidase deficient) show that only L-ethionine is effective as an inducer and inhibitor. D-ethionine is inverted (in strains not carrying the oxD mutation) to L-ethionine before acting. (See also report no. 101 in this volume by B. Eberhart and N. H. Horowitz.)

The foregoing results are in line with the long known effect of sulfur deprivation in inducing tyrosinase synthesis in growing cultures. Besides sulfur deficiency, nutritional deficiencies for nitrogen, carbon, and (to a smaller extent) biotin will also cause Neurospora to produce tyrosinase.

The general conclusion from these findings is that tyrosinase synthesis is favored under conditions in which general protein synthesis is inhibited. This suggests the possibility that tyrosinase is synthesized over a special pathway, or from a special pool of precursors.

Publications:


100. INCORPORATION OF LABELED VALINE INTO TYROSINASE

Investigators: Yasushi Watanabe, N. H. Horowitz

Support: International Cooperation Administration-
National Academy of Sciences
National Science Foundation

Since the tyrosinase of Neurospora is induced under conditions in which general protein synthesis is inhibited, it was important to learn whether induction involves synthesis of the enzyme from amino acids or merely reorganization of a pre-existing, enzymatically inactive protein. An experiment was therefore carried out in which tyrosinase was induced in mutant ty-1 in the presence of DL-valine-1-C14. The results of this experiment lead to the following conclusions: (1) tyrosinase is synthesized de novo from amino acids following induction, (2) some incorporation of valine occurs into the general soluble proteins during induction, but to a lesser extent
than into tyrosinase, (3) the addition of an inducer (D-phenylalanine) to resting mycelium causes a significant dilution of the radioactive valine in the pool. This indication of a greater net breakdown of proteins in induced than in non-induced cultures is consistent with other evidence that induction inhibits general protein synthesis.

101. SCREENING METHODS FOR TYROSINASELESS MUTANTS OF NEUROSPORA

Investigators: Bruce M. Eberhart, N. H. Horowitz
Support: National Science Foundation

The aim has been to obtain mutant strains of Neurospora which are deficient in the ability to produce tyrosinase. Particularly desirable are mutations at the T locus, which controls the structure of tyrosinase. The procedure involves irradiation of Neurospora conidia with ultraviolet light and their growth in liquid culture under conditions where each colony becomes a separate sphere. These colonies are tested for tyrosinase by the addition of dopa. Colonies which possess the enzyme turn dark from their production of dopachrome and then melanin, while mutants remain light under these conditions. In order to maintain colonial growth, conidia from colonial mutant strain C102 are used and grown at 34°C. Carboxymethyl cellulose has been found to be an ideal agent to keep colonies from fusing during their growth. As the experiment proceeds the carboxymethyl cellulose is removed by the action of Neurospora cellulase and at the end of this growth period colonies are washed by decantation and placed in induction medium for maximal enzyme production (usually sodium phosphate pH 6). Several strains have been obtained by this procedure which are lower in tyrosinase than their parent strain. Their allelic relationships are being determined.

A second method has been developed to test potential mutants on petri plates by the addition of ethionine as an enzyme inducer followed by the addition of dopa. This method is used as a check of mutants obtained by the liquid culture methods.

A related procedure has been developed for the qualitative screening of inducers of tyrosinase on plates. This method led to the observation that oxD mutants (D-amino acid oxidase less) are inducible by L-ethionine rather than D-ethionine. This is an exception to the rule that the D-form of an inducer has a greater activity than the form for tyrosinase induction (see Biology, 1960).

102. FURTHER STUDIES ON D-AMINO ACID OXIDASE DEFICIENT MUTANTS OF NEUROSPORA

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

As was reported last year, a D-amino acid oxidase deficient mutant—designated oxD1—shows, in addition to a defect in the ability to synthesize the mentioned enzyme, a block in the utilization of sulfate. Genetic experiments showed that these two characters are very closely linked, if not allelic. In order to determine whether the loss of D-amino acid oxidase activity is the cause of the block in sulfate reduction, a new series of D-oxidase deficient mutants were produced by ultraviolet irradiation, and their ability to utilize sulfate was examined. Four such mutants, all deficient in the oxidase, have been tested and found to be normal in their ability to use sulfate as the source of sulfur for growth. These mutants appear to be allelic with oxD1, although the evidence on this point is not very extensive. In any case, it is clear that a D-amino acid oxidase deficiency does not of necessity lead to a defect in sulfate metabolism, and it is likely therefore that the oxD1 mutation involves the coincident mutation of two closely linked genes.
103. D-ALANINE METABOLISM IN NEUROSPORA

Investigator: Yasushi Watanabe
Support: International Cooperation Administration-
National Academy of Sciences
National Science Foundation

The cell wall of Neurospora does not contain D-amino acids (see Biology 1960). It was found, however, that labeled D-alanine added to the culture medium is converted to two conjugated compounds that are the same as, or similar to, those known to be intermediates in bacteria which contain D-amino acids in their cell wall.

One of the compounds was identified as D-alanyl-D-alanine. The other is probably a sugar complex in which the nitrogen of D-alanine is attached to a sugar carbon atom. These compounds are accumulated in the free form both in the medium and mycelium, but they do not seem to participate in cell-wall structure.

104. SUBSTRATE SPECIFICITY OF S AND L TYROSINASES OF NEUROSPORA

Investigator: Alfred S. Sussman
Support: National Science Foundation
General Funds

The differences between a thermostable ($T^S$) and thermolabile form ($T^L$) of tyrosinase from Neurospora crassa have been studied by means of a spectrophotometric technique for assay of the enzyme using ascorbate or ferrocyanide as reducing agents. No significant differences in substrate specificity have been found in a survey of over 50 substances which included mono-, di-, tri- and conjugated phenols. Catechol was found to be a substrate, in contrast to previous findings. Michaelis-Menten constants for the two forms of enzyme acting on L-tyrosine and D- and L-dopa also were found to be similar, as were the pH optima on L-dopa and phenyl 4-catechol, and the response to several inhibitors. On the other hand, the activity of the thermolabile enzyme was lost more rapidly than that of the thermostable one after incubation for 5 to 15 minutes in high concentrations of urea and formamide. Above pH 9.6 the autoxidation of ascorbate was markedly arrested by the thermostable enzyme but was little impaired by the thermolabile one. These data are interpreted as support for the conclusion of Horowitz and Fling (1956) in that the two forms of the enzyme differ mainly in their secondary and tertiary structures and not in their active centers.

Another problem concerned the mechanisms of dormancy in ascospores of Neurospora. The mechanism proposed to explain the phenomenon involves the activation of an enzyme system concerned with the dissimilation of trehalose. With this in mind a search was undertaken for mutants with impaired ability to utilize trehalose as an energy source. Several putative mutants were obtained, whose content of trehalase is now under investigation.

105. MAPPING A TYROSINASELESS MUTANT OF NEUROSPORA

Investigator: Paul A. Walker
Support: General Funds

A prime objective of this sabbatical leave was the learning of various techniques in experimentation with Neurospora. This was effected not only by means of the undertaking of a small research problem, but also by the reading of a very large percentage of the published literature on this organism contained in the Biology Library of the California Institute of Technology.
The problem pursued was the mapping of the ty-1 (tyrosinase-less) gene in Neurospora crassa. After having made some "classical" dissections of asci, unordered tetrads were obtained through the use of a modification of the method of Strickland.

Crosses were made between ty-1 stock 91383A, supplied by Dr. N. H. Horowitz, and three other mutant strains: inos (inositol-less) 89601a; sc (scumbo) 5801R10a (both also supplied by Dr. Horowitz); and tyr (tyrosine-less) 52a (supplied by Neurospora Stock Center, Dartmouth College). No linkage was observed with inos (linkage group V), but linkage was clearly demonstrated between ty-1 and sc (linkage group III), with a crossover percentage of about 37% in the 248 unordered tetrads studied. The choice of the tyr mutant was made for two reasons: (a) Barratt, et al., and Perkins and Ishitani had shown the tyr gene to be located about 32.3 map units to right of the centromere (which is located just to the left of the sc locus) and thus the tyr gene could be close to ty-1; (b) it seemed that tyr and ty-1 might conceivably prove to be closely linked since both are involved with tyrosine metabolism.

This cross (tyr x ty-1) proved to be the least fertile of the three crosses studied, with only about 56.5% germination of the isolated ascospores; however, on the basis of the 130 unordered tetrads obtained a crossover percentage of approximately 6% seems indicated. Thus, tyr and ty-1, although they lie close to each other in linkage group III, do not seem to be allelic.

The order of the three genes investigated in linkage group III seems, therefore, to be: sc (0.9); tyr (32.3); ty-1 (374). Furthermore, the gene, tyr-1, appears to be the most distally located of any gene yet investigated on the right arm of this chromosome.

References:

106. COMPLEMENTATION STUDIES WITH ISOLEUCINE-VALINE MUTANTS OF NEUROSPORA

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

In previous reports (Biology 1960, Nos. 76 and 77), studies on complementation among isoleucine-valine (i-v) mutants were summarized. This work has now been extended.

A total of 612 mutants capable of growth on a medium supplemented with isoleucine and valine, but not on minimal medium, were obtained through the use of 9 different mutagens. On the basis of heterokaryon complementation tests all of the mutants could be allocated to five groups. These groups were designated val-1, val-2, iv-1, iv-2 and iv-3. Mutants in the val-1 and val-2 groups required valine as the sole supplement. Members of the iv-1 group are probably blocked in the dehydrase step of isoleucine-valine biosynthesis, and are characterized by slow growth between 4 and 6 days after inoculation on minimal medium. An extensive program of complementation testing was performed among mutants within both the iv-2 and iv-3 groups.
The results of these tests allowed, in each case, the formulation of a complementation map in which sets of mutants could be represented as single linear segments. However, three iv-3 mutants were found which were not compatible with a linear pattern. An interesting feature of the iv-3 studies, and to some extent of the iv-2 studies, was the finding that among successive mutants the same patterns of complementation interaction often recurred. Mutants with the same complementation behavior constitute a complementation subgroup. Each of these subgroups usually contained mutants representative of several different mutagenic treatments. This observed clustering may reflect an intrinsic property of the genetic locus—namely, that each locus has associated with it a unique pattern of discrete complementation subgroups.

Publication:

107. PYRUVIC ACID METABOLISM IN NEUROSPORA

Investigators: Alexander Miller, Frances Hardin
Support: National Institutes of Health, Public Health Service

In a previous report, a group of isoleucine-valine requiring mutants (iv-3) was described. It has been found that extracts of these mutants lack the ability to condense two moles of pyruvate to give α-acetolactic acid, an intermediate in valine biosynthesis. Furthermore, these mutant extracts catalyzed no measurable amount of C¹⁴O₂ liberation from pyruvate-1-C¹⁴. Equal activity for pyruvate-dependent DPN reduction was demonstrated in mutants and wild strains. No pyruvate oxidase activity could be demonstrated in either mutant or wild strains. These results suggest that in Neurospora, pyruvate is not directly oxidized via the tricarboxylic acid cycle, in contrast to what is found in many other organisms.

Reference:

108. LOCALIZATION OF ENZYMES IN NEUROSPORA

Investigator: Alexander Miller
Support: National Institutes of Health, Public Health Service

It has been shown that the Neurospora mutant poky has altered mitochondrial morphology. This mutant, in young cultures, also lacks succinic oxidase and cytochrome oxidase—typical mitochondrial enzymes. It was of interest to examine other enzymes in Neurospora, which in other organisms are associated with mitochondria.

Enzymes assayed in crude extracts of unfrozen 24-hour-old Neurospora mycelia were glutamic acid dehydrogenase, malic acid dehydrogenase, glucose-6-phosphate dehydrogenase, 6-phosphogluconic acid dehydrogenase, fumarase, isocitric acid dehydrogenase, aconitase and pyruvic oxidase.

No activity was found for the last two enzymes and the isocitric acid dehydrogenase activity was low and variable. All other activities were easily measurable. In particular, the glutamic acid and malic acid dehydrogenases were exceptionally active.
Experiments were prepared in a variety of suspension media and using several different disruption techniques. In all cases, activity was found only in the supernatant fluid after centrifugation at 100,000 x g. Also, no indication was found that these activities are present in the particulate fraction in latent form.

These results raise the possibility that the distribution of enzymes in Neurospora differs markedly from that found in organisms more usually studied by biochemists. However, it cannot be excluded that procedures sufficiently severe to disrupt cell walls invariably destroy the integrity of Neurospora mitochondria.

References:

A. Miller. Biology 1958, No. 9, p. 17.

109. BASIS OF SUPPRESSOR ACTION ON THE Pryr-3a MUTANT OF NEUROSPORA

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

The Pryr-3a mutant of Neurospora is problematic, in that it is allelic with other Pryr-3 mutants which lack aspartic transcarbamylase, yet it appears to have normal activity for this enzyme. Direct attempts to identify other enzymatic blocks which might be responsible for the pyrimidine requirement have been unrewarding.

A suppressor gene has been found, however, in the laboratory of H. K. Mitchell, which, when combined with Pryr-3a eliminates the pyrimidine requirement. It is unlinked to the Pryr-3 locus. The suppressed mutant (Pyr-3a, s) is inhibited in its growth by low concentrations of arginine. It has recently been found that the suppressor gene leads to a 98% reduction in the activity of an arginine-synthesizing enzyme, ornithine transcarbamylase (OTC), without, however, creating a deficiency for arginine. These and other data suggest strongly that the basis of the mutant phenotype of Pryr-3a is the sensitivity of an early step in pyrimidine synthesis to endogenous arginine or an arginine derivative. The suppressor gene, in effect, leads to a reduction in the concentration of this inhibitor through its effect on OTC.

References:


110. GENETIC AND CYTOLOGICAL STUDIES OF COMMON-B HETEROKARYOSIS AND MUTATIONS AFFECTING THE INCOMPATIBILITY FACTORS IN SCHIZOPHYLLUM

Investigator: Yair Parag
Support: National Institutes of Health, Public Health Service

In the mushroom Schizopyllum commune, a heterothallic, tetrapolar Basidiomycete, sexual fertility depends upon heterozygosity of two incompatibility factors, A and B; e.g., A'B is incompatible with A'B, A'B, and A'B, but with
$A^2B^2$ it gives a fertile dikaryon, $(A^1B^1 + A^2B^2)$. A long series of alleles exists for each factor. The dikaryotic mycelium typically has binucleate cells and clamp connections; the latter regulate the distribution of nuclei following their conjugate division in such a way that each daughter cell receives two nuclei, one of each mating type. On this mycelium the fruit bodies usually develop.

It has been found in previous studies that when $A^1B^1$ is mated with $A^2B^1$, a common-B heterokaryon ($A^1B^1 : A^2B^1$) can be selected, which typically has incomplete clamp connection, termed pseudoclamps. Mycelia of such heterokaryons have now been studied by means of phase microscopy. It has been found by this study that, although there is variability among different strains, it is common to all common-B heterokaryons that nuclear division and initiation of pseudoclamps mimic in several details those of the dikaryon, until a very significant step: the pseudoclamp invariably fails to fuse with the sub-apical cell, as is the case with the clamp connections of the dikaryon. The result is the capture of one of the daughter nuclei in the pseudoclamp and the resulting sub-apical cell becomes uninucleate. However, both side-branches from sub-apical cells and the growing pseudoclamps retain the morphology of the heterokaryon. Efforts are now being made to isolate single, uninucleate cells to test whether they are genetically heterokaryotic, though they apparently start as uninucleate.

The $B$ factor is comprised of at least two sub-units, $a$ and $\beta$, separable by crossing over; e.g., $B^{41}$ has two subunits, $a_3$ and $\beta_2$, and it is therefore written as $B_3^{32}$. A mutation has been found changing $B^{41}$ to $B^m$ (m designating a mutation--to distinguish from naturally found alleles), which is compatible with $B^{41}$, with 58 other $B$ alleles and apparently with itself. Among about 600 segregants that were isolated from two crosses, $A^{41}B^m \times A^{43}B^{43}$ and $A^{41}B^m \times A^{39}B^{32}$, only one $B$ recombinant, $B_3^{3-1}$, was obtained, and that from the first cross, suggesting that the mutation occurred within the $B$ factor, and probably changed the $\beta$ subunit: $B_3^{3-2}$ mutation $\rightarrow B_3^{3-m}$

Mycelia of strains carrying $B^m$ convert very frequently to dikaryon-like mycelia with fertile fruit bodies, and the interpretation was that these mycelia resulted from a mutation in the $A$ factor leading to complete self fertility. Later J. R. Raper (unpublished) found that it is a result of a suppressor, $A_{sup}$, unlinked to the $A$ factor. Parallel to genetic experiments by Raper at Harvard, phase-microscope observations have been done here on heterokaryons carrying $A_{sup}$ in one or both of their components. These heterokaryons were found, very surprisingly, to have exactly the same morphology as the common- $B$ heterokaryon, and the suppressor was found to be partially dominant both in aping the common-B and in suppressing the $A$ factor. The meaning of this mimicry of the function of the $B$ factor by the suppressor of the $A$ factor is not yet understood, but its frequent emergence from strains having $B^m$ suggests a peculiar relation between $B$ and $A_{sup}$. These mutations, altering the effect of the incompatibility factors, are very promising in helping to elucidate the mode of action of the incompatibility factors.

References:


The advent of hybrid corn in the 1930's brought about revolutionary changes in agricultural practices in the corn belt states. Among these changes was the concentration of hybrid seed production in large isolated fields where hybridization was accomplished by detasseling the plants of the female parental line and allowing the plants of the male parental line to pollinate all plants in the field. Usually two rows of male parents alternated with 6 or 8 rows of female parents. As both parents were themselves extremely vigorous single crosses, the height of the plants made detasseling difficult from ground level. Special tractor equipment was designed to straddle rows and bring the personnel above the level of the plants. These operated well on firm soil but could bog down in mud. So any prolonged rainy spell could prove disastrous. The cost of detasseling added several dollars per bushel to the cost of producing commercial hybrid seed, about one-fourth or one-third of the total cost.

The use of cytoplasmic pollen sterility in the female parent to reduce or eliminate the need of detasseling in commercial seed production has become prevalent, but has not always been reliable. The pollen sterility, being cytoplasmic, is transmitted through the female only, but when pollinated by certain male parents, pollen fertility may be partially or wholly restored. With the commonly used T cytoplasm Duvick (1956) has shown that complete restoration requires the simultaneous presence of at least two major dominant genes. One of these, Rf₂, is present in most corn belt lines. The other, Rf₁, occurs in only a few sources and is the major factor in the so-called "restorer" lines. Since either Rf₁ or Rf₂ alone (or with minor modifying genes) may give a variable amount of partial pollen fertility, the pollen sterile female lines should be homozygous recessive for both of the restorer genes (rf₁ rf₁ rf₂ rf₂). This likewise requires that the male lines be homozygous for both of the dominant restoring genes (Rf₁ Rf₁ Rf₂ Rf₂). Thus in a standard four-way hybrid (A x B) x (C x D) the composition of the four inbred lines should be.

| Line A | cytoplasmic pollen sterile | T | rf₁ rf₁ rf₂ rf₂ |
| Line B | normal cytoplasm | rf₁ rf₁ rf₂ rf₂ |
| Line C | normal cytoplasm | Rf₁ Rf₁ Rf₂ Rf₂ |
| Line D | normal cytoplasm | Rf₁ Rf₁ Rf₂ Rf₂ |

To render practicable the breeding of inbred lines which would give a reliable means of eliminating the detasseling operation in hybrid seed production,
genetic studies on the restorer genes are under way in cooperation with Dr. D. N. Duvick of the Pioneer HiBred Corn Co. These studies involve the use of gene-translocation techniques. The restorer gene Rf₁ has been located in the proximal portion of the long arm of chromosome 3, not far from the gene for tassel seed. A report is now in press (Duvick, Snyder and Anderson, 1961). The localization of Rf₂ required the synthesis of special tester stocks. This has been done and the testing is now under way.

In its practical application to hybrid seed corn production, the selected inbred lines must be converted to the genetic compositions given above. For the restorer lines C and D only present routine methods are required plus testing for homozygosis of both Rf₁ and Rf₂.

For the three recessive lines, the cytoplasmic pollen sterile A, the pollen fertile A and B₄, the process is more difficult. The conversions may be greatly facilitated by a chromosomal technique, and for this a knowledge of location of rf₂ is essential.

References:


112. SERIAL DUPLICATIONS IN MAIZE CHROMOSOMES

Investigator: John R. Laughnan
Support: National Science Foundation
Guggenheim Foundation
General Funds

Studies on the Aᵇ complexes in maize indicate that they represent adjacent, serial duplications whose members have retained synaptic homology and in which reside the genetically identified alleles designated alpha and beta. Certain of the alpha isolations from these complexes that occur as nonrecombinants for marker loci have been considered anomalous because their occurrence is not explained according to conventional patterns of recombination. Our analyses indicate that these alpha isolations are not the result of (a) gene mutation of the adjacent beta element, (b) multiple exchanges within localized pairing regions (negative interference), or (c) recombination at the side-chain level. Rather, they suggest that the anomalous alpha derivatives arise as a result of intrachromosomal recombination. According to this model the adjacent, homologous members of the complex may pair with each other to form a double-loop configuration within which an occasional crossover should lead to controlled loss (or gain) of one complete member of the duplication.
113. PROPERTIES AND STRUCTURE OF NUCLEIC ACIDS IN ORGANIC SOLVENTS

Recently much interest and activity have focused on the problem of the secondary structure of nucleic acids as indicated by the studies of denaturation, renaturation and estimations of helical content of nucleic acids under varied conditions. A considerable amount of attention has also been given to the chemical modification of nucleic acids. It is believed that the new systems described below in which nucleic acids were dissolved in organic solvents will provide fresh and fruitful approaches to the above problems.

Although the method of optical rotatory dispersion has been found since 1956 to be useful in studies of the secondary structure of proteins this technique was not applied to the problems of nucleic acids to any large extent until last year. The technique is especially adapted to study of the secondary structures of nucleic acids in organic solvents. A considerable amount of work has been done in this laboratory to develop the approach to the fullest extent.
I. DENATURATION OF NUCLEIC ACIDS IN ORGANIC SOLVENTS

Investigators: George Helmkamp, Ingelore Melvin, Paul O. P. Ts'0o
Support: National Institutes of Health, Public Health Service

Calf thymus DNA and pea microsomal RNA lose all their formal secondary structure when dissolved in formamide or dimethylsulfoxide. The following properties of such nucleic acid solutions provide 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 in water 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.

Publications:


II. STABILITY OF TMV RNA TO SOLUTION IN FORMAMIDE

Investigator: F. William Studier
Support: National Science Foundation
General Funds

Formamide is coming into general use as a solvent for nucleic acids. To test whether the solvent itself has deleterious effects on intact nucleic acid molecules, the time course of possible changes in the infectivity and the sharpness of the sedimentation boundary of TMV RNA (both very sensitive to degradation) with time of solution in formamide was followed. Samples of TMV RNA dissolved in formamide at room temperature for periods of time ranging from 30 minutes to one day had all essentially identical infectivities and sedimentation patterns, indicating that storage in formamide at room temperature does not in itself degrade the TMV RNA. However, compared to the original, untreated TMV RNA, the samples which had been dissolved in formamide had 30 to 40% lower infectivities and a smaller proportion of the total material sedimenting with the sharp boundary characteristic of intact TMV RNA. The cause of this slight degradation in the treated samples has not been established.

III. DENATURATION OF TRANSFORMING DNA BY FORMAMIDE

Investigator: P. O. P. Ts'0o
Support: National Institutes of Health, Public Health Service

The denaturation of DNA by formamide at a given ionic strength and temperature occurs at a critical concentration of formamide and this concentration is similar to that observed by optical rotation. Denaturation has been followed by the loss in transforming activity of D. pneumoniae DNA and by the demonstration of strand separation of N14N15 labeled hybrid E. coli DNA. Formamide denatured transforming DNA can be made to regain its biological activity upon heating the formamide treated DNA at temperatures below the melting temperature.

This work was done at Harvard University in collaboration with J. Marmur.
IV. OPTICAL PROPERTIES OF NUCLEOTIDES AND NUCLEIC ACIDS

Investigators: Paul O. P. Ts'o, George Helmkamp, Ingelore Melvin, C. Sander
Support: National Institutes of Health, Public Health Service

Comparative information about the optical rotatory dispersion (from 340 mµ to 600 mµ) and optical absorbancy of nucleic acids has been obtained through a systematic investigation of solutions of these materials in formamide and in aqueous systems of varied pH and temperature conditions. The increases of specific rotation and the decreases of absorbancy of nucleic acid polymers as compared with their monomeric units were found to be only slight in formamide solution. Under this condition, these polymers are presumed to be devoid of all formal secondary structure. In aqueous solution, on the other hand, the difference between the optical properties of the monomer and polymer pairs was often large and dependent on secondary structure of the polymers. Preliminary results suggest that nucleic acids in formamide in the absence of salt exist in the conformation of an extended chain.

V. RELATIONSHIP OF OPTICAL PROPERTIES TO CONFORMATION

Investigators: George Helmkamp, P. O. P. Ts'o
Support: National Institutes of Health, Public Health Service

Data on the absorbancy and optical rotation of various nucleic acids were analyzed on the assumption that these optical properties can be represented by the algebraic sums of the contributions of three factors: the intrinsic properties of the constitutive monomeric units, the interaction of the groups due to the primary structure, and the interaction of the groups due to the secondary structure. These contributions were evaluated for a number of nucleic acids by varying the pH, the temperature, and the solvent. Comparison of the contribution of secondary structural interaction based on optical rotation data and absorbancy data indicates that the absorbancy of the polymers is less sensitive and the rotation more sensitive to the segment size of the helical region. It was also found that the plot of ([n]_polymer - [n]_monomer) versus wave length follows a one term Drude equation.

Publication:


VI. STRUCTURE AND PROPERTIES OF THYMUS DNA IN FORMAMIDE IN THE PRESENCE OF ELECTROLYTES

Investigators: P. O. P. Ts'o, G. Helmkamp, I. Melvin, F. William Studier
Support: National Institutes of Health, Public Health Service

Previous work has shown that DNA is completely denatured when it is dissolved in pure formamide. Experiments were performed to determine what happens
when DNA is dissolved in formamide containing various concentrations of salt. Samples of thymus DNA were dissolved for 5 to 10 days in formamide containing salt at various concentrations, then precipitated and redissolved in aqueous solution for study. Data from optical density versus temperature, cesium chloride density gradient and viscosity measurements indicate that DNA previously dissolved in 0.4M potassium formamide possesses the same properties as the original native material. However, properties of an aqueous solution of a thymus DNA sample, previously dissolved in formamide of intermediate salt concentration (0.1-0.3M), are intermediate between those of native and fully denatured DNA. These intermediate states appear to be fully stable, the degree of denaturation depending only on the salt concentration at a given temperature, and not changing over a period of several days. These data lend themselves to several interpretations but the evidence from work on T-7 DNA (see following report) suggests that the most likely interpretation is that the partially denatured samples of thymus DNA consist of a population of molecules some of which have been completely and irreversibly denatured and others of which remain fully native. Thus this procedure recommends itself as a possible practical method for fractionation of DNA. The optical and physical properties of formamide solution of DNA with salt have also been investigated. DNA in this condition has higher optical rotation and optical density and lower viscosity than DNA in the comparable aqueous system, suggesting that the DNA in formamide in the presence of high salt concentration may exist in other types of ordered conformation.

VII. STUDIES ON DENATURATION AND RENATURATION OF T7 DNA

Investigator: F. William Studier
Support: National Science Foundation
General Funds

Phage T7 DNA was chosen for study because it is a very homogeneous DNA which contains only "normal" DNA bases with no extra sugars attached. The denaturation and renaturation behavior of T7 DNA upon heating and cooling in salt solutions of varying ionic strength was followed by optical density measurements. In agreement with reports on DNAs from other sources it was found that the melting temperature, the sharpness of the transition and the degree of renaturation upon cooling all increase with increasing ionic strength, and the amount of renaturation at a given ionic strength increases with DNA concentration. Under favorable conditions denatured T7 DNA can be fully renatured so that the melting profile of the renatured sample is indistinguishable from that of a native sample.

Phage T7 DNA, because of its homogeneity, has been used to elucidate the molecular details of denaturation of DNA in FAM (formamide) solutions at various salt concentrations and to clarify the interpretation of the data obtained for thymus DNA. Preliminary results from kinetics of denaturation in FAM-salt as followed by melting profile and density gradient patterns of the reisolated samples in aqueous solution indicate that denaturation of T7 DNA by FAM is irreversible and that no species other than fully native and fully denatured molecules are stable in aqueous solution after this treatment. The rate of denaturation decreases with increasing salt concentration at a given temperature. These findings suggest that the stable, partially denatured states attained by dissolving thymus DNA in formamide at various salt concentrations are due to population heterogeneity. Thus a portion of the molecules in the population of thymus DNA become fully and irreversibly denatured while the remainder of the population remains native, the relative proportions being dependent on salt concentration and temperature. This leaves the interesting question of what factors control the order of denaturation of the molecules in the population. This problem is being pursued by comparing the rates of denaturation of other phage DNAs with that of T7 DNA.
VIII. BROMINATION OF DNA IN FORMAMIDE

Investigator: F. William Studier
Support: National Science Foundation
General Funds

Chemical bromination of DNA is being investigated as a possible means for preparing heavy labeled molecules in vitro. Since adenine is the only base which does not react with bromine it may also be possible to separate complementary strands of a brominated DNA in a density gradient if the two strands contain appreciably different quantities of adenine. A system for bromination of DNA in formamide has been worked out on thymus DNA and appears to give almost complete bromination of guanine and cytosine and an as yet undeterminate amount of bromination of thymine. Due to the heterogeneity of the thymus DNA it cannot be said with certainty that the brominated product is undegraded; bromination of a more homogeneous DNA should answer this question. The brominated thymus DNA does, however, band in the density gradient (around 1.84) and it does not appear to have significant amounts of trailing material in its sedimentation pattern, indicating that wholesale chain breakdown does not occur.

114. CHEMICAL INTERACTION BETWEEN NUCLEIC ACIDS AND NUCLEIC ACID BUILDING BLOCKS (BASES, NUCLEOSIDES, OLIGONUCLEOTIDES, ETC.)

Recent researches on the synthesis of DNA and RNA in vitro with simple enzyme systems and with the nucleoside triphosphates as substrates indicate that both syntheses require a DNA primer. The newly synthesized deoxyribo-polynucleotide or ribo-polynucleotide is similar in base composition and probably in sequence to that of the primer DNA. Studies on the chemical interaction of the nucleic acids and their building blocks are therefore clearly of interest. The binding constants, specificities, the groups and the forces involved in these interactions may give us some insight into the chemical mechanism of the replication of nucleic acids in particular and the replication of the biopolymers in general.

I. INTERACTION BETWEEN BASES AND NUCLEOSIDES--MONOMER-MONOMER INTERACTION

Investigators: Alfred Olson, Paul O. P. Ts'o
Support: U.S. Department of Agriculture
National Institutes of Health, Public Health Service

The purine bases, adenine and guanine, are soluble in water at 25°C only to a very limited extent (1 x 10^-2M to 1 x 10^-5M). The nucleosides of the pyrimidines on the other hand are soluble up to concentrations of 0.4 to 0.8M. If a water soluble complex could be formed between a purine base and a pyrimidine nucleoside, the solubility of the purine in water should be increased by the presence of the pyrimidine nucleoside. Preliminary experiments indicate that in the presence of 0.5M uridine, the solubility of the adenine can indeed be increased up to 3.2 fold. Further experiments are being conducted on the stoichiometry and specificity of this complex formation. Since these results are probably not due to artifacts of solid solution or to changing of water activity, the equilibrium constant and free energy of the reaction can be obtained. Assuming a one to one stoichiometry of the adenine-uridine complex, these preliminary data indicate an equilibrium constant of 3 to 6, and free energy of about one kilocalorie.
II. INTERACTION OF NUCLEIC ACIDS WITH NUCLEOSIDES AND THEIR DERIVATIVES—POLYMER-MONOMER INTERACTION. A. STUDIES BY THE METHOD OF OPTICAL ROTATION

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

DNA and helical poly A (poly-riboadenylic acid) undergo helix-coil transitions within a narrow temperature zone designated as melting temperature (Tm) at a given pH and ionic strength. This melting behavior of the helical polymer can be conveniently and accurately studied by the change of optical rotation versus temperature especially in the presence of highly absorptive materials. The melting temperatures of poly A and DNA at various pH and ionic strength, and in the presence of nucleosides and related compounds maintained at a given pH and ionic strength, were studied by the method of optical rotation measurement.

The effect of the following compounds at various concentrations (0.05M to 0.6M) on the Tm of helical poly A has been studied: uridine, cytidine, thymidine, pyrimidine, adenosine, deoxyguanosine, inosine, purine, adonital, 1-methyl ribose, urea, and phenol. The results obtained so far suggest the following conclusions:

a. The poly A nucleoside interaction as indicated by lowering of Tm (-3° to -22°C) does not follow the Watson-Crick hydrogen bonding scheme of A to T, etc.

b. Substituted functional groups (amino and keto groups) are not necessarily involved since the effect of lowering the Tm of poly A by purine and pyrimidine is the same as that produced by adenosine, inosine, uridine and cytosine.

c. There is no clear indication that hydrogen bonding is involved since compounds having more hydrogen bonding sites are not more effective than compounds with no hydrogen-bonding sites. Urea is 1/15 as effective as pyrimidine and as 1/45 as effective as purine.

d. Compounds with a conjugated double ring system are more effective than compounds with only a single ring. Aromatic and planar character seems to be essential.

e. Sugars are not effective and are not required.

Preliminary investigations with these compounds on the Tm of DNA indicate that the above conclusions are also applicable.

II. B. STUDIES BY THE METHOD OF EQUILIBRIUM DIALYSIS

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

The method of equilibrium dialysis was adopted to study the binding of adenine-C14 to polyuridylic acid and of thymine or uridine-C14 to poly A. A high molar ratio of the polymer (per monomeric unit) to the bases, i.e., 50-5000 to 1, was used in an attempt to demonstrate the binding. The difference of concentration inside and outside of the dialysis were less than 1%, which was within the variation of experiments. This preliminary data indicate that the free energy of binding is much less than 500 calories. This apparent lack of interaction of monomer with polynucleotides and the reported strong interaction between oligonucleotides and the
polynucleotides suggest that a cooperative effect must be involved and this effect may be of importance to the functioning of nucleic acids.

III. INTERACTION OF ADENINE OLigonuCLEOTIDES

Investigators: Richard Squires, George Helmkamp, Paul O. P. Ts'o
Support: National Institutes of Health, Public Health Service

Following the approach to the interaction between nucleic acids outlined above, experiments have been conducted to investigate the interaction of adenine oligonucleotides of varying sizes from \((\text{Ap})_2\) continuously to \((\text{Ap})_{10-12}\). These oligonucleotides were prepared by partial alkaline hydrolysis of poly A followed by fractionation on a DEAE column using a gradient salt elution. The interaction was studied spectrophotometrically by (1) measuring the increase in optical density (hyperchromicity) due to heat or hydrolysis, and (2) measuring the change in the position, bandwidth, and shape of the characteristic absorption band, as a function of temperature, salt concentration, and pH.

1. Intra-reaction at pH 14. Hyperchromicity ranging from 10\% of \((\text{Ap})_2\) to 44\% of \((\text{Ap})_{10-12}\) and 47\% of poly A was obtained after hydrolysis of these oligonucleotides. This hyperchromicity also appears to be a smooth continuous function of sizes and approaches a limiting value at \((\text{Ap})_{9-10}\). Information from the spectrum as well as this high pH suggested that the hyperchromicity is due to intra-reaction of residues in the same molecules and not to inter-reaction between molecules.

2. Interaction at pH 3.0. 0.01M citrate buffer. It is known that the stability of the poly A helix increases with decreasing pH and decreasing ionic strength. The condition of pH 3.0 is the limiting case which can be conveniently used without fear of depurinization. In this condition, then, all the adenine oligonucleotides larger than \((\text{Ap})_6\) appear to exist mostly in helical conformation at room temperature. The helical conformation of \((\text{Ap})_{12}\) was so stable at this pH that there is essentially no increase in optical density upon heating up to 99\°C.

3. At intermediate pH, such as 4.85, 0.2M salts, the oligonucleotides of \((\text{Ap})_{12}\) at room temperature still cannot resume the helical conformation, while the poly A had a helical conformation until heated to 62\°C, the melting temperature.

115. NUCLEOTIDE COMPOSITION AND MELTING TEMPERATURE OF VARIOUS RIBOSOMAL RNAs

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

The RNA of ribosomes may carry genetic information related to the sequence of amino acids in the soluble proteins synthesized by these particles. The ribosomal RNA of reticulocytes is specially interesting since the bulk of the protein made in the reticulocytes is of a single type, hemoglobin. A comparison of the ribonucleotide compositions of ribosomes from plant and rabbit reticulocyte has already been reported (Biology 1960).

The ribonucleotide composition of reticulocyte ribosomes from three sheep, two of which were homozygous for the common hemoglobin, HbI, and the third which was homozygous for the rare hemoglobin HbII, is shown in table 1. Published data indicate that these two hemoglobin types are determined by a single gene and that there is a difference of about 30 to 36 amino acids between these two hemoglobins, which contain a calculated total of 590 amino acid residues.
Ribonucleotide Composition of Sheep Reticulocyte Ribosomes

<table>
<thead>
<tr>
<th>Sheep</th>
<th>Hb type</th>
<th>U</th>
<th>G</th>
<th>A</th>
<th>A</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Hb II</td>
<td>0.180 ± 0.004</td>
<td>0.310 ± 0.006</td>
<td>0.188 ± 0.005</td>
<td>0.322 ± 0.003</td>
</tr>
<tr>
<td>2</td>
<td>Hb I</td>
<td>0.193 ± 0.007</td>
<td>0.327 ± 0.006</td>
<td>0.178 ± 0.005</td>
<td>0.302 ± 0.013</td>
</tr>
<tr>
<td>3</td>
<td>Hb I</td>
<td>0.173 ± 0.003</td>
<td>0.344 ± 0.008</td>
<td>0.180 ± 0.006</td>
<td>0.303 ± 0.002</td>
</tr>
</tbody>
</table>

The differences in composition between individuals of the two hemoglobin types are not significantly greater than the differences between individuals of the same hemoglobin type. It is concluded that the nucleotide compositions of these two types of sheep reticulocyte ribosomes are quite similar.

The ribosomal RNA from sheep contain 63 to 64\% G + C, while the ribosomal RNA from peas contains only 53\% G + C. The $T_m$ (temperature for the 50\% increase of the total hypochromic effect) of sheep RNA (69°C) is higher than that of pea RNA (66°C). The magnitude of the increase of the $T_m$ (3°C) is also similar to that for DNA having a difference of G + C to A + T of 9.5\% (Marmur and Doty, Nature 183, 1427, 1959). The magnitude of the hypochromic effect of the sheep RNA having high G + C is also lower than that of the pea RNA, as was expected from the DNA results. The data suggest that base pairing similar to that in DNA may also occur in RNA.

Publications:


116. BINDING OF SILVER IONS TO RNA

Investigator: F. William Studier
Support: National Science Foundation
General Funds

The effect of silver ions on the sedimentation of TMV RNA and on the change of optical density with temperature of pea ribosomal RNA has been studied. In 0.1M acetate solutions it was found that the presence of silver ions in molar ratios of silver ions to RNA bases of as little as 1:10 affects the melting profile of pea RNA by lowering the relative increase of optical density with increase in temperature, and that at a ratio of one silver ion to one base the optical density of the solution does not change at all from 30 to 90°C. The sedimentation behavior of TMV RNA in solutions of 0.1M sodium acetate at silver to base molar ratios of from 1:10 to 10:1 was studied. With no silver present the TMV RNA sediments as a single component of about 30s, but upon addition of silver ions aggregated material appears. However, the bulk of the material sediments as one fairly sharp component at silver ratios of 1:10 to 2:1, the sedimentation coefficient of the major component gradually increasing with silver to base ratio from 30s with no silver present to 50s at a 2:1 molar ratio. At a 10:1 silver to base composition most of the material is aggregated and there is no single main component.
117. PURIFICATION AND FRACTIONATION OF ISOLATED PEA NUCLEI

Investigators: Max L. Birnstiel, Joon H. Rho, Alan Huber, Robert J. Hayes
Support: National Academy of Sciences
National Science Foundation
National Institutes of Health, Public Health Service

Fractionation of pea nuclei into cytologically, chemically and functionally characteristic fractions was achieved by disintegrating the nuclei with sodium citrate and subsequent differential centrifugation.

Nuclei were isolated from young pea stems or from 36-hour-old pea seedlings according to Rho, et al. The nuclei were separated from starch and cytoplasmatic contaminants by centrifugation in a two-layer sucrose system. Nuclear suspensions were homogenized in an Omnimix in the presence of citrate (0.2-10mM; pH 7.2). liberating 60 to 80% of the nucleoli as intact particles. Centrifugation at increasing speeds sedimented sequentially: starch, unbroken nuclei, RNA rich nucleolar apparatus and DNA rich chromatin-like particles. In the submicroscopic range, a fraction containing most of the nuclear DNA and a ribosome-like, RNA rich sediment was recovered. This leaves principally proteins and a little RNA (5%) and DNA (3%) in the supernatant.

With increasing citrate concentrations increasing RNA/DNA ratios were obtained in the nucleolar and ribosomal fractions. Excess of citrate decreased the RNA content of the nucleolar apparatus, partially disintegrated the chromatin and released up to 20% of the total RNA and 8% of the total DNA into the supernatant.

118. CYTIDINE INCORPORATION INTO RNA BY ISOLATED NUCLEI

Investigators: Joon H. Rho, Margaret I. H. Chipchase, Robert J. Hayes
Support: National Academy of Sciences
National Institutes of Health, Public Health Service
General Funds

Since it is in general difficult to obtain intact nuclei from ground tissue, a new method for the liberation of the nucleus from the cell contents has been devised. This method consists of passing the plant tissue through a set of spring-loaded counter-rotating rollers and the simultaneous addition of tris buffered isotonic sucrose solution containing calcium ions. A semi-automatic machine has been constructed to perform this operation on a rather large scale and in a rapid way. The plant tissues are first chopped into one millimeter sections and then carried through to the rollers. The pressure exerted by the rollers pops the cells gently open, allowing the nuclei to be liberated uninjured. The nuclei thus collected in buffered medium are quickly purified by filtration and centrifugation.

Such nuclei rapidly incorporate tritiated cytidine into RNA under the suitable condition. The additions of adequate amounts of calcium ions and neutral pH, conditions which are required for the stability of the material, are essential for the optimum activity of isolated nuclei. The incorporation of cytidine into RNA is almost wholly dependent upon the presence of the other three nucleoside triphosphates and is increased by the addition of an energy-regenerating system.
119. THE SITE OF RNA SYNTHESIS IN THE ISOLATED NUCLEUS

Investigators: Joon H. Rho, Robert J. Hayes
Support: National Academy of Sciences
National Institutes of Health, Public Health Service
General Funds

Although there is increasing cytological evidence that a substantial portion of the cytoplasmic RNA is synthesized in the nucleus and subsequently migrates to the cytoplasm, the site of RNA synthesis in the nucleus is yet variously proposed to be the nucleolus, the chromatin or both.

In the present work nuclei isolated from pea tissue by a new method which consists in passing the tissues through a set of counter-rotating rollers and simultaneous addition of sucrose medium were used for a study of RNA synthesis in the subnuclear components. By fractionation of cytidine incorporated nuclei it was found that uptake of cytidine into RNA took place in two regions of nuclei, namely, chromatin and nucleoli. Cytidine uptake by chromatin at the early time intervals was at a much faster rate than other fractions, whereas incorporation of tritiated cytidine into nucleoli showed a gradual increase following a prolonged lag period.

When nuclei were first exposed to cytidine containing medium for a short period and then the radioactive RNA precursor pool was diluted with an excess amount of unlabeled cytidine triphosphate, a decrease of radioactivity in chromatin RNA occurred much earlier than in the case of nucleolar RNA, so that the increase in the labeling of the nucleoli actually corresponded to the decrease in the labeling of the chromatin materials. Therefore, it appears that the labeled RNA was first synthesized in the chromatin particles and transferred to the nucleolus.

120. INCORPORATION OF C$^{14}$-L-LEUCINE BY A CELL-FREE PREPARATION OF PEA NUCLEI

Investigators: Max L. Birnstiel, Margaret I. H. Chipchase, Robert J. Hayes
Support: National Science Foundation
National Institutes of Health, Public Health Service

Nuclei prepared from 4-day-old pea stems or from 36-hour-old pea seedlings actively incorporated labeled L-leucine into proteins.

Nuclei were prepared in a sucrose-calcium chloride-tris medium according to Rho, et al., and were incubated with C$^{14}$-L-leucine. It has been found that the presence of 0.003M calcium chloride and 0.3M sucrose and pH 7.0 (0.02M tris) are essential to maintain the structural integrity and maximal activity of the nuclei. Incorporation of leucine is independent of the presence of added pH 5 fraction or of pH 5 supernatant. Addition of a complete amino acid mixture enhanced incorporation of the labeled leucine by 25%. The addition of an adenosine triphosphate regenerating system similarly doubled such incorporation. Sodium ions were found to increase incorporation slightly, but potassium, manganese and magnesium ions exerted no effects. TPNH, DPNH and glutathion are also ineffective. The presence of a complete mixture of the nitronucleosidetriphosphates failed to give increased incorporation of leucine. Thus RNA synthesis is not essential to leucine incorporation in these nuclei.

Under optimal condition, incorporation by these nuclei lasts for 25 to 40 minutes at 37°C and 100 µmmoles C$^{14}$-L-leucine/mg nuclear proteins are recovered in hot TCA stable, 1M sodium hydroxide resistant compounds.
121. A SITE OF PROTEIN SYNTHESIS IN PEA NUCLEI

Investigators: Max L. Birnstiel, Margaret I. H. Chipchase, Robert Hayes
Support: National Science Foundation
National Institutes of Health, Public Health Service

Incubation of isolated nuclei and their subsequent fractionation into subnuclear components (as described above in report No. 117) reveals that the nucleolar apparatus is the principal site of incorporation of tritiated leucine into acid and base resistant compounds, presumably protein.

Nuclei incubated in tritiated leucine for 1.5 minutes at 37°C were found to contain labeled protein predominantly in the nucleolar fraction. The chromatin and ribosomal fractions were found to be but slightly labeled. After a longer (5 minutes) incubation, the activity in the chromatin fraction attained a level equal to that in the nucleoli. To confirm that this pattern is the result of transfer of labeled material from nucleolar apparatus to chromatin and ribosomal fractions, cold leucine was added in approximately 1,000 fold excess after 5 minutes of preincubation with H^-DL-leucine. It has been found that after such addition the specific activity of the protein in the nucleoli decreases, while that of the remaining nucleoplasm (predominantly that of the chromatin) gained, indicating transfer of material.

122. S-RNA IN PEA NUCLEI

Investigators: Margaret I. H. Chipchase, Max L. Birnstiel, Robert J. Hayes
Support: National Institutes of Health, Public Health Service
National Science Foundation

Carbon-14 labeled amino acids have been fed to isolated pea nuclei in vitro and a labeled amino acid soluble RNA complex subsequently extracted from them. Fractionation of the nuclei, as described in report No. 117, had shown that the soluble RNA is bound to the particulate fractions. It can be extracted from them by the phenol method and purified by 2M sodium chloride. Approximately 40% of the total RNA is solubilized by the phenol method and both nucleolar and ribosomal fractions are rich in this small molecular RNA.

Nuclei incubated for four minutes with tritiated cytidine contain labeled small molecular RNA only in association with the DNA rich chromatin. During longer incubations the chromatin fraction increases in specific activity and only a minor proportion of labeled small molecular RNA appears in other nuclear fractions. These experiments show that the chromatin is a major site of small molecular RNA synthesis in the nucleus.

123. STREPTOMYCIN INHIBITION OF NUCLEAR ACTIVITY

Investigators: Margaret I. H. Chipchase, Robert J. Hayes
Support: National Institutes of Health, Public Health Service

Experiments in this laboratory have shown that isolated nuclei from pea embryos can incorporate tritiated thymidine under conditions similar to those described for cytidine incorporation (report No. 118). Under these conditions the incorporation of thymidine is only one tenth that of cytidine by identical nuclei.

Incorporation of thymidine, cytidine and amino acids has been used as a relative measure of DNA, RNA and protein synthesis in the presence or absence of
streptomycin. In this way it has been shown that streptomycin inhibits protein synthesis to an inconsiderable extent and DNA synthesis by 45% whereas RNA synthesis is inhibited by approximately 90%.

This inhibition will be used to study the interrelationships of the various nuclear syntheses. The first conclusion is that nuclear protein synthesis is not dependent on concurrent RNA synthesis.

124. SYNTHESIS OF RIBONUCLEIC ACID BY PARTICULATE PREPARATIONS FROM NORMAL AND TMV-INFECTED TOBACCO

Investigators: S. Maheshwari, R. S. Bandurski
Support: National Institutes of Health, Public Health Service
National Science Foundation
General Funds

An attempt has been made to obtain in vitro biosynthesis of tobacco mosaic virus (TMV) RNA. Since it is possible that the cell nucleus may be involved studies were directed primarily at RNA synthesis by nuclei. Homogenization of leaf tissue in a meat grinder followed by differential centrifugation yields preparations in which the ratio of chloroplasts to nuclei has been reduced from an initial 500:1 to approximately 5:1. Incubation of these preparations in a reaction mixture containing C14-ATP, UTP, CTP, GTP, phosphoenolpyruvate, fructose diphosphate, and pyridine nucleotide results in the incorporation of C14 radioactivity into RNA. The reaction is dependent upon the presence of all four nucleoside triphosphates and is augmented by the addition of fructose diphosphate and pyridine nucleotide. The rate of incorporation is approximately 250 µmoles/mg protein/hour, for nuclei prepared from either normal or TMV infected leaves. Approximately 1% of the phenol extractable infectious RNA of the homogenate is associated with the nuclear fraction and this infectivity does not increase during incubation of nuclei in the above described reaction mixture.

125. THE ENZYMATIC SYNTHESIS OF RNA

Investigators: Ru-chih C. Huang, Nirmala Maheshwari, James Bonner
Support: National Institutes of Health, Public Health Service
Frasch Foundation

The enzyme system with which the present work is concerned possesses the property of synthesizing RNA, using the four riboside triphosphates as the substrates. This enzyme system is composed not only of protein, but of DNA, and is in fact the chromatin fraction of the cell. This laboratory has developed a large-scale procedure for the isolation of such chromatin from pea embryos. The homogenized embryonic tissue is first filtered through miracloth and then centrifuged at 4,000 x g. The gelatinous chromatin of the pellet is scraped from the underlying starch and purified by recentrifugation.

Synthesis of RNA by this purified and dialyzed preparation is dependent upon the presence of the DNA, since degradation of the latter by RNAase inactivates the RNA synthesizing ability. It is dependent too upon the presence of an enzyme or enzymes, since the deproteinized DNA is similarly inactive. The two moieties are however closely associated in a nucleoprotein complex, which is not dissociated even in media of high ionic strength.

RNA synthesis by this preparation is dependent upon the simultaneous presence of the four riboside triphosphates. If but one is omitted, RNA synthesis is
abolished. Although the amount of RNA synthesized by the complete system is sufficient to detect by spectroscopic means, use has routinely been made of ${{}_{14}C}$ labeled adenosine triphosphate and followed the incorporation of such label into RNA, as a measure of RNA synthesis.

The system catalyzes a further reaction, namely the exchange of $p^{32}$ labeled pyrophosphate into the residual riboside triphosphates. This reaction also is completely dependent upon the simultaneous presence of the four riboside triphosphates. We have found in addition that the amount of pyrophosphate equilibrated into the riboside triphosphates bears a stoichiometric relationship to the amount of nucleotide incorporated into RNA. Approximately four moles of pyrophosphate are exchanged for each mole of base thus incorporated.

These facts suggest that RNA synthesis proceeds in the present instance from the riboside triphosphates with the elimination of pyrophosphate. It further suggests that some sort of random trial followed by acceptance or rejection is the mechanism at work in production of the proper base sequence in the newly synthesized RNA.

Publications:


126. THE PHYSICAL STATE OF NEWLY SYNTHESIZED RNA

Investigators: Ru-chih C. Huang, Nirmala Maheshwari, James Bonner
Support: National Institutes of Health, Public Health Service Frasch Foundation

The enzymatic synthesis of RNA by a DNA-containing chromatin fraction of pea embryos has previously been described. The RNA thus newly synthesized is not, however, released as free RNA but remains attached to the chromatin with which it is sedimented at 10,000 x g. The newly synthesized RNA possesses the interesting property, while in this association, which is referred to as complex, of complete resistance to attack by RNAase. That the protection of RNA from RNAase is due to the DNA of the chromatin is indicated by the fact that degradation of the DNA by DNAase renders the RNA susceptible to RNAase attack.

The newly synthesized RNA may also be released by heat. Thus the RNA is sharply and completely liberated from the complex, in the sense that it is caused to become susceptible to RNAase, by brief heating to 50–60°C (in 0.015M sodium chloride). The melting profile of the newly synthesized RNA-chromatin complex is to be contrasted with that of the DNA of the chromatin nucleoprotein which (when deproteinized by the Sevag procedure) melts at 71°C (in 0.015M sodium chloride). The newly synthesized RNA is thus bound in a complex of stability less than that of the DNA itself.

A substantial portion of the newly formed RNA accompanies the DNA through deproteinization by the Sevag procedure. Such RNA is again resistant to attack by RNAase. That the newly formed RNA is thus protected by association with DNA is
again indicated by the facts that (a) it is liberated by the action of DNAase and (b) it is liberated by solution of the preparation in formamide, a solvent in which secondary structure of nucleic acid is abolished.

It is concluded therefore that the enzymatically synthesized RNA is produced in close association with the DNA, which constitutes an essential component of the system. That this association is attributable to hydrogen bonding is evidenced both by the melting behavior of the complex and by its disruption in formamide.

127. POLYNUCLEOTIDE PHOSPHORYLASE

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

Various plant materials have been examined for the presence of polynucleotide phosphorylase. This enzyme, which catalyzes the synthesis of polynucleotides from the nucleoside diphosphates ADP, GDP, CDP and UDP was found to be abundant in the cells of the green alga Chlorella. It has been purified and some of its properties studied. Preparations with high specific activity were obtained from particle-free extracts by means of several precipitations with ammonium sulfate and ethanol after inert proteins had been previously removed. With adenosine diphosphate as substrate synthesis of polyadenylic acid was catalyzed by such purified preparations; when polyadenylic acid was used as substrate in the presence of radioactive phosphate, labeled adenosine diphosphate was formed. A requirement for the presence of primer in the polymerization reaction could not be demonstrated.

128. PROTEIN SYNTHESIS

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

These investigations have to do with the direction and manner of growth of the peptide chains of hemoglobin. It has been found that separation of the α and β chains of rabbit hemoglobin is best accomplished after alkaline oxidation of the globin which is then passed through a short column containing a cation exchange cellulose, Cellex-P. Sharp, reproducible peaks are eluted by 5, 6, and 8M urea solutions. The peptide chains so separated are recovered in 70 to 92% yields by acid-acetone precipitation. The material released by 5M urea appears pure; that released by 8M urea is slightly contaminated by the first. The material eluted by 6M urea is a mixture of the two but can be rechromatographed.

Hemoglobin was obtained from rabbit reticulocytes incubated for 15 minutes at 15°C with Borsook's amino acid mixture containing arginine-C14. Chain separation was carried out as above and the separated chains hydrolyzed with trypsin. The peptides were then separated by electrophoresis and the arginine-containing peptides subjected to paper chromatography (n-butanol:acetic acid: water = 57:14.5:28.5). There are two such peptides per chain: 5M urea elutable chain: Rf 0.46-0.47 (5L) and Rf 0.54-0.57 (5H); 8M urea elutable chain, Rf 0.24-0.25 (8L) and Rf 0.51-0.59 (8H). The arginine-containing peptides were then hydrolyzed with hydrochloric acid. The individual arginines, separated by paper chromatography (ethanol:water:diethylamine = 77:22:1) possessed specific activities (counts per minute per micromole) of 5L, 36.6 + 4.4; 5H, 26.3 + 1.6; 8L, 23.3 + 0.5; and 8H, 16.2 + 1.7. Thus the individual peptide chains exhibit non-uniform labeling of a type to be expected on the basis of sequential chain growth.

It would be elegant and helpful if peptide chains could be split only at arginine residues. The present investigation has revealed that there are three
arginine residues per chain; this being the case, cleavage at arginine alone should result in only four peptides. Trypsin is known to specifically attack lysyl and arginyl bonds. Hence it is only necessary to mask the lysines. This has been done by reacting the chain with carbobenzoxy chloride which reacts with the epsilon amino group of lysine. The chain is then hydrolyzed with trypsin, the carbobenzoxy group removed, and the soluble fraction separated by means of starch block electrophoresis at pH 3.6. The peptides were easily separated, but recoveries of only 30 to 40% were obtained. Electrophoresis on a vertical polyurethane felt column has overcome this difficulty and yielded almost complete recovery.

129. FRACTIONATION OF RIBOSOMES

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

It is desirable to be able to fractionate that portion of the ribosome directly involved in the synthesis of protein from the inactive portions of the ribosome. Such fractionation should not involve high salt concentration or use of strong acid or alkali. A suitable system should have good solvent capacity for ribosomes but be without effect on chemical bonds involved in protein synthesis. Solution of the ribosome is first of all necessary for clear-cut fractionation. Since the organic solvent formamide is miscible with water, is essentially neutral, and possesses other unique properties, its effectiveness for fractional extraction of ribosome has been investigated.

Three-times recycled ribosome was homogenized in water. Formamide and water were then added to the homogenate to obtain a set of serial dilutions of formamide. The most striking effect was produced by 66% formamide. This completely solubilizes the ribosome to yield a water-clear solution. Upon centrifugation at 41,000 x g, a small pellet is sedimented. Repeated extraction of the pellet yields little material. On the basis of extractable phosphorus, 66% formamide solution extracts about 78% of ribosomal RNA. Upon removal of the water from the extract by lyophilization, the bulk of the protein is precipitated while the RNA remains in solution. The precipitate is insoluble in 95% formamide. Thus, this method offers three ribosomal fractions for closer scrutiny. Fractionation of labeled ribosomes has therefore been undertaken.

Rabbit reticulocytes were incubated for 1) 15 minutes at 15°C, 2) 5 minutes at 37°C, and 3) 180 minutes at 37°C in Borsook’s amino acid mixture containing arginine-Cl4. Ribosomes isolated from the above incubation were radioactive. When such ribosomes were extracted with 66% formamide, all the radioactivity was solubilized from (1), 78% from (2), and 72% from (3). When water was removed from the 66% formamide extracts, 34% of the activity of (2) remained in supernatant; 40% of (3). Since ribosomal protein is not synthesized in reticulocytes, the radioactivity is due to incorporation of arginine-Cl4 into the growing peptide chain. Hemoglobin is not soluble in formamide so it is reasonable to suppose that the unextractable fraction is composed of large, almost completed protein. In the case of ribosomes, obtained from incubation of actively growing pea shoots with valine-Cl4, and extracted in a similar manner, only 50 to 60% of the radioactivity was solubilized. Since pea shoots synthesize ribosomes, it is not unreasonable to suppose that part of the valine-Cl4 is incorporated into the structural protein. Therefore, it is also possible that the insoluble fraction of reticulocyte ribosome contains some structural protein. Short peptides are quite soluble in formamide so the smaller peptides remain in solution when water is removed. Hence there is a concentration of short peptides in the 99% formamide solution and as a consequence the specific activity of such peptides should be very high.
The three fractions obtained by solution of ribosomes in formamide might well represent: (1) fraction not extractable with 66% formamide—RNA probably bound to some structural protein containing almost completed chains; (2) fraction insoluble in 99% formamide—bulk of structural protein and medium-sized chains; (3) fraction soluble in 99% formamide—bulk of RNA and small peptides.

130. NATURE OF THE MAJOR COMPONENTS LOST DURING PURIFICATION OF RIBOSOMES

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

The rate of protein synthesis by ribosomes in vitro is but a fraction of that in vivo. In addition, as ribosomes are purified the rate of protein synthesis falls drastically. Is the decreased synthetic ability due to loss of cofactor, loss of certain aspects of the template necessary to initiate and continue peptide synthesis, or a combination of the above?

In order to learn whether substances essential to protein synthesis are lost during the preparation of clean ribosomes, a systematic investigation has been made with pea shoots and rabbit reticulocytes. Pea ribosomes represent a heterogeneous population which synthesizes several different proteins. Reticulocyte ribosomes on the other hand are essentially homogeneous since hemoglobin is the principal product.

Valine-\(^{14}\)C and leucine-\(^{14}\)C are readily incorporated in vivo into the ribosomes of pea shoots and of reticulocytes. When ribosomes pelleted at 105,000 \(\times\) g are resuspended, the insoluble removed by centrifugation at 41,000 \(\times\) g (pellet designated L) and the supernatant again pelleted at 105,000 \(\times\) g (pellet designated H), pellet L is always much more radioactive than pellet H. As the ribosomes are further purified by resuspension and recentrifugation, the specific activity of the 105,000 \(\times\) g pellet continues to decrease. This is not due to loss of adsorbed C\(^{14}\)-amino acid. Pellet L of each clarification step is always higher in radioactivity than that of the corresponding pellet H. It appears that peptides of high specific activity are being lost to pellet L in the purification procedure. Paper chromatography and electrophoresis reveal many ninhydrin positive bands from pellet L, but few from pellet H.

In addition to its higher specific activity, pellet L also has a higher phospholipid (12%) content than pellet H (4%). Nevertheless, pellet L has 16% RNA content, whereas pellet H has 35 to 40% (about 50% in reticulocyte ribosomes). On the basis of extractable phosphorus, 95% of the RNA of pellet L is solubilized with 40% formamide, while pellet H loses only 12%.

It is as yet uncertain whether RNA and phospholipid are chemically bonded to the growing peptide. It does appear, however, that pellet L contains the majority of the shorter peptides together with other components essential to protein synthesis. Furthermore, the nature of the RNA in pellet L appears to be different from that in pellet H. Whether the type of RNA being lost to pellet L is essential to protein synthesis has not been elucidated.
131. THE BIOSYNTHESIS OF TERPENOIDS

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

A cell-free enzyme system from peas which converts mevalonic acid to neutral lipids has been previously described (Biology 1959, page 82). The identification of several intermediates and products of these reactions has now been accomplished. Thus, the pathway mevalonic acid \( \rightarrow \) mevalonic acid-5-phosphate \( \rightarrow \) mevalonic acid-5-diphosphate \( \rightarrow \) isopentenyl pyrophosphate \( \rightarrow \) dimethyl allyl pyrophosphate \( \rightarrow \) geranyl pyrophosphate \( \rightarrow \) trans-farnesyl pyrophosphate \( \rightarrow \) nerolidyl pyrophosphate has been established for the pea enzyme system. Further evidence suggests that the same system is found in germinating mung beans, mature whole Kentucky Wonder beans and green peppers.

The reactions described above are identical to those found by other workers in studies on the biosynthesis of cholesterol. In the plant systems, however, the final products represent pivotal points from which branch pathways for the biosynthesis of carotenoids, terpene hydrocarbons, gibberellins and components of the essential oils—products not produced by animal tissues, as well as the pathway leading to the steroids.

132. POLYSACCHARIDE SYNTHESIS

Investigators: Alfred C. Olson, Clifford J. Pollard
Support: U. S. Department of Agriculture
U. S. Atomic Energy Commission

The objective is to find out how cell wall polysaccharides are synthesized by studying the incorporation of labeled monosaccharides into polysaccharides in vitro. Carbon-14 labeled glucose and fructose is incorporated into hot water soluble polysaccharide (I) and hot water insoluble polysaccharide (II) by a particulate preparation from germinating mung beans. Efficiency of incorporation is high, amounting to 5 to 30% of the labeled monosaccharide supplied. Both I and II are insoluble in boiling 80% ethanol and are immobile on paper chromatograms in several different solvent systems.

Incorporation into I is dependent on the age of the seedling and on the presence of a small amount of inorganic phosphate in the incubation medium. The following unlabeled materials do not dilute the incorporation of labeled glucose into I: ATP (with or without the addition of magnesium chloride), GTP, CTP, UTP, DPN, TPN, xylose, sucrose, arabinose, glucolactone, glucuronic acid, glucosamine, fructose, or glucose-1-phosphate. Addition of unlabeled glucose or glucose-6-phosphate does reduce incorporation of labeled glucose into I.

According to Hassid and co-workers the path of glucose incorporation into polysaccharide begins as follows: glucose \( \rightarrow \) glucose-6-phosphate \( \rightarrow \) glucose-1-phosphate \( \rightarrow \) UDP-glucose. Polysaccharide formation may use either glucose-1-phosphate or UDP-glucose as substrate. The failure of glucose-1-phosphate to reduce incorporation of glucose and of adenosine triphosphate and magnesium chloride to increase incorporation suggests that the biosynthetic path to I may not involve any of the steps in this scheme.

Glucose is apparently incorporated intact since uniformly labeled glucose, glucose-1-C\(^{14}\) and glucose-6-C\(^{14}\) are incorporated to the same extent.
Product I is resistant to dilute acid hydrolysis at 100°C for short periods of time and to prolonged treatment with crystalline alpha and beta amylases. More severe acid hydrolysis or overnight treatment with commercial alpha and beta amylase, pullidase, malt diastase or pectinase yields products which resemble oligosaccharides in chromatographic behavior and in addition a monosaccharide which does not appear to be glucose. The structure of both I and II is still under investigation.

Reference:

133. STUDIES OF AN ANTHOCYANIN-DEGRADING ENZYMATIC SYSTEM FROM IVY

Investigator: J. E. Scheibe
Support: National Institutes of Health, Public Health Service

One striking difference between the adult (flowering) and juvenile forms of Hedera helix (ivy) is that the juvenile is more or less colored with anthocyanins while in the adult these pigments are absent. Slices of the young adult stem were found to decolorize anthocyanin extracts of ivy berries. Red cabbage extracts were also decolorized but extracts of grape and ripe olives were not. Further, these inert extracts when mixed with the Hedera pigment preparation completely inhibited its decolorization by ivy stem slices. The principal anthocyanin of Hedera was tentatively determined to be idaein (3-galactoside of cyanidin) by chromatographic comparison with the pigments of Aralia japonica, which are also decolorized by the system under investigation.

Activity was retained in a protein fraction of Hedera prepared by acetone and ammonium sulfate precipitations. The enzyme possesses ordinary pH optimum and thermolability characteristics. Since the substrate could not be supplied in pure form, little could be learned about the reaction itself. However, the decolorization is not a simple glycosidation since the anthocyanidin cannot be recovered from the mixture after the reaction has been completed. Specificity appears not to be restricted to the aglycone portion of the molecule since the olive anthocyanin, which is not attacked by the enzyme, has an identical ring structure, differing only in the sugar attached at the 3-position. Specificity is not, however, restricted to idaein, since the red cabbage anthocyanin component, which has markedly different absorption spectra, is an equally suitable substrate. Thus the present evidence indicates that (1) decolorization is the result of an attack upon the aglycone portion, (2) the nature of the sugar residue in the 3-position is one of the factors determining whether or not the reaction can take place, and (3) the reaction may be inhibited by anthocyanins which are not substrates.

The enzyme was found to be present in juvenile tissue in approximately the same concentration as in the adult. If this system is responsible for the difference in pigmentation between the two forms, its activity must be curtailed in the juvenile. So far, it has not been possible to demonstrate the presence of an inhibitor.

134. CHEMICAL AND MECHANICAL ANALYSIS OF ROOT GROWTH

Investigator: D. James Morre
Support: National Institutes of Health, Public Health Service
Frasch Foundation

It is known that indoleacetic acid (IAA) in concentrations which promote shoot growth normally inhibits root growth. The mechanical and chemical responses of maize roots to IAA have therefore been compared with those of Avena coleoptiles.
The nature of the inhibition is unknown but some insight into the process has been gained from measurements of growth rate. The progress curve of root growth is characterized by an initial rapid increase in rate followed by an abrupt cessation of growth. Although IAA in sufficiently high concentration may reduce the growth rate of maize roots somewhat, its primary effect is to shorten the period over which such roots elongate.

The initial responses of roots to application of growth substance have been examined in detail. Measurements of the deformability of the rapidly elongating region under artificially imposed load have revealed a concentration-dependent, IAA-induced plasticity component absent in untreated roots. Half-maximal plasticity after one hour is induced by around $5 \times 10^{-7}$M IAA (a concentration comparable to that which elicits half maximal plasticization of Avena coleoptiles). The response is transient and is replaced (after about two hours of incubation in IAA) by the complete rigidity associated with growth inhibition.

Associated with increased plasticity is rapid formation of a carbazole-positive, cold water soluble, 70% ethanol insoluble material (presumably containing a polygalacturonic acid moiety), designated as cold water soluble pectin (CWSP). Normally undetectable in elongating root sections, its concentration approaches that in non-elongating tissue following IAA treatment. Incubation of root sections for one hour with the IAA concentration necessary to induce half maximal plasticity does not alter the concentration of the cold water insoluble pectic substances associated with the cell wall, i.e. the classical pectin fraction solubilized in hot water and the remaining residual pectic substances extractable in hot 0.5% EDTA.

Although technical difficulties preclude a simple assessment of IAA-induced growth rate, changes in fresh weight associated with IAA treatment indicate a substantially accelerated growth rate during the first hour. This would be expected because of the concurrent increase in cell wall deformability. The initial responses of roots to IAA therefore resemble those of Avena coleoptiles both qualitatively and quantitatively. The end result, however, is to effectively shorten the period over which the root can elongate.

135. THE METABOLISM OF A KININ, BENZYLADENINE-C14

Investigators: Dennis R. McCalla, D. James Morre, Daphne J. Osborne
Support: National Research Council of Canada
National Institutes of Health, Public Health Service
Rockefeller Foundation
General Funds

While the physiological effects of kinetin (6-furfurylaminopurine) and kinetin analogs have been the subject of considerable investigation, there is no published information on how these compounds are metabolized. Results with senescing Xanthium leaves suggest that 6-benzylaminopurine (benzyladenine or BA), which possesses physiological properties similar to those of kinetin, is converted to benzyladenine riboside (BAR) and benzyladenine ribotide (BAMP) as well as into small amounts of adenylc, guanylic, and inosinic acid, adenine and hypoxanthine. Authentic BAR was synthesized enzymatically and compared with the compound from Xanthium. The presence of BAMP is inferred from the fact that phosphomonoesterase treatment of a component from the Xanthium extract yields a compound having properties similar to those of the riboside. With respect to the above compounds, the metabolism of BA appears to be similar to that of other purines.

A degradative pathway for purines also appears to be operative in Xanthium leaves since a number of urea derivatives were found to contain label. One of these,
tentatively identified as allantoic acid, is present in relatively large amounts while urea and allantoin are present in smaller amounts. No incorporation of BAMP into polynucleotides could be found in three systems, Xanthium, E. coli and maize root tips, although in view of the low specific activity of the BA used and the necessarily low concentrations employed, the data are equivocal. In Xanthium, the upper limit of incorporation of BA into RNA is of the order of one molecule of BA for every 10 molecules of RNA of molecular weight two million. Results with E. coli and maize root tips provide further evidence that BA does not appear to be incorporated into nucleic acid in any substantial amount.

In contrast to results with the previously mentioned systems, bean leaves form a single major metabolite following BA treatment. Although the bean metabolite has not been identified, it appears to be different from any of the compounds produced in Xanthium. It is of interest in this connection that both in Xanthium and in bean, the metabolism of BA by rapidly growing leaves is similar to that of senescent leaves.

136. THE ROLE OF KINETIN WITH RETARDATION IN LEAF SENECEENCE

Investigator: Daphne J. Osborne
Support: Rockefeller Foundation

When leaves of any age are detached from the plant and kept with their petioles in water they speedily undergo a series of biochemical changes which resemble those of the normal yellowing, senescence and death of mature and attached leaves. If, however, a detached leaf forms root-initials along its petiole these changes may be indefinitely delayed and the leaf remains green.

A feature of senescence in either attached or detached leaves is the declining ability of the cells to synthesize new protein in spite of an ample supply of nitrogenous intermediates. This results in a fall in the level of total protein. When a detached leaf forms roots the ability to synthesize protein is restored, suggesting that the roots supply a factor which is necessary for protein synthesis in leaf cells. In a number of species the "root-factor" has been shown to be replaced by treatment of the leaf-blade with a plant hormone, such as kinetin or 2,4-dichlorophenoxyacetic acid.

Kinetin is effective in delaying senescence in excised leaves of Xanthium pennsylvanicum. Experiments with both entire leaves and discs of leaf blade in this species have shown that the fall which occurs in the level of chlorophyll is temporarily retarded in the presence of kinetin, while the levels of protein and RNA may be temporarily increased by this treatment before eventually declining to the control levels.

The incubation of leaf-discs with 14-C-labeled leucine or orotic acid in the presence or absence of kinetin has shown that both the incorporation of leucine into protein and orotic acid into RNA, and the specific activities of the isolated protein and RNA, are increased in the presence of kinetin.

Results suggest that the role of kinetin in the retardation of leaf senescence may be mediated through its action on nucleic acid and protein metabolism.

Reference:

RAPID BIOASSAY FOR KINETIN AND KININS

Investigators: Daphne J. Osborne, D. R. McCalla
Support: National Research Council of Canada
Rockefeller Foundation
General Funds

Attempts to isolate kinetin-like compounds (kinins) from natural sources have been hampered by lack of a simple, rapid, sensitive and reliable bioassay for these materials. Kinins not only promote cell division, but also retard the loss of chlorophyll in detached Xanthium (cocklebur) leaves. The latter effect has been used as a measure of kinin activity over the range 0.05 to 5 µg kinetin equivalents. Leaf discs are placed in a small petri dish on a piece of filter paper moistened with 0.5 ml of the extract to be tested. The small petri dish is then enclosed in a larger one containing damp filter paper. The plates are incubated in the dark at 25°C for about three days. After this time the remaining chlorophyll is extracted with 80% ethanol, the extract made up to a standard volume and the optical density measured at 665 mµ. The procedure is calibrated with a series of known kinetin concentrations.

Publication:

PHYSIOLOGICAL STUDIES ON A PROGRESSIVE NECROTIC MUTANT OF CORN

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

The chemical CMU [3-(p-chlorophenyl-)l,1-dimethylurea] specifically inhibits photosynthesis of corn seedlings by about 90%. CMU treatment delays the onset of necrotic symptoms in plants homozygous for the necrotic rd gene (Biology 1960, 1959) and in addition, reduces the rate at which the remaining leaves become necrotic.

This finding together with earlier data suggests that the basic lesion in necrotic rd corn lies in a reaction associated with photosynthesis, which leads to the accumulation of one or more substances toxic to the cell membranes. The necrotic phenotype appears to result from breakdown of cell compartmentalization.

RIBONUCLEIC ACID SYNTHESIS IN THE BUD: AN ESSENTIAL COMPONENT OF FLORAL INDUCTION IN COCKLEBUR

Investigators: James Bonner, Jan A. D. Zeevaart
Support: National Science Foundation
Frasch Foundation
Netherlands Organization for the Advancement of Pure Research (Z.W.O.)
General Funds

The cocklebur flowers only if the leaves are exposed to at least one dark period longer than 8-1/2 hours. Application of 5-fluorouracil (5-FU) at the beginning of such an inductive dark period inhibits subsequent floral development. The
Present studies are concerned with the mechanism by which 5-FU exerts its flower-inhibiting effect.

By application of labeled 5-FU either to the leaf or the apical bud, it has been shown that 5-FU is rapidly translocated from leaf to bud during a 16-hour night, but not in the reverse direction. This indicates that 5-FU exerts all of its inhibition effects in the apical bud.

5-FU is incorporated into bud RNA. Moreover it inhibits both RNA and DNA synthesis as measured by the incorporation of labeled orotic acid.

Inhibition of flowering by 5-FU can be reversed by simultaneous application of orotic acid. Kinetic studies have shown that the inhibition has become completely irreversible after 8 hours of darkness. At the same time 5-FU application in the middle of a 16-hour inductive dark period is almost without effect, so that all the bad effects of 5-FU are exerted during the first half of a 16-hour dark period.

A specific inhibitor of DNA multiplication, 5-fluorodeoxyuridine (5-FDU), also powerfully inhibits development of floral primordia, but in contrast to the effect of 5-FU, this inhibition can still be fully reversed by thymidine at the end of a 16-hour dark period. This demonstrates that DNA multiplication in the bud during the inductive dark period is not essential for induction.

It has been concluded, therefore, that RNA synthesis in the bud is the essential dark period process which is inhibited by 5-FU and which is related to photoperiodic induction.

Reference:

RNA and DNA synthesis in Pharbitis buds, as measured by net increase, is inhibited by 5-FU. 5-FU is also incorporated into RNA.

5-Fluorodeoxyuridine (5-FDU), which is known to inhibit the methylation of deoxyuridylic acid to thymidylic acid, exerts a flower-inhibiting effect similar to that of 5-FU but in lower concentration. The effects of both inhibitors are reversed by thymidine, thymidylic acid, 5-methyldeoxycytidine, deoxycytidine, and deoxyuridine, provided that the application is made to the bud before the inhibitory effect has been exerted. The substances which antidote flower-inhibition suggest that the latter is based on competitive inhibition of the enzyme thymidylate synthetase.

The floral stimulus begins to move out of the cotyledons immediately after a 16-hour dark period, as was demonstrated by removal of the cotyledons at various times. This provides some clue as to the lifetime of the floral stimulus in the bud, since the inhibitors suppress DNA multiplication only for 2 to 3 days. The results suggest that the floral stimulus is perceived by the bud and results in floral initiation only if the bud DNA is multiplying.

141. THE NATURE OF PHOTOPERIODIC INDUCTION IN PERILLA LEAVES

Investigator: Jan A. D. Zeevaart
Support: National Science Foundation
Frasch Foundation
Netherlands Organization for the Advancement of Pure Research (Z.W.O.)

Perilla crispa, the red perilla, is a short-day plant which requires several long nights for flowering. It has been shown previously that induction of Perilla leaves is an irreversible process. Even three months after the leaves are transferred to non-inductive conditions they are still able to supply floral stimulus to a receptor plant in grafting experiments. It has therefore been concluded that an induced leaf continues to produce the floral stimulus even after transfer to non-inductive conditions.

Recent experiments in which Perilla plants were exposed to various numbers of long nights have shown that threshold induction also remains preserved in leaves after transfer to non-inductive conditions. These results lend support to the idea that various degrees of the induced state can exist in the leaves, the degree depending upon the number of long nights to which the leaf had been exposed.

Some investigation has been made concerning the nature of induction in the Perilla leaf. Neither application of 5-fluorouracil nor 2-thiouracil exerts any inhibitory effect on the induction process, although the higher concentrations of the material cause severe damage to the leaves. The same has been found to be true of ethionine, an inhibitor of protein synthesis.

5-Fluorouracil does, however, cause inhibition of floral bud development, but this inhibition in the bud has not been studied in detail.

Chemical analysis has shown that there is a net synthesis of RNA and DNA that takes place in fully expanded leaves during induction, but that a similar increase in nucleic acids takes place in comparable non-induced leaves.

It is concluded that neither nucleic acid nor protein synthesis is directly involved in photoperiodic induction of Perilla leaves.

Reference:
In many plants with a long-day or a cold requirement for flower formation, gibberellin application results in shoot elongation and flowering under "non-flowering" environmental conditions. It therefore was interesting to investigate the part which endogenous gibberellins may play in natural shoot elongation, flower induction and development. In the present study, two varieties of black henbane (Hyoscyamus niger) were used, one requiring long-day treatment only and the other requiring cold-treatment followed by long days. Extractions were made from non-induced plants and from plants exposed to different durations of inductive conditions. The extracts were made with methanol, purified by means of ethyl-acetate partitioning and then fractionated on a charcoal-celite column by elution with increasing concentrations of acetone. The fractions were tested for activity in a dwarf-pea bioassay (see report No. 144). In both varieties there was one gibberellin activity elutable with 60% acetone present in both vegetative and flower-induced plants. After 10 to 20 days of induction, when the plants either initiated flowers or possessed microscopic flower buds, four additional activities (elutable in 50, 70 and 90% acetone and after prolonged washing with 100% acetone) were present; at a still later stage the entire activity level decreased sharply, the 60% acetone fraction being the only one present.

Thus flower initiation in both varieties of Hyoscyamus niger is associated with substantial quantitative as well as qualitative changes in the gibberellin content of the plants. These changes follow the same pattern both in long-day and low-temperature-induced plants. Preliminary studies with another test plant (the d-5 dwarf mutant of maize) indicate, however, that there are still other gibberellin-like materials in the plants, not detectable with peas. The behavior of these gibberellins must be studied further in order to obtain a more complete picture of the relations between flower formation and gibberellin content of Hyoscyamus.

Reference:

Cucumber plants pass through a definite pattern of flowering, consisting of three successive stages. The first nodes of the main stem carry only staminate (male) flowers. This is followed by a mixed phase in which both male and pistillate (female) flowers are produced, and a final female phase in which only pistillate flowers are formed. This flowering pattern is controlled by genetical, environmental and chemical factors. Plants carrying a certain gene (st) may completely lack the first two phases, starting their flowering with the last, purely female phase. Another gene (m) may cause the formation of bisexual (hermaphrodite) flowers. Minor shifts
in sex expression are also caused by a group of polygenes. Long days and high temperatures extend the male phase, the opposite conditions the female phase. Applied gibberellins also extend the male phase; applied auxins hasten—although to a relatively lesser degree—the onset of the mixed and female phases.

The present study was started with two approaches. One was to investigate the content of native gibberellins in cucumber plants in order to determine whether there exists a relationship between sex expression, as determined by genotype and environment, and the gibberellin content of the plants. Plants or parts of them were extracted and tested by the methods worked out in this laboratory (see preceding report). Added gibberelin A\textsubscript{3} (gibberellic acid) was recovered at a very high percentage, proving that this method is suitable for cucumbers. Results so far clearly indicate the existence of gibberellins in cucumber plants. However, the activity levels were quite low, and attempts are being made to improve the results by use of other assay plants.

Another approach is to investigate sex determination in vitro, by culturing excised floral buds or stem tips which produce flower buds. Cucumber flowers are initiated as bisexual organs; male and female flowers originate by failure of the female and male parts (pistils and stamens), respectively, to develop beyond a certain, early stage. It is clearly interesting to know whether the environmental and chemical factors which regulate sex expression act directly upon the young flower or whether their action is mediated through the apex or through larger parts of the whole plant. So far, it has been possible to grow male flowers from a sexually indeterminate to a clearly recognizable male stage, although the anthers were smaller than in comparable flower buds in situ. The medium used was White's mineral medium with addition of 2% saccharose, 0.8% agar, amino acids, vitamins and kinetin. All these supplements gave better and more extended growth than did the basic medium (minerals and sugar). Additions of the auxin indoleacetic acid (IAA) and gibberellin (gibberellic acid, GA\textsubscript{3}) to the medium caused considerable further increase in development as compared to the controls. The ratio of the two regulators seemed to be more critical than the absolute levels, combinations of 3.0 mg/l GA\textsubscript{3} and 0.3 mg/l IAA or 1.0 GA\textsubscript{3} and 0.1 mg/l IAA giving better growth than any other combinations.

Reference:

144. AN IMPROVED DWARF-PEA BIOASSAY FOR GIBBERELLINS

Investigators: E. Reinhard, A. Lang
Support: National Science Foundation
General Funds

Since gibberellins are present in higher plants only in very small quantities it is not possible to detect these substances by any reliable chemical means. Therefore a variety of bioassays has already been developed, using different test materials such as seedlings of single-gene dwarf mutants of maize, dwarf pea varieties and leaf sections from wheat and oat seedlings. However, most of these assays suffer from one drawback or another. The plant material for some is available only in limited quantities, prohibiting use on a routine scale, the sensitivity of others is relatively low and limited to a narrow concentration range; still others require relatively long periods of time.

The following bioassay lacks most of these drawbacks. The test plant is the dwarf variety of Progress No. 9 of Pisum sativum; other dwarf varieties are probably also suitable. The peas are germinated in darkness at 23°C, selected for
uniformity after four days and transplanted to nutricultures with half-concentrated Hoagland solution. When about 2 cm high, the plants are treated with test or standard solutions of gibberellin by applying drops (0.005 ml) per plant; thereafter, they are kept in red light. Measurements of total height are made 5 days after treatment. The lower sensitivity limit of the test is 0.0004 µg gibberellin A3 (gibberellic acid) per test plant. The dose-response curve for gibberellin A3 is linear over a range of 0.001 - 0.1 µg per plant, when using a log-log plot. The test is highly sensitive to gibberellin A3. The other gibberellins which are so far known give the same growth-response if applied at levels about 10 times (A1, A7), 100 times (A2, A4, A5), 1,000 times (A6, A8, A9) as high as A3.

145. A CUCUMBER SEEDLING BIOASSAY FOR GIBBERELLIN A4

Investigators: E. Galun, Yvonne Jung
Support: National Science Foundation

The bioassay methods for gibberellins which are presently available and which are based on the use of dwarf varieties of peas, maize and other plants or of sections of leaves from oat or wheat seedlings are adequate for gibberellin A3 (gibberellic acid) and several of the other known gibberellins. But there seems to be no appropriate, equally sensitive bioassay for gibberellin (GA4). Utilizing the already known specific sensitivity of cucumber seedlings to GA4, an assay was developed for this substance, based on the effect of GA4 on the elongation of the hypocotyl of three-day-old seedlings. The seedlings are raised on moist tissue in plastic boxes in the dark; the test itself is done in light. Results can be recorded 22 hours after application. The threshold of sensitivity is at 0.001 µg per test plant, the response seems to be linear in the range of 0.001 to 0.01 µg GA4. The bioassay is apparently specific for gibberellins, as indoleacetic acid, applied alone or in combination with GA4, had no effect on the growth of the seedlings.

146. CONTROL OF DIRECTION OF CELL DIVISION IN ROSETTE PLANTS

Investigators: M. Negbi, Aurora Agatep, A. Lang, Kurt S. Anderson
Support: National Institutes of Health, Public Health Service
National Science Foundation
American Cancer Society
General Funds

The purpose of this project was to study factors which control the orientation of cell division in the plant shoot, and thus help to insure organized development of the latter. The first stages of this study were reported in Biology 1960 (p. 90; No. 115). Since then, the following results were obtained.

A. Hyoscyamus niger (black henbane). Application of gibberellic acid (GA) increased the mitotic activity in the subapical meristematic tissue by 5 to 10 fold (24 hours after application). The majority of the divisions thus induced are "transverse" division, i.e. their spindle axis is parallel to the longitudinal axis of the plant. It is clear that this orientation of cell division is an essential factor in normal shoot development. Complete defoliation (with application of GA) reduced somewhat the number of cell divisions at the first 24 hours, but the orientation of the spindle was greatly reduced. Experiments with partial defoliation showed that one young leaf of 10 mm in size or one fully expanded leaf was sufficient to re-establish a significant portion of spindle orientation. This suggests that there is practically no "wound-effect" caused by defoliation. With respect to maximal mitotic activity a lag of about 48 hours was found in the defoliated plants. The different defoliation
treatments with the lag in maximal mitotic activity suggest that some influence from the leaves is necessary for normal orientation of the subapical cell divisions. Preliminary experiments indicate that addition of auxin (3-indoleacetic acid) is able to maintain at least part of the normal orientation of these divisions in GA-treated defoliated plants.

B. Crepis parviflora (biennial hawksbeard). This long-day rosette plant was chosen because of its karyotype. It has fewer \(2n = 8\) and larger chromosomes than Hyoscyamus \(2n = 34\). This makes the scoring of mitotic activity easier and faster. The experiments so far confirmed the basic findings concerning two other rosette plants, Hyoscyamus and Samolus parviflorus (Sachs, et al. 1959), and the present study concerning Hyoscyamus. Bolting and flowering was induced by repeated applications of GA in plants kept under short days. The bolting is due mainly to increase in the mitotic activity in the subapical meristem, though so far the extent of the increase was not the same as in Hyoscyamus. Defoliation affected orientation of the spindle much as in Hyoscyamus; it lowered it in the control plants from 50 to 25\%, and in the GA-treated ones from 75 to 35\%. However, the reproducibility of the results in Crepis was so far not satisfactory, and more tests are needed to determine whether the species will be suitable for this type of study.

C. Raphanus sativus (radish). Preliminary trials with a pronounced biennial variety of this rosette plant showed that it is very responsive to GA, even as seedling with only the cotyledons unfolded. Single applications of GA caused bolting in young seedlings as well as in established rosettes. Since the plant is considerably faster-growing than Hyoscyamus and Crepis, it may be a superior material for further work.

Reference:


147. PHYSIOLOGY OF SUGAR PINE EMBRYOS

In common with many other pines, seeds of sugar pine (Pinus lambertiana) require several months of incubation in a cold, wet environment before they will germinate. However, embryos isolated from untreated seeds can be cultured without benefit of cold treatment. A study of some aspects of the physiological behavior of embryos during the first few hours after removal from dry seeds was undertaken to provide information upon which further work can be based.

Embryos do not undergo a respiratory increase as a direct result of removal from the endosperm. Respiration is almost immeasurably slow until after hydration. Embryos absorb water very rapidly and reach an equilibrium weight of about twice the initial weight in less than an hour. There is no further net water uptake for at least 24 hours. Respiratory oxygen uptake lags behind water uptake and requires about 4 hours to reach a steady state. Dry embryos presented with a solution of glucose-\(\text{C}^{14}\) hydrate activate metabolic systems rapidly enough to produce readily measurable amounts of \(\text{C}^{14}\text{O}_2\) within 10 minutes. Rapid initial water uptake cannot be used to sweep large amounts of labeled sugars into the embryonic tissue. Sugar uptake is more rapid after hydration and activation of respiration has been completed.
Leaching of metabolites into the ambient solution during hydration is very appreciable and in 3-hours may exceed half the water-soluble dry weight of the embryos. The leachate includes bases, acids, and sugars. Passive movement of sugars into the tissue is minimal and when labeled glucose is fed labeling of the free glucose pool is minor. All data are compatible with the concept that glucose is phosphorylated during active uptake.

The respiration of 3-hour hydrated embryos is only slightly inhibited by malonate even though labeling of intermediates suggests activity of the Krebs cycle. The ratio of C\textsubscript{14}O\textsubscript{2} release from glucose-1-C\textsubscript{14} to that from glucose-6-C\textsubscript{14} is initially about 5 to 1, indicating that the pentose phosphate cycle probably mediates such a large fraction of the respiration that inhibition of the Krebs cycle with malonate cannot greatly reduce O\textsubscript{2} consumption.

148. DORMANCY AND GROWTH CONTROL IN WOODY PLANTS

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

An extensive literature review and problem analysis has been made of dormancy, morphogenesis, and growth control in woody plants. This will be the basis of experimental work on the biochemical mechanisms controlling dormancy and growth. Publication of the review and analysis as a monograph is anticipated.

149. STUDIES ON SEEDLING GROWTH OF CONIFERS

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

Increased emphasis on reforestation programs has increased the demand for knowledge concerning requirements for good seedling establishment and growth. The present study concerns these matters.

The growth response of Jeffrey pine seedlings to temperature has been found to be different for different characters. The seedlings were grown for six months under 15 combinations of day and night temperatures in the artificial light rooms of the Earhart Plant Research Laboratory.

Maximum total dry weight production was obtained with about 350 total daily degree-hours. The maximum was obtained under cool nights and warm days as well as under warm days and cool nights provided only that the total daily degree-hours requirement was met. Distribution of dry weight between tops and roots was however affected by the day-night temperature combination. A warming of the day, a cooling of the night, or a combination of both favored top production while the reverse conditions, a cooling of the day, a warming of the night, or a combination of both, favored root growth.

Secondary needle formation was also increased by high day and low night temperatures.
150. INTERACTION OF TEMPERATURE AND PHOTOPERIOD UPON SHOOT AND NEEDLE ELONGATION OF COULTER PINE

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

Previous work with Coulter pine and other coniferous species has suggested that the effects of temperature and of photoperiod upon elongation of shoot and needles are interrelated. Furthermore, night temperature appears to be the more critical.

Dormant seedling of Coulter pine were therefore placed under long (16 hours) or short (8 hours) photoperiod at three night temperatures. All plants received natural light and 26°C for 8 hours and either 26°C, 17°C, or 7°C for 16 hours in total darkness or with 8 hours of supplemental light. The temperature-controlled greenhouses and rooms of the Earhart Plant Research Laboratory were used for the study.

Shoots elongated under both long and short photoperiods. However, under the long photoperiod elongation was about twice that under the short photoperiod at all three temperatures.

Needles also elongated under both long and short photoperiods but a photoperiodic response was elicited only under the highest temperature. With the 26°C night the needles under long photoperiod were almost double the length of those grown under short photoperiod. The needles under short photoperiod elongated at the same rate as did the needles at the 17°C under both long and short photoperiods, and better than the cold needles.

Thus, it appears that shoot elongation for Coulter pine is both photoperiodic and temperature controlled over a range of temperatures while needle elongation responds to photoperiod only under temperatures optimal for needle growth.

151. EFFECTS OF AIR AND ROOT TEMPERATURE ON ROOT PENETRATION OF PONDEROSA PINE SEEDLING

Investigator: M. M. Larson
Support: U.S. Forest Service
General Funds

Throughout the range of ponderosa pine, droughts commonly occur after seed germination and new seedlings must therefore quickly produce a penetrating taproot in order to survive the drying out of the upper soil layers. This study concerns the influence of various soil and air temperatures on initial root penetration by pine seedlings.

Ponderosa pine seedlings were grown in 1-inch diameter by 20-inch long glass tubes filled with vermiculite. The tubes were immersed in controlled temperature water baths at 7°C, 15°C, 23°C, and 31°C to provide different root temperatures. Different air temperatures were obtained by placing one set of water bath units in each of the 7°C, 15°C, 23°C, and 31°C constant temperature rooms in the Earhart Plant Research Laboratory.

Results to date indicate best root penetration when seedling tops are kept at 7°C or 15°C and roots at 23°C. Maximum root penetration amounted to more than 40 cm in a 6 weeks period. Roots at 7°C developed poorly regardless of the top temperature and penetration was less than 10 cm in 6 weeks. Roots were also greatly retarded at all root temperatures when the tops were kept at 31°C. Best top development occurred in the 23°C air temperature.
152. INTERACTIONS BETWEEN GIBBERELLIN AND VARIOUS ENVIRONMENTAL FACTORS

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

The interactions between gibberellin and various environmental factors on stem elongation of greenhouse-grown Phaseolus vulgaris have been studied in steady-state growth systems. The action of gibberellin was found to be independent of rate of accumulation of photosynthetic products, root activity, temperature, water availability and both ultraviolet and X-radiation. It was also shown that gibberellin interacts completely with red/far-red radiation given at the end of the daily light period. This interaction is complete even when maximum growth rates are experimentally reduced by limiting various independent factors. These results are interpreted as demonstrating that red/far-red radiation controls rate of stem elongation in greenhouse-grown plants through an effect on the endogenous gibberellin system of the plant. None of the other environmental factors studied acts through the gibberellin system.

Publication:

153. MECHANISM OF THE INFLUENCE OF SHADE ON STEM ELONGATION

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

It has already been reported (Biology 1960) that the photo-inhibition of stem elongation of beans at high light intensities is caused by an influence of light on the gibberellin metabolism of the plant.

Pea varieties come in two sizes, dwarf and normal. The normal, tall varieties respond very little to gibberellin, indicating they are nearly saturated with respect to endogenous gibberellin, while dwarf varieties are very deficient in gibberellin as demonstrated by their spectacular response to added hormone. If high intensity light acts on stem elongation by affecting gibberellin metabolism, dwarf plants should be much more sensitive to high intensity light than normals. Experiments with dwarf and normal peas confirmed this prediction.

In further experiments dwarf (d1) Zea mays, which is dwarfed due to insufficient natural gibberellin, responded markedly to shading. That is, in shade it was much less dwarfed than in full sun. The normal segregates (D1), which respond little to gibberellin, show very little response to shading. Similarly, d1 plants when treated with saturating doses of gibberellin also show very little response to shading.

Further experiments with beans demonstrated that high-intensity light and red/far-red radiation act on the same step in the gibberellin metabolism system.

Publication:
Among the environmental factors which influence plant growth, temperature is of major importance. However, the mechanism by which temperature exerts its action is, in general, obscure. The hypothesis has been made that temperature affects the production or destruction of one or more essential metabolites. If this idea is correct, it should be possible to prevent temperature damage by supplying from an external source the substances which limit growth. Experiments have been designed to test the hypothesis. Such experiments can at the same time reveal which substances limit growth under particular unfavorable temperature conditions and, therefore, which biochemical processes are modified by particular temperatures in a particular plant species.

Instances which support the above idea have been reported in previous years (Biology 1959, 1960). Further examples have now been found. Application of a mixture of the ribosides (3 x 10^{-4} M of each) increases the dry matter accumulation of eggplant grown in suboptimal temperatures by 50 to 60% over a period of three weeks as compared with plants treated with water. Increases in dry weight of 40 to 50% are obtained by application of a vitamin B mixture or of the mixture of ribosides to lupin plants (var. nanus blue) grown in supra-optimal temperatures. In these cases the substances have been applied regularly as a foliar spray. Other modes of application may be effective too. For example, soaking tomato seeds in a 100 mg/l nicotinic acid solution for 24 hours before planting causes a 30% increase in dry weight production during the first three weeks of growth when the plants are subsequently grown in suboptimal temperatures (20°C day temperature, 14°C night temperature). The nicotinic acid treatment does not however affect day of first flower, fruit set or fruit maturation. Seed soaking followed by regular spraying of the plants with 50 mg/l causes a 50% increase in dry weight, but does not influence flowering or fruit set.

The evidence obtained clearly indicates that the chemical prevention of climatic injury is a reality, even though complete prevention of any injury is rare. The fact of the chemical cure of climatic lesions suggests that climate and in particular temperature influence growth through direct effects on specific biochemical processes of the plant.

The question has been asked whether periodic changes in the environment are required for normal plant development. Consideration of this question will be restricted here to the light-dark periodicity in constant temperature. It has already been shown that tomato plants exhibit an absolute requirement for a daily dark period. In the absence of such a dark period the new leaves are chlorotic and the plants eventually die. Because of the similarities between the syndrome caused by periodicity and that caused by lack of synchronization, it may be suggested that both are caused in the same way, namely by interference of the environment with the internal periodicity.
The behavior of the tomato plant is not however representative of that of the plant kingdom generally. Thus, pea, peanut and soybean plants do not show any injury when grown in continuous light. These plants grow as well in continuous light as in any regime with alternating light and dark periods. Peas flower earlier and stop growing earlier in continuous light. The dry matter production of all three plants is directly proportional to the length of the light period between 8 and 20 hours light per 24-hour cycle. Increasing the length of the photoperiod beyond 20 hours either does not affect dry matter accumulation at all or is relatively ineffective in doing so. In general, however, the absence of periodic alternation of light and dark is in no way injurious to pea, peanut or soybean plants.

Cycle lengths other than 24 hours do however cause reduction in growth of these plants, as in tomatoes. Again peas, peanuts and soybeans do not show injury other than growth reduction, while tomatoes do. The effect of abnormal cycles on soybeans is rather small. Because these plants do respond to cycle length and because the optimal cycle length is 24 hours, it may be concluded that proper synchronization between external and internal periodicities is required for normal development. This leaves the effect of continuous light on tomatoes an unexplained oddity—either the injury is not related in any way to disturbances in the internal periodicity, or continuous light affects the internal periodicity of tomato plants in a way different from that of other plants. It is possible that the effect of continuous light on the growth of tomatoes is secondary.

156. EFFECT OF CONTINUOUS LIGHT AND ABNORMAL LIGHT REGIMES ON THE GROWTH BEHAVIOR OF PEAS

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

Experiments reported in Biology 1960 and at the Cold Spring Harbor Symposium 1960 showed that there was a carry-over effect of the light regime in which peas were grown. These experiments are being continued. While these experiments have shown an immediate effect, i.e., that plants grown in continuous light formed seeds which in the next generation were superior to seeds formed in a light regime of 16 hours light, 8 hours dark, it was not known whether this effect would be apparent for more than one generation. Experiments are now in progress to determine whether the carry-over effect does indeed last for more than one generation.

Plants are also being grown in abnormal light regimes such as 24 hours light, 24 hours dark, and in continuous light to study the carry-over effect of such light regimes as well as the effect of these light regimes on the endogenous clock system of peas.

Publication:

157. CARRY-OVER EFFECT OF THE PREVIOUS ENVIRONMENT ON THE GROWTH OF PEAS

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

It has been reported that there may be a carry-over by the pollen of environmental influences on the pollen parent. These studies of the relationship between the previous environment and the effect on the progeny are being continued with special emphasis on the relative contribution of the male and female plants. Crosses have been made both between and within environments, reciprocally, and the progeny of these crosses are being tested at various environments.

Publication:


158. INTERRELATIONS OF DIURNAL RHYTHMS AND PHOTOPERIODIC RESPONSES IN THE GENUS CESTRUM

Investigator: Wesley O. Griesel
Support: American Academy of Arts and Sciences

Various species of the genus Cestrum show rhythmic as well as photoperiodic responses in relation to their blooming. Cestrum nocturnum (night-blooming jasmine) exhibits a partial photoperiodic response to floral initiation and a diurnal rhythm in flower opening and odor production. Cestrum diurnum (day-blooming jasmine) exhibits an apparently complete photoperiodic response in flowering and a diurnal odor rhythm. Cestrum purpureum does not have either of these responses.

These three species have been grafted in combinations and to date have shown no effect on each other in flower induction or in altering or inducing diurnal rhythms of odor and flower opening.

The effects of auxin on flowering of the two species that show photoperiodic responses have been studied. Plants whose natural auxin supply or distribution have been altered by removal of the terminal bud, bending of the main stem, etc., show an increase in vegetative activity and an accompanying decrease in floral initiation and ability to mature flowers. In both species the increase in vegetative activity occurs in regions in which the auxin decrease would be expected to be the greatest in accordance with our present knowledge of auxin distribution and migration. Day-blooming jasmine shows a more polarly oriented response than does night-blooming jasmine.

The data suggest that the three species studied differ in the systems which control floral initiation, odor production and flower opening. It is of interest that photoperiodically-induced floral initiation is not transmissible by grafting in Cestrum as it is in most other photoperiodically sensitive plants.
Growth regulators are necessarily involved in geotropism since geotropic curvature is caused by different growth rates of the two sides of a plant organ. A difference in growth regulator activity on the two sides of a geotropically stimulated organ has been frequently found. The question remains as to whether the geotropic role of growth substances is restricted to the final growth reaction. Are growth regulators involved in the perception of the gravitational stimulus and how does the unequal distribution of growth regulator activity come about?

A start has been made in the investigation of these matters. Sunflower seedlings have been used. Growth substances are produced by the tip of the seedling and the production center can be easily removed by decapitation. Such decapitated plants do not lose their ability to carry out geotropic curvature without external supply of auxins even after 5 days in constant darkness. The nature of the geotropic reaction does change, however. The minimum stimulation time increases from a few minutes for freshly decapitated plants to 1 hour for plants kept in darkness for 4 days after decapitation. Geotropic curvature reaches a maximum and then reverses itself when intact plants are placed in the vertical position after stimulation. Such reversal is slower and incomplete in decapitated plants. Time lapse movies have clearly revealed these differences.

The finding that decapitated plants can continue to carry out the geotropic reaction is at variance with other data reported in the literature. Further examination has shown that the results depend on the variety of sunflowers used. Tall varieties, such as Russian Mammoth, are able to curve 5 days after decapitation. Shorter varieties may lose this ability 1 to 2 days after decapitation. Apparently the growth rate at the time of decapitation is decisive. High growth rate indicates that the ability to carry out curvature will be maintained for a long time after decapitation.
160. VISUAL DISCRIMINATION LEARNING IN SPLIT-BRAIN MONKEYS; FURTHER STUDIES OF DOUBLE LEARNING WITH CONFLICTING TASKS

Investigator: Colwyn B. Trevarthen
Support: Arthur McCallum Fund

Reports made in Biology 1959 and 1960 describe a method for presenting two contradictory visual discrimination tasks simultaneously to split-brain monkeys with the aid of plane-polarized light. This "balanced learning" technique enables one to test for independence of learning in the two separated visual systems, and also to investigate factors which affect the outcome when two conflicting learning processes interact at deeper levels.

In a number of cases the two visual tasks have been learned simultaneously as if by completely independent brains, one connected to each eye. In others, however, there has been some interaction leading to partial or complete suppression of learning by one eye. This selection within learning must be the result of some internal attentional set, because there are no external signs that one eye is not in use. When vision is forced to the inattentive eye by covering the other eye there are erratic shifts in the level of performance, or else the performance stays at an intermediate level. These effects could result from a swinging of attention back and forth between the favored, but covered, eye and the free one. There is a general tendency for eye and hand of opposite sides of the body to be used together. Spontaneous changes of hand-use precede the visual learning, as if the developing habit to respond in a particular way sets the visual system for learning by a particular eye. With practice the monkeys show less bias towards contralateral eye-hand pairing, and eventually they settle into a habit for consistent use of one hand regardless of which eye is in use. The split-brain monkeys used in these experiments were operated upon by Dr. H. L. Arora.

161. CROSS-INTEGRATION OF VISUAL AND TACTILE PERCEPTION

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

Almost nothing is known about the brain processes underlying any form of mental association. In the present experiment the split-brain approach is being
utilized to learn something about the brain events involved in a perceptual task that requires interaction between visual and tactile sensation. Specifically the test requires the monkey to pull one of two levers, the handles of which differ in roughness or shape or size, etc. The levers remain out of sight and the one that is correct on a given trial depends on which of two visual patterns are presented with it. The right-left placement of the levers as well as the two visual patterns are both interchanged on a random schedule.

The results to date indicate that this close and mutually dependent association of visual and tactile cues does not necessarily involve a close topographic association of the neural processes for touch and vision in the cerebral cortex. The performance succeeds under conditions of the most extreme separation where the visual cues are fed into one hemisphere, the tactile into the other and the two hemispheres are separated surgically by division of the brain down through the quadrigeminal plate and cerebellum.

162. CROSS-INTEGRATION OF BASIC PERCEPTUAL ATTRIBUTES

Investigators: Colwyn B. Trevarthen, R. W. Sperry
Support: Arthur McCallum Fund
National Institutes of Health, Public Health Service
Frank P. Hixon Fund

Cross-integration of visuomotor and visuotactile activities has been demonstrated in split-brain animals. The nature and means of the cross-communication remain to be worked out. Analysis is being continued in the visual sphere where clear separation of the input is more readily assured. Tests for size discrimination have been started using pairs of open circles of different sizes, with one of each pair projected to each eye via polarizing filters. Three split-brain subjects, one with the brain divided through the quadrigeminal plate, have succeeded in discriminating for size under these conditions. Further tests will be made involving other basic perceptual properties like height, number, sharpness, curvature, intensity, obliqueness to see first if there are differences in the brain levels at which these different perceptual attributes can be cross-communicated.

163. TESTS FOR PRESENCE OF TWO ACTIVE PERCEPTUAL PROCESSES IN SPLIT-BRAIN MONKEYS

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

The demonstration of double simultaneous learning of contradictory perceptual tasks in split-brain monkeys suggests the presence of two separate active perceptual learning and memory processes running simultaneously in parallel in the separated hemispheres. The alternative is a rapid alternation of attention and learning from one hemisphere to the other from trial to trial during the learning session or even more rapidly within a period of several seconds in a single trial.

The aim of the present experiment is to test further for a doubling of perceptual activity under conditions that restrict more rigorously the possibilities for rapid alternation in use of the divided hemispheres. The perceptual task involves the correct selection of one or two visual stimulus patterns, the correct one being determined by its match with a preceding visual signal.
The visual signal is presented tachistoscopically through projectors with gradual reduction of the exposure time to the minimum threshold. At present one split-brain monkey is down to $1/10$ second in the training involving both eyes. The critical tests will involve restriction of the input to one or the other or both hemispheres on a random schedule to find out if both or only one hemisphere is attentive and perceiving at any given moment.

164. INTEROCULAR TRANSFER OF BRIGHTNESS AND COLOR DISCRIMINATION LEARNING IN SPLIT-BRAIN MONKEYS

Investigator: Colwyn B. Trevarthen
Support: Arthur McCallum Fund

Both brightness and color discrimination learning have been found to transfer in the monkey from one half of the brain to the other after midline section of the optic chiasm, anterior and habenular commissures, and corpus callosum. Primitive visual structures of the roof of the midbrain remain intact after this surgery and it is possible that simpler visual information can cross at a midbrain level.

This has been tested in two cases with additional midline surgery in which Dr. Harbans Arora divided the posterior commissure and the roof of the midbrain. Color discrimination learning, which transferred more weakly in the earlier cases with forebrain parts separated, failed to show any transfer in either of these animals. Both cases, however, showed strong transfer of the brightness discrimination task.

Though the transferred habit could be reversed, and both directions of choice could be retained together after extensive training, both subjects showed recurring transfer in both directions. In one case, transfer took place more strongly when the monkey was forced to react very suddenly as in a reflex reaction. When the stimuli were presented only when the subject was ready and looking to the response screens, transfer became much weaker.

165. BINOCULAR DEPTH DETECTION IN MONKEYS

Investigator: Colwyn B. Trevarthen
Support: Arthur McCallum Fund

Normal binocular depth detection involves slight differences in parallax between the visual fields of the two eyes, and hence requires central interaction between the visual projection systems of the two eyes. This function is being tested with projected polarized stimuli arranged to give an illusion of a difference in position. A first normal case has learned to choose the one of two spots which appears closer. Progressive midline surgery, beginning with chiasm section to eliminate crossed optic projection, will be applied in stages in an attempt to delineate the minimal requirements for this fundamental visual function.

166. SPLIT-BRAIN SURGERY IN MONKEYS

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

During the past year techniques have been developed for achieving increased surgical separation of the two halves of the brain. The present improved technique extends the midline sections to all fore and midbrain structures, excepting the
tegmentum. The cerebellum has also been divided in the median plane, usually in a separate operation. Thus the two halves of the brain are completely disconnected from each other except for the midbrain tegmentum, pons, and medulla. Recovery from division of the tegmentum in cat indicates it may be feasible to divide this part of the brain stem in the monkey also.

167. CENTRAL NERVOUS PATHWAYS INVOLVED IN CONDITIONING

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

The present series of experiments is the continuation of a project aimed at determining the critical neural structures involved in the performance of a conditioned withdrawal of one forelimb to a flashing light signal.

It has been demonstrated that animals can perform at high levels of accuracy when the sensory input is restricted to one hemisphere and the motor output is restricted to the opposite hemisphere, even though all the dorsal commissures, as well as the thalamus, have been separated by midline section. This suggests that the sensory information which signals the onset of the motor response may be crossing the midline by way of deeper undivided pathways. The midbrain surgery has been advanced to include bisection of the midbrain tegmentum caudally into the rostral half of the pons. Tests are now under way to determine whether or not the conditioned response is eliminated subsequent to this complete separation of all midbrain as well as forebrain structures.

168. NEURAL MECHANISMS MEDIATING INTERHEMISPHERIC PERCEPTUAL INTEGRATION IN CATS

Investigators: John S. Robinson, Theodore J. Voneida
Support: National Institutes of Health, Public Health Service

Cats were trained under normal visual conditions to select the brighter of two lighted windows. An attempt was then made to determine the capacity of various commissures to mediate perceptual integration of brightness inputs to the separate eyes. Initially, the inputs to the two eyes were separated by using red and blue filters over the light sources and in goggles over the subjects' eyes. Both inputs could still enter each side of the brain, however, since some of the optic fibers from each eye cross to the opposite side via the optic chiasm. Inputs to each hemisphere were then separated by sectioning the optic chiasm. Finally, brain bisection was carried out in two stages, the first involving the corpus callosum and the habenular and hippocampal commissures; and the second the thalamus, anterior and posterior commissures, and the commissures of the superior and inferior colliculi.

Up to the second stage of brain bisection the animals performed at a high level. There was, however, a marked change in accuracy and consistency of performance following the last operation. The subjects' ability to integrate the two inputs, i.e., to "compare" the brightness, was not eliminated—they could still perform at the 90 to 95% level, but only for one or two consecutive sessions (60 trials per session). Performance inevitably declined to the near-chance (50 to 60%) level in subsequent sessions. Thus, their performance curves for an extended period of time exhibited cyclic fluctuations. In sharp contrast to this reduced efficiency, the subjects performed accurately and consistently when they were run without filters, presumably because the brightnesses could once again be compared with relative ease, since either hemisphere received both inputs.
A control animal with all the above-enumerated interhemispheric connections sectioned, but with the chiasm intact demonstrated no such fluctuation, showing essentially preoperative accuracy and consistency. This control and the performance of subjects run without filters suggest that the final operation had a significant disrupting effect on central neural structures mediating integration of information to the separate hemispheres, but that sufficient pathways of integration remained to mediate occasional high level performance.

169. INTERACTION BETWEEN CORTICAL AND SUBCORTICAL STRUCTURES IN THE PERFORMANCE OF A TACTILE DISCRIMINATION PROBLEM

Investigators: Theodore J. Voneida, D. B. Webster
Support: National Institutes of Health, Public Health Service

Previous experiments have demonstrated that the callosum-sectioned cat can learn and retain discrete tactile discriminations following massive unilateral cortical removals, including all but a small frontal remnant of cortex in the hemisphere being used.

Callosum-sectioned animals with similarly isolated cortical remnants are being utilized in the present experiment. In addition to the cortical removal, a stepwise elimination of subcortical structures is being carried out with the aim of discovering the essential combination of cortical-subcortical centers and pathways needed for tactile discrimination learning and retention. To date the following structures have been removed (on the same side of the brain as the cortical removal): hippocampus, frontal third of the thalamus, head of the caudate nucleus, superior and inferior colliculi. The midline sections have been extended to include the anterior and posterior commissures, commissures of the superior and inferior colliculi and the thalamus. The caudate nucleus and hippocampus are the only structures which, when removed, have resulted in an appreciable impairment of the learning ability.

170. RECENT MEMORY TESTS IN SPLIT-BRAIN MONKEYS WITH UNILATERAL TEMPORAL LOBE ABLATION

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

An impairment in memory for recent events is reported in humans who have undergone a radical bilateral temporal lobe ablation which includes the hippocampal zone. In monkeys analogous operations produce a deficit in discrimination of visual forms and memory for spatial cues. The present experiment is an effort to study these and related effects using unilateral temporal lobe ablation in split-brain monkeys.

The experimental procedure consists of presenting the subject with one of two visual stimulus patterns projected on a screen. This signal S disappears when the subject makes an appropriate response and is followed after a controlled time delay by a pair of patterns one of which is the same as that presented earlier. The subject is rewarded for selecting, from the pair, the pattern that matches the preceding signal stimulus. The technique and a balanced experimental design provides comparison between hemispheres and different eye-hemisphere combinations.
171. **DIVISION OF EMOTIONAL BEHAVIOR PATTERNS IN SPLIT-BRAIN MONKEYS**

**Investigators:** J. Bossom, R. W. Sperry, H. Arora  
**Support:** National Institutes of Health, Public Health Service

Bilateral ablation of the temporal lobes including the hippocampus, amygdala and uncus produces a syndrome of dramatic behavioral changes in the rhesus monkey. The syndrome described by Kluver and Bucy consists of changes in emotional behavior, in the direction of increased tameness, visual agnosia, a tendency to examine all objects orally, hypersexuality and obesity. A pilot study recently completed indicates that the emotional changes, oral tendencies, and visual agnosia can be produced from one hemisphere in a split-brain animal. The symptoms are hemisphere-specific when elicited by visual stimuli. The sight of a "fear producing object" such as a rubber snake produces startle and withdrawal behavior only when the normal hemisphere has vision. When the experimental hemisphere is presented with the same visual stimuli the monkeys react to the "fear producing objects" as though they were food.

The monkey wears binocular contact lens filters that transmit wavelength bands which are different for each eye. Vision is available to the animal through either the right, left or both eyes, simply by switching the color of the ambient illumination.

The contact lenses are designed to be made from high grade plexiglass, which is obtained to color specifications. They are made with flanges of various angles so that each pair will fit a large number of animals.

172. **TRANSFER OF VISUAL-MOTOR COORDINATION IN SPLIT-BRAIN MONKEYS**

**Investigator:** Charles R. Hamilton  
**Support:** National Institutes of Health, Public Health Service  
**General Funds**

Several types of sensory discriminations may be learned only by the trained hemisphere of a split-brain animal, the untrained half remaining ignorant of the problem. This demonstrates a high degree of functional autonomy of the hemispheres. It is also true that these animals are not complete split-brains since motor coordinations, brightness discriminations, and some forms of interhemispheric comparisons are relatively unaffected by section of the various fore and midbrain commissures. It is obviously desirable to know what types of functions are separated in the split-brain preparations. Those that are split may be further analyzed within one hemisphere by additional surgery.

It is already known that human subjects, when looking through wedge prisms, readjust their visual and/or motor kinesthetic interpretations of the world and thus re-establish their visual-motor coordination. This represents a type of learning probably somewhat more complex than a motor coordination yet less than sensory discriminations. An investigation of adaptation to prisms in normal and split-brain cats and monkeys has been started. It has been found that normal monkeys adjust well to the deviating prisms and that transfer from exposed to unexposed eye occurs with 100% efficiency. Transfer from practiced to unpracticed hand occurs with about 60% efficiency. These values correspond well with the human data. Tests for transfer in split-brain cats and monkeys are still in progress. Further surgical intervention should help establish what brain structures are involved.
173. ANALYSIS OF SINGLE UNITS IN THE OPTIC TRACT AND TECTUM OF GOLDFISH

Investigator: J. Cronly-Dillon
Support: National Institutes of Health, Public Health Service

Recordings have been made of action potentials within single units of the optic tectum in goldfish. Metal microelectrodes with a tip diameter of approximately 5μ were used for recording. The fish is immobilized with Tubocurarine injected intramuscularly, and respiration is maintained by circulating aerated tap water past the gill clefts. The preparation secured in a clamp lies almost completely immersed in water. The eye, from below water level, looks through a window at a white screen upon which are presented the visual stimuli. Alternatively, in experiments to determine the topographical representation of the retina on the surface of the tectum, the stimulus was provided by a flashing pinpoint of light that could be placed at any point of the visual field by moving it along a hemi-spherical perimeter.

The projection of the retina onto the surface of the tectum in intact fish was obtained by recording the evoked potential at the surface. Some preliminary results have been obtained on the characteristics of tectal neurons. Several units were found deep in the tectum which would not respond to diffuse illumination or even to the switching on or off of the light. One type of neuron appeared to require that an edge or contour move over its visual receptive field in order to elicit a discharge. Another type would not respond to diffuse illumination, to the switching on or off of the light, or even when a contour was moved over its visual field; however, if a contour was moved over the visual field and the light was then switched off this caused a prolonged and extremely regular discharge that was immediately erased as soon as the light was switched on again.

174. REGENERATION OF OPTIC TRACT IN GOLDFISH

Investigator: J. Cronly-Dillon
Support: National Institutes of Health, Public Health Service

Recent experiments by Attardi and Sperry (1960) show that neuronal specificities operate to assure orderly development in the patterning of nerve fiber pathways within the central nervous system. It is proposed to extend these studies and to examine the details of the synaptic connections which regenerating optic tract fibers establish within the tectum when their paths are diverted by surgical interference. Specifically, it is wished to learn if excising a portion of the tectum will divert regenerating optic fibers and induce tectal neurons in neighboring regions to articulate with some of the displaced fibers in addition to carrying their normal complement of afferent innervation.

The problem is being approached electrophysiologically and a preliminary analysis is being made of the characteristics of fibers in the optic tract and of neurons in the optic tectum. (See preceding section for a preliminary report of these results.) It is hoped that by comparing the size of the receptive field of tectal neurons in intact and operated fish it may determine whether some of the displaced optic fibers have established synaptic connections with tectal neurons in regions neighboring the lesion. In addition, the characteristics of tectal neurons in operated fish will be examined to see if those neurons with abnormal input display any selectivity with respect to the function or retinal locus subserved by the optic fibers with which they synapse.
175. LOCALIZATION OF LEARNING AND MEMORY IN THE BRAIN OF FISHES

Investigators: H. L. Arora, R. W. Sperry
Support: National Institutes of Health, Public Health Service
Frank P. Hixon Fund

It has been reported earlier in the cichlid fish Astronotus that extirpation of the forebrain or the cerebellum following color discrimination training had little or no effect upon a previously learned color discrimination. Neither did combined removal of both forebrain and cerebellum produce any impairment of the trained color associations. This leaves the midbrain, particularly the optic lobes, as the most likely location of the learning and memory changes involved. In the present experiment color discrimination training was given to one eye only, the monocular blinding was effected by section of the optic nerve. After training, the optic tectum directly involved was extirpated and the color discrimination was tested with the untrained eye. In all but one case, the trained color response transferred to the untrained side and was not impaired by the removal of the trained optic tectum. Such interocular transfer may be due to the establishment of two memory trace systems, one on each side of the brain, or to the drawing by the untrained side upon the traces established in subtectal centers on the trained side.

176. AN ANATOMICAL STUDY OF DEGENERATED VISUAL PATHWAYS FOLLOWING SECTION OF THE OPTIC NERVE, OPTIC CHIASM AND OPTIC TRACT IN CATS

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

The Nauta-Gygax staining technique is being utilized in an attempt to trace primary visual pathways in the cat, particularly unmyelinated components. This technique allows for the staining of nerve tracts which have heretofore been only poorly demonstrated but which may be of considerable importance in visual learning.

Publications: (July 1, 1960 - June 30, 1961)


Attempts to induce virus release from polyoma virus transformed cells were made with some non-virus-releasing transformed hamster cultures and with some non-virus-releasing transformed clonal mouse cultures. A number of different agents were employed; some of these are known to induce virus release from "inducible" strains of lysogenic bacteria; others are able to do so with "non-inducible" strains. All the attempts were negative. They are listed here in some detail.

Attempts were carried out by using X-rays, treatment with mitomycin, treatment with aminopterin followed by thymidine. Furthermore, on the hypothesis that induction of virus release may depend on metabolic imbalance, the cells were exposed to starvation procedures of various kinds, such as delaying fluid change, or withdrawing from the medium a required factor such as serum or an amino acid; in other experiments the cells were exposed to azaguanine or to chloramphenicol. Another series of tests was based on the assumption that the increased resistance of the transformed cells to superinfection with polyoma virus is due to a mechanism similar to that causing immunity of lysogenic bacteria. Therefore it was tested whether several of the above mentioned inducing conditions would induce in the cultures an increased sensitivity to superinfection.

By taking advantage of the occurrence of two mutant types of polyoma virus differing in plaque size, both of which cause neoplastic transformation, in vitro experiments were carried out in an attempt to demonstrate the existence of the plaque size marker of the transforming virus in the transformed cells: cells transformed with virus of one plaque type were superinfected with polyoma virus of the other type, and the plaque marker of the transforming virus was looked for in the progeny. The experiments were carried out in the two possible combinations. Although these experiments are not exceedingly sensitive, the presence of 1% of the minority plaque type could have been detected.
In consideration of the fact that the neoplastic transformation might be caused by non-cytocidal mutants of polyoma virus, extracts of non-virus-releasing transformed cultures were tested for the ability to cause the transformation of normal cultures.

In several of these experiments tests were made not only for the production of infectious virus, but also for the production of viral antigen as detectable by using fluorescent antibodies, and of infectious polyoma virus DNA. These attempts were also unsuccessful.

178. ANALYSIS OF THE CONDITIONS CAUSING PERSISTENT VIRUS RELEASE IN CULTURES OF MOUSE OR HAMSTER EMBRYO CELLS TRANSFORMED TO THE NEOPLASTIC STATE BY POLYOMA VIRUS

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

The in vitro transformation of normal cultures by polyoma virus has been described. It was observed that many such transformed cultures continue indefinitely to release virus of small plaques (sp) type, even when infected by virus of large plaque (lp) type. This continuous release seemed at first sight to support the hypothesis that these transformed cells carry the virus in a state similar to that of lysogenic bacterial cells.

A study of this phenomenon brought the following results: all transformed mouse cultures continued to release virus indefinitely; of the transformed hamster cultures only about 10% did so; the rest persisted as non-virus-releasers. The virus was released by a few cells in a population; these cells released a yield of several hundred plaque-forming units of virus and were then presumably killed. By examining these cultures after staining with fluorescent antiviral gamma globulin one finds a proportion of fluorescent nuclei well corresponding to that of virus-releasing cells.

Many cells from such virus-releasing cultures were cloned with special precaution to reduce possible superinfection of the clones with free virus. With the best precaution (use of antiviral serum, use of a feeder layer of transformed hamster cells unable to reproduce the virus) all clones were non-virus-releasing.

The transformed cells always displayed an enhanced resistance to superinfection with PY virus; however, the non-virus-releasing transformed cells of the clonal mouse cultures could be superinfected. These superinfected cultures became persistent virus releasers and showed the substitution of lp with sp virus as in the primary transformed cultures.

These results thus show that the persistent virus release of some transformed cultures is due to a "virus carrier state." There is therefore no relationship of the persistent virus release with an hypothetical lysogenic state of the virus in the cells.
An attempt has been undertaken to improve and standardize the procedures and methods which are employed in the study of polyoma mediated neoplastic conversion of Syrian hamster cells in vitro. For this purpose cell cultures were derived from whole embryos and from several organs of animals of different ages and the effects of numerous different conditions and experimental treatments on time sequence and frequency of conversion of these cultures were studied. At present, methods are available that give rise to the production of neoplastically converted cultures of embryonic cells within 22 days, and of the organs within 8 to 20 days after infection. Newly converted cells grow progressively in vivo; this constitutes strong evidence for the neoplastic properties of the converted cells.

Detailed karyotype analysis has been undertaken in the course of the polyoma virus mediated neoplastic conversion in vitro and in vivo. Since normal chromosome constitutions predominated in both systems it is very unlikely that karyotype instability is causally related to the neoplastic conversion caused by polyoma virus.

Di Mayorca, et al. (1959) extracted a subviral infective agent (SIA) with phenol from mouse embryo culture cells infected with polyoma virus. The SIA reproduced the cytopathic effect of the intact virus on mouse embryo cells as well as its carcinogenic action in hamsters.

A satisfactory plaque assay method for the quantitative determination of the SIA has been worked out. From the linearity of the dose-response curve it is inferred that a single particle of the SIA is able to produce a plaque and that the number of susceptible cells is not limiting. The number of plaques formed represents only a fraction of the number of potentially infective particles present in the phenol extracts. The proportionality factor however remains unknown.

The plaques initiated by the SIA show the same features as those developed by the respective parent virus, both in time of appearance, size, and in the genetic control of plaque size.

The efficiency of plating, defined as the ratio of the number of plaques formed by a phenol extract to the number of plaques formed by the virus source extracted, is of the order of $10^{-4}$.

The subviral infective agent is inactivated by pancreatic deoxyribonuclease. It is resistant to ribonuclease and to $\alpha$-chymotrypsin. The infectivity is fully preserved and often slightly increased after treatment of phenol extracts with 85 to 95% v/v formamide. The buoyant density of the SIA in the cesium chloride density gradient is approximately 1.707 gm cm$^{-3}$ and it is essentially unchanged after treatment with formamide. Mouse deoxyribonucleic acid shows under the same experimental conditions changes which are thought to reflect the loss of the secondary structures. About 60% of the infectivity can be recovered under conditions of heating
and cooling where E. coli DNA (density = 1.708 gm cm\(^{-3}\)) is fully melted out. Slowly cooled phenol extracts show a markedly lower infectivity than rapidly cooled aliquots. A large fraction of the SIA after heating shows a marked increase in buoyant density. Subsequent treatment of heated phenol extracts with formamide, however, reconstitutes the density distribution of the SIA approximately to that found in unheated or in only with formamide treated extracts. The experimental evidence is compatible with the assumption that the SIA is mostly, if not exclusively, deoxyribonucleic acid of an unusual nature.

Publications:


181. STUDIES OF POLYOMA VIRUS INFECTION IN MOUSE CELLS

Investigator: Robert A. Weisberg
Support: American Cancer Society
National Institutes of Health, Public Health Service

A. Nature of the Polyoma Infected Mouse Culture in the Initial Stages

The oncogenic transformation of cultures of mouse embryo cells by polyoma virus described by Vogt and Dulbecco in Biology 1960 is being studied in greater detail. An attempt is being made to decide whether polyoma-infected cultures, in the stage before transformation has occurred, are "carrier cultures," in which only a small proportion of the cells are infected, or whether the initial infection produces virus-cell complexes whose progeny give rise to the transformed cultures. Preliminary studies indicate that cells taken from cultures up to a week after infection either fail to reproduce or give rise to untransformed clones.

It is also of interest to know whether a population of cells taken from a whole mouse embryo is homogeneous or heterogeneous in its response to polyoma. One culture, derived from a single cell, has been divided into two parts. The first part was infected with polyoma, the second served as a control. The first culture showed the normal sequence of events after polyoma infection and is now a typical transformed culture. The control culture continued to grow, but is quite different from the infected culture in cell morphology, acid production, ability to grow under crowded conditions and growth rate; in respect to these characters the control culture is more similar to a normal culture. This experiment is being repeated on a larger scale. In addition, these two cultures are being studied intensively in order to discover other possible differences.

B. Analysis of the Resistance of Polyoma Infected Mouse Embryo Cultures to Vesicular Stomatitis Virus (VSV)

It has been confirmed that polyoma virus infected mouse embryo cultures show little cell degeneration and VSV multiplication upon infection with VSV. These cultures continue to produce polyoma. The effect is dependent on the physiological state of the culture: crowded polyoma infected cultures are resistant to VSV but sparse cultures are sensitive. Persistence of resistance after transfer of once challenged cells seems to depend on keeping the culture crowded. It has been found that the resistance inducing principle is not separable from the virus by sedimentation in the ultracentrifuge. The possibility that resistance is induced by extracellular rather than intracellular polyoma is being investigated.
182. PURIFICATION AND PROPERTIES OF ROUS SARCOMA VIRUS

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

The physical and chemical properties of Rous sarcoma virus purified by density gradient centrifugation were studied. The purity and specific infectivity (physical particles per infectious unit) of the preparation were determined by electron microscopy; the particles to infectious units ratio was found to be 750 or greater. The virus particle appears to contain approximately $9.5 \times 10^6$ molecular weight units of ribonucleic acid. The base composition of the RNA was determined; however, material was available only for a single estimation. It was found that this RNA contains a high proportion of adenine and cytosine.

References:


183. AN INHIBITOR OF CELLULAR TRANSFORMATION BY ROUS SARCOMA

Investigator: John P. Bader
Support: National Institutes of Health, Public Health Service

Rous sarcoma virus characteristically produces a morphological transformation of chick embryonic cells in culture. A substance which is capable of interfering with such cellular transformation has been found in the fluids of cultures infected with Rous sarcoma virus. The inhibitor is not associated with virus particles, since centrifugation at $10^5 \times 9 \times g$ for one hour sediments most of the virus, leaving the inhibitor in the supernatant. The inhibitor is stable at $56^\circ C$ for 30 minutes and withstands dialysis at pH 2. It is destroyed by pepsin and partially inactivated by trypsin, but DNAase has no effect on its inhibitory activity. In these characteristics the inhibitor is similar to interferon.

Exposure of cells to inhibitor prior to infection is necessary for maximum interference, but some effect is noted if inhibitor is added as late as two hours after infection. Removal of inhibitor just before infection reduces the efficiency of the inhibitor but does not eliminate its action.

The mechanism of action of the inhibitor, its relation to other viral inhibitors, and its effect on tumor production are currently under investigation.

184. THE APPEARANCE OF RESISTANCE TO SUPERINFECTION IN CHICKEN EMBRYO CELLS INFECTED BY ROUS SARCOMA VIRUS

Investigator: Robert C. Y. Ting
Support: National Institutes of Health, Public Health Service

A study of the interaction between mutants of the Rous virus infecting the same cell should lead to a better understanding of the state of the virus in the cells transformed by the virus itself. To this purpose host range mutants, previously
isolated by Temin, are well suited because of ease and reproducibility of scoring. Of these mutants, the h+ (wild-type) virus plates with low efficiency (less than $10^{-6}$) on duck embryo cultures, the h^d virus plates on the same cells with high efficiency ($3-8 \times 10^{-1}$).

Cultures of chicken embryo cells were infected by h^+ virus at high multiplicity and challenged at various time intervals with a low multiplicity of the h^d mutant. The supernatants were harvested five days after the superinfection and the amount of each type of virus was determined by titration on duck cells as well as on chicken cells. The results showed that the production of h^+ virus was constant in all cases, whereas that of h^d virus decreased as the time interval between h^+ infection and h^d challenge increased. The effect became first noticeable after a 4-hour interval; after an interval of 12 hours the production of h^d virus was only 1/50 of that found after simultaneous infection.

Whether this effect is due to the production of an interferon or to an immunity comparable to that of lysogenic bacteria is now being investigated.

185. STUDIES ON ENCEPHALOMYOCARDITIS VIRUS

Investigator: Clyde R. Goodheart
Support: American Cancer Society
National Institutes of Health, Public Health Service

Encephalomyocarditis virus (EMC) is one of a group of small (27-30 mµ) neurotropic viruses containing only RNA and protein which grows to high titers in many types of mouse cells. It is easily purified and crystallized, it has a high ratio of infective physical particles, and an extractable infectious RNA. This combination of properties suggests that EMC should be a useful tool for studying various aspects of RNA and protein synthesis and metabolism. Therefore, work has been started to develop EMC into a truly quantitative system.

It has been found that EMC can be easily labeled with P^32; chromatography on CaHPO_4•2 H_2O columns yields a preparation which is free of non-viral P^32, and virtually free of non-viral protein. The virus bands in a cesium chloride density gradient at a density of 1.33; it has the same size and RNA:protein ratio as polio virus (density 1.34). The density difference probably reflects a different degree of solvation of the two viruses, in turn reflecting a difference in the protein shells. A further difference in the protein is found in the different Arrhenius heat of inactivation, it being 68,000 cal/mol for polio virus and about 20,000 cal/mol for EMC.

At the completion of these preliminary studies, it is planned to exploit the useful properties of EMC to the fullest and investigate the possibility of transfer of RNA from the infecting virus to the progeny virus, as well as other studies of the fate of the infecting RNA within the cell.

186. RIBOSOMES OF POLIO-VIRUS-INFECTED HEla CELLS

Investigators: G. Attardi, J. D. Smith
Support: National Institutes of Health, Public Health Service
National Foundation

Cytoplasmic extracts of polio-virus-infected HeLa cells incorporate amino acids into protein having the immunological specificity of the virus (Attardi, Biology 1960). Most of the newly synthesized protein is attached to ribosomes, suggesting that these are the sites of virus protein synthesis. Studies were made to observe whether the infected cell synthesizes any distinct type of ribosome-bound RNA which might be concerned in the synthesis of virus protein.
The purification and a preliminary characterization of the ribosomes of these cells was undertaken. A procedure was developed whereby reasonably pure ribosome preparations could be obtained from normal cells and from cells infected with polio virus.

The ribosomes were isolated from cell homogenates (prepared in 0.05M tris buffer pH 7.6, 0.25M sucrose, 0.025M potassium chloride, 0.0025M magnesium chloride and freed of nuclei) by treatment with 0.5% sodium desoxycholate, followed by differential centrifugation, precipitation of high magnesium concentration (5 x 10^{-2}M) and resuspension at low magnesium concentration (2.5 x 10^{-3}M).

Ultracentrifuge studies of purified ribosome preparations from normal HeLa cells, with schlieren and UV optics, in 0.05M tris buffer pH 7.6, 0.025M potassium chloride, 0.0025M magnesium chloride, revealed the presence of a major component having a sedimentation coefficient of 68s and 74s respectively in the schlieren and UV absorption pattern (values corrected for temperature only), with smaller proportions of a faster and a slower component (101s and 50s respectively in the schlieren diagram).

No appreciable difference was observed between the sedimentation pattern of ribosomes from normal HeLa cells and that of ribosomes from cells infected with polio virus (six years after infection). A fast component with a sedimentation coefficient of 150s, which was present in the schlieren diagram of a crude ribosome preparation from infected cells before magnesium precipitation, was not detectable in the final purified preparation. This component represents in all likelihood polio virus. Reconstruction experiments made with P^{32}-labeled polio virus and infectivity assays have in fact shown that polio virus is not precipitable by magnesium at a concentration of 5 x 10^{-2}M, so that less than 1% of the virus is present in the purified ribosome preparations from infected cells. Complete removal of the virus was achieved by sucrose density gradient zone centrifugation of the purified ribosomes.

Sedimentation analysis in sucrose gradient of HeLa cell ribosomes at various magnesium concentrations has shown a substantial similarity in behavior to ribosomes from other sources as regards the magnesium controlled stability.

Total HeLa cell ribosomes and purified 74s particles have a RNA/protein ratio of approximately unity.

Newly synthesized RNA was followed by P^{32} orthophosphate labeling of intact HeLa cells. The ribosomal RNA components synthesized between two and six hours after polio virus infection differed from those of similarly labeled uninfected cells in the following respects: (1) their "apparent base composition" (as measured by the proportion of P^{32} nucleotides after alkaline hydrolysis) was quite different from the composition of HeLa ribosome RNA and more closely resembled that of polio virus RNA. (2) After dissociation of the 74s ribosomes in low magnesium ion concentrations into 50s and 30s particles a much higher proportion of the labeled RNA from ribosomes of infected cells sedimented with or more slowly than the 30s ribosome components. (3) A high proportion of the labeled RNA isolated from ribosomes of infected cells sediments more slowly than the major ribosome RNA components.

**RNA AND PROTEIN SYNTHESIS IN E. COLI EXTRACTS**

Investigators: R. Rolfe,* J. D. Smith
Support: National Institutes of Health, Public Health Service

About 60% of the activity of E. coli extracts incorporating amino acids into protein is sensitive to treatment of the extract with deoxyribonuclease, suggesting that
a limited synthesis of active "messenger" RNA may occur in the cell-free extract. Preliminary experiments have shown that E. coli extracts incorporate C\textsuperscript{14} adenosine triphosphate and P\textsuperscript{32} uridine triphosphate into an RNA fraction which sediments with the 70s and 100s ribosome components when these are isolated in 10\textsuperscript{-2} M magnesium ions. Over 90\% of this activity is destroyed on treating the extract with deoxyribonuclease. After dissociation of the 70s ribosomes into 50s and 30s particles in 10\textsuperscript{-4} M magnesium ions most of the newly synthesized RNA is dissociated from and sediments more slowly than the ribosomes while a part sediments with the 30s ribosome particles. The possible role of this RNA in protein synthesis is being investigated.

* Division of Chemistry and Chemical Engineering, California Institute of Technology.

188. AN INVESTIGATION OF THE CO\textsubscript{2} -SENSITIVITY FACTOR IN FLIES

Investigator: Robert Seecof
Support: National Institutes of Health, Public Health Service

Carbon dioxide is lethal to fruit flies after they have been infected with sigma, a virus-like agent which is transmitted through the eggs and sperm of infected flies. The pattern of sigma-infection inheritance suggests that the relationship between the hereditary material of sigma and the cells of the fly may be more intimate than is recognized for other virus-multicellular animal relationships, and may be similar to the lysogeny phenomenon in bacteria. The previous work on sigma has recently been reviewed by Ph. L'Heritier in "Advances in Virus Research V," Academic Press, N.Y., 1958.

A procedure for isolating sigma from extracts of infected flies is being developed in order to confirm the virus nature of sigma and to study its properties. Filtration, differential centrifugation, gradient density centrifugation and ammonium sulfate fractionation have yielded purified and concentrated extracts. Sigma has been tentatively identified in electron micrographs as a roughly spherical particle about 3,000 Å in diameter.
PART B.—BACTERIAL VIRUSES

STAFF:
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Visiting Professor: Eduard Kellenberger
Research Associate: J. J. Weigle
Assistant Professor: R. S. Edgar
Instructor: Charles M. Steinberg
Senior Research Fellow: Matthew S. Meselson
Research Fellows: Sidney Brenner, Francois Jacob, H. E. Johns, Ulrich Winkler
Graduate Students: Juan Bozzini, John R. Menninger, Michael Nesson, Seymour Rapaport, Ann Roller, Millard Susman
Student Assistants: Marcia Bourgin, Peter A. Gail, Richard B. Süßler, Francisc C. Wilson
Research Assistant: Ilga Lielausis
Research Aide: Bertha E. Jones
Laboratory Assistants: Johanna Feyen, Betty A. Joyner

189. DENSITY MUTANTS OF PHAGE LAMBDA

Investigator: J. J. Weigle
Support: National Foundation

Two mutants of phage lambda with altered density have been studied. The density of both is less than that of wild type, and, in each case, a single genetic marker is responsible for the altered density. One marker, called b5, is located in the c region. The density change is associated with a change of immunity, that is, the mutant grows in bacteria lysogenic for the wild type and conversely. However, the mutant is not an inductor of the wild-type prophage and neither is the wild-type phage an inductor for the mutant prophage. The mutant prophage is located on the chromosome of the lysogenic bacteria at the same place, within the experimental error, as the wild-type prophage. The mutant also forms double lysogens with the wild type. The plaques formed by the mutant on certain indicator stains differ in appearance from those formed by the wild type.

The other marker, called b2, is located on the genetic map in the region m5-h. This marker behaves in crosses like any other genetic marker of lambda. Wild-type lambda produce either a lytic or a stable lysogenic response in the bacteria they infect. The mutant b2, however, either lyses the bacteria or establishes an "abortive" lysogenization. In the latter case, the phage remains in the bacterium without multiplying while the bacterium divides. During the growth of the abortively lysogenized bacteria, the phage passes to only one of the daughter cells, which can be induced to lyse by irradiation with UV light. The altered density of b2 has been found to be due to an altered amount of DNA contained in the phage. The wild-type particle has about 18% more DNA than b2.

This work was done in collaboration with G. Kellenberger and M. Zichichi, Laboratory of Physics, University of Geneva.
190. EXCHANGE OF DNA IN GENETIC RECOMBINATION OF PHAGE LAMBDA

Investigator: J. J. Weigle
Support: National Foundation

In genetic crosses between lambda phage of different densities (see preceding report) the recombinants can be physically separated from the parents by density gradient centrifugation. The crosses involve two density markers, $b_2$ and $b_5$, with one of the parents labeled with $P^{32}$. Most of the $P^{32}$ transferred to the progeny is associated with the genotype of the parent originally labeled. A significant fraction, however, is associated with recombinant genotypes. The fact that much less is associated with the genotype of the parent which was originally unlabeled indicates that random breakdown of DNA and reutilization of the $P^{32}$ is negligible.

The simplest model accounting for these facts is one in which recombinants are formed by breaks and reunion of parental material.

This work was done in collaboration with G. Kellenberger and M. Zichichi, Laboratory of Biophysics, University of Geneva.

191. GENETIC RECOMBINATION BY CHROMOSOME BREAKAGE IN BACTERIOPHAGE

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

If bacteriophages with several different hereditary traits are allowed to propagate in the same host cell, the progeny are found to include genetically recombinant types. The genetic material of bacteriophages is known to be DNA and it is commonly assumed that the genetic information resides in the linear sequence of nucleotides which comprise the DNA molecule. Under this assumption, genetic recombination in bacteriophage corresponds to the production of a nucleotide sequence derived partly from one parental line and partly from another. This may be imagined to occur in either of two ways:

1. **Copy choice:** The recombinant sequence is synthesized de novo by copying first one parental sequence, then another.

2. **Breakage:** The recombinant sequence is formed by association of DNA fragments from different parental lines.

Recombinants produced by copy choice would be free of segments of parental DNA while breakage could result in the appearance of portions of parental DNA in recombinant phages.

Experiments have been performed with the bacteriophage lambda aimed at detecting parental DNA in recombinant phages. Density gradient centrifugation was utilized to determine the distribution of labeled parental DNA among both parent and recombinant genotypes emerging from a two-factor cross between unlabeled lambda and lambda heavily labeled with the isotopes $C^{13}$ and $N^{15}$. Results demonstrate the presence of discrete portions of parental DNA in recombinant phages and support the following conclusions.

1. Recombination by chromosome breakage occurs in bacteriophage.

2. Distances along the genetic map are proportional to amounts of DNA in the bacteriophage chromosomes.
3. Breakage occurs when DNA is in the two-strand condition.

4. A bacteriophage chromosome need not replicate in order to undergo recombination.

A molecular model is proposed to account for these findings.

Publication:

Genetic recombination by chromosome breakage in bacteriophage.
P.N.A.S., in press.

192. TRANSFER OF PHAGE DNA FROM PARENT TO PROGENY

Investigator: Ann Roller
Support: National Institutes of Health, Public Health Service
         Arthur McCallum Fund
         General Funds

When a bacteriophage particle infects a bacterium, its DNA enters the host cell. The purpose of the present research has been to learn more about the mechanism of replication of the parental DNA and its distribution among progeny phage.

Parental phage in which the nitrogen and carbon were replaced by the stable heavy isotopes N\textsubscript{15} and C\textsubscript{13} were used to infect unlabeled bacteria. DNA molecules containing heavy parental atoms show a corresponding increase in density; separation is achieved by density gradient centrifugation. At various times after infection, aliquots of the culture were removed and lysed; finally, progeny phage were harvested and lysed. Most heavy parental atoms transferred to progeny phage are found in DNA of light density. Following fragmentation of this DNA, DNA containing parental atoms is found to have shifted to densities between those of light and half-heavy DNA. In the replicating pool, half-heavy DNA is also found. It is concluded that segments of parental DNA are incorporated into molecules which are, for the most part, newly synthesized. The parental atoms may comprise a part of one strand of duplex DNA molecules.

An analysis was made of the density distributions of the viable progeny of a mixed infection with T4 of \( r \) and \( r^+ \) genotypes, one of which was labeled with N\textsubscript{15} and C\textsubscript{13}. At densities greater than that of the average progeny, relatively more progeny of the genotype of the labeled parent are found. Thus, there is a class of progeny phage with parental DNA which is specifically associated with parental genotype. These progeny contain a variable amount of parental isotope; few if any contain more than about 25% parental atoms. It is concluded that the \( r \) marker is not found in a chromosome which is transferred to progeny without fragmentation, as has been previously suggested.

193. EVIDENCE FOR THE CIRCULARITY OF THE LINKAGE MAP OF T4D

Investigator: R. S. Edgar
Support: National Foundation
         National Institutes of Health, Public Health Service
         Commonwealth Fund

Other workers have shown that all of the markers of T4D are located on a single linkage structure. To demonstrate this fact, sensitive tests for linkage must be utilized in order to establish linkage and order of markers which are only slightly
linked. This sensitivity is obtained by performing a three-factor cross of the type abc x +++ in which a and b are moderately linked (10 to 20%) and c is distantly linked to a and b. Linkage and order is established from the relative frequencies of c and c+ phage among recombinants for a and b. The randomizing effects of multiple rounds of mating can be minimized by using premature lysis and unequal input multiplicities of the two parental phages. Experiments of this type have been performed, testing for linkage between what had previously been considered terminal markers on the linkage structure. Unambiguous linkage has been detected; the order of the markers is that expected if the linkage structure of T4D were circular. This project has been carried out in collaboration with Dr. G. Streisinger and Miss G. Harrar of the University of Oregon.

194. ABSENCE OF A GENETIC GAP BETWEEN THE rII A AND B CISTRONS

Investigator: Lee Hartwell
Support: National Foundation
National Institutes of Health, Public Health Service

The rII region of phage T4 consists of two non-overlapping functional units, the A and B cistrons. Due to the phenotypic properties of heterozygous phage, intercistronic crosses give artificially high recombination values resulting in an apparent "genetic gap" between the two cistrons. The object of this experiment was to determine whether a "gap" of appreciable length actually exists. A number of rII mutants, shown by qualitative tests to be near the region between the cistrons, were obtained from Benzer. These mutants were mapped and ordered within their respective cistrons. A cross was then made involving the mutants found to be nearest to the edge of each cistron. By means of a special technique it was possible to measure the true recombination value without the complication formerly caused by the heterozygous phage. The map distance between the two cistrons was found to be 0.38 map units. This value is very small in comparison to the lengths of the A and B cistrons, which are 11 and 6 units respectively.

195. AMBER MUTANTS--GENETIC CHARACTERIZATION

Investigators: R. H. Epstein, C. M. Steinberg, F. Wilson
Support: National Foundation
National Institutes of Health, Public Health Service

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). To date, more than 100 such mutants have been isolated, the great majority having been obtained from stocks treated with nitrous acid or grown in the presence of 5-bromodeoxyuridine. After infection of strain B with an amber mutant, few of the bacteria yield any infective particles. The fraction of bacteria yielding at least one infectious particle varies from 0.5 for "leaky" mutants to about 10^-4 for "non-leaky" mutants. Only 79 of the mutants are sufficiently non-leaky to permit the plating of large numbers of the mutant on strain B; of these, all but one (see below) have been shown to be capable of reversion to an apparently wild-type state.

Mixed infection of strain B with an amber mutant and wild type leads to productive infection, usually with a normal yield of progeny phage. Mixed infection of strain B with two different am mutants can lead either to a productive infection ("complementing" mutants) or to a non-productive infection ("non-complementing" mutants). Complementation tests have been performed with 79 mutants in all possible combinations. These mutants fall into 30 complementation groups or "cistrons,"
such that members of a group are mutually non-complementing but all pairs of mutants from two different cistrons do complement. There is one exceptional mutant which does not complement with any mutant in two groups (known from mapping experiments to be widely separated on the genetic map); the exceptional strain also does not revert and is thus presumed to be a double mutant.

An extensive series of crosses between pairs of am mutants have been performed, involving about 24 mutants, each from a different cistron. The linkage map prepared from these data (given below) shows the markers to be distributed into three large segments. It has not yet been possible to demonstrate linkage between markers from different segments, but all relevant crosses have not been completed. Seven am mutants have been crossed to known morphology mutants and in this manner the relationship between the linkage map prepared from am x am crosses and the known linkage structure of T4 has been partially resolved: one segment is essentially linkage group III, another segment linkage group II, while the third segment is as yet unlocated.

The smallest measured interval between two complementing mutants is about 5 map units. In eleven crosses between mutants belonging to a single cistron, the largest interval observed was 2 map units. In one am x am cross, the double mutant was isolated and shown to be am in phenotype; the double mutant and wild-type recombinants occurred in about equal frequencies.

![Linkage Map](image)

20 map units (map is roughly to scale)

**196. AMBER MUTANTS—NATURE OF THE BACTERIAL FACTOR**

Investigators: R. H. Epstein, J. J. Weigle  
Support: National Foundation  
National Institutes of Health, Public Health Service

Many substrains of E. coli K-12 from the stock collection of J. J. Weigle were tested for their ability to support the growth of amber mutants. There were marked differences in this respect, even among closely related strains. Indeed, in some cases strains which were recorded to be different in their history only by a single transductional event for the galactose locus had lost the ability to plate am mutants. Further studies of the nature of these differences are incomplete, but the following results have been obtained:
1. The am plating factor is mutationally distinct from the galactose fermentation property. Out of 12 independent, UV-induced mutations from gal+ to gal−, none lost the ability to plate am mutants.

2. The amber plating factor is recombinationally distinct from the gal locus. Among independent clones transduced from gal− to gal+ by phage lambda and 56 independent clones transduced by phage P1, none had acquired the ability to plate am mutants.

3. All of the strains from our collection which plate am mutants also plate the hd mutants of phage lambda, and vice versa.

Results (2) and (3) taken together are difficult to understand. Campbell, using bacterial crosses, found that the hd plating factor is a single marker lying between the gal locus and the lambda prophage site.

197. AMBER MUTATIONS--PHYSIOLOGICAL CHARACTERIZATION

Investigators: C. M. Steinberg, E. Kellenberger, R. H. Epstein
Support: National Foundation
National Institutes of Health, Public Health Service
General Funds

The genetic experiments reported above show that the am mutants are distributed in a large number of cistrons, which in turn are distributed over a large portion of the phage genome. This suggested that the am phenotype (i.e., ability to grow on K but not on B) is not the result of K supplying a function which B is unable to supply. Rather, it is likely that the intracellular environment in K differs from that in B and that a "B-sensitive" mutation can occur in almost any cistron of the phage. Thus we would have a means for obtaining mutants blocked in a variety of functions without a priori knowledge of what these functions are. Such mutants would be quite valuable for studying the biosynthesis of phage in a manner analogous to the use of "biochemical mutants" in elucidating synthetic pathways of various biologically important substances. Preliminary experiments amply confirm this view.

To date the work has been concerned with developing and applying methods which are sufficiently simple to be used in preliminary screening of large number of mutants. More intensive study of selected mutants will be undertaken later. Methods used and results obtained so far are:

1. Nuclear Breakdown. Upon suspending bacteria in a gelatin solution whose refractive index matches that of the bacterial cytoplasm, nuclear bodies, "nucleoids," become visible in the phase-contrast microscope. Within ten minutes after infection with a wild-type T4 (at 30°C), the "nucleoids" become dispersed in a characteristic fashion. All of the amber mutants tested so far (representing 24 cistrons) also give this nuclear breakdown.

2. Pool Formation. After nuclear breakdown has occurred, there are further cytological changes associated with the formation of the pool of vegetative phage. These changes can also be observed with the phase contrast microscope. Again representatives from 24 cistrons have been examined. All but 4 behave essentially like wild type. Two mutants (am 3, am 116) show the formation of bodies which vaguely resemble the original nucleoids of the bacteria; this phenomenon is referred to as "reorganization." One mutant (am 54) gives rise to infected cells which become very transparent (revealing many small "dots"); this suggests a loss of material from the cell, and this phenomenon is referred to as "leakage." Another mutant (am 15) seems to be arrested in its development at nuclear breakdown.
3. DNA Synthesis. Representatives of 24 cistrons have been tested. Of these, 20 have made at least 50% as much DNA as wild type at 30 minutes after infection, one is doubtful, and 4 show no detectable increase (actually, a small decrease is usually observed). One of the negative mutants is am 15 which is also abnormal in its cytological development.

4. Lysis. Out of 24 mutants, 5 do not lyse the host cell. Others might be slightly precocious or delayed. The 5 mutants which fail to lyse include the 4 which do not synthesize DNA.

5. Synthesis of "serum blocking protein." The phage tail proteins which are involved in serum-inactivation can be easily determined by a "serum blocking" test. Only 7 mutants have been tested. One mutant synthesizes essentially no such protein; this mutant (am 52) is closely linked to a host-range mutant.

6. Precipitable antigen synthesis. Several agar diffusion methods are being tested for use in determining material antigenically related to phage structural protein. Unfortunately, none of the methods are very quantitative. However, there is evidence that some am mutants make more such antigen than the wild type. It is speculated that these antigens actually measured are not major structural subunits of the phage (such as entire heads) but rather are the molecular subunits out of which the structure is built; thus a mutant which is blocked in the polymerization of the monomer protein would yield a larger amount than wild type.

198. A NEW CLASS OF HOST SPECIFICITY MUTANTS OF PHAGE T4

Investigator: Harris Bernstein
Support: Commonwealth Fund

A class of T4 host specificity mutants has been found which resembles the rII mutants in their ability to plate on bacterial strain S/6 but not on 112 lambda. They differ from the rII's in plaque morphology on S/6 and by their low efficiency of plating on 112. These mutants have been called azures. The four azures studied complemented with rdf41, which is a long deletion for both the A and B cistrons. The data from crosses of the azures to rB45, a point mutant in the rII B cistron, indicate that azure mutants may arise as a result of defects at sites that are distant from the r region and also widely separated from each other.

199. HEAT SENSITIVE MUTANTS OF PHAGE T4D

Investigators: R. S. Edgar, I. Lielausis
Support: National Foundation
National Institutes of Health, Public Health Service
Commonwealth Fund

T4D wild-type phage can form plaques on plates incubated at 25°C or at 42°C. Mutants have been isolated which form plaques at 25°C but which do not form plaques at 42°C. Some 30 nitrous acid induced and 30 2-aminopurine induced mutants have been isolated. The frequency of such heat sensitive mutants is comparable to the frequency of r mutations. The mutants vary in their heat sensitivity; some mutants form plaques at 37°C while other do not. Of these 60 mutants only with four has it been found that the phage particles themselves are more heat sensitive than the wild type. Thus, in the majority of cases, the heat sensitive step affected by the mutation must occur during the growth of the phage.
Pairwise complementation tests have been performed with the 60 mutants. They fall into about 40 discrete functional groups (cistrons). Preliminary mapping experiments show that the mutants are distributed widely over the known linkage structure of T4D.

**200. INHIBITION OF BACTERIOPHAGE T4 BY 9-AMINO ACRIDINE**

Investigator: M. Susman  
Support: Arthur McCallum Fund  
National Institutes of Health, Public Health Service  
National Foundation  
General Funds

In the presence of an inhibitory concentration of 9-amino acridine (9AA), infection of bacteria with bacteriophage T4 results neither in the synthesis of infective progeny phage nor in the lysis of the infected cells. DNA is synthesized in inhibited phage-cell complexes at about the same rate as in control cultures. Condensed, phage-like bodies of DNA are seen in electron micrographs of thin sections of the inhibited complexes. Lysates of these cells contain only empty head membranes and detached tails.

The inhibited complexes partially recover when 9AA is removed by dilution. With increasing times of exposure to 9AA, decreasing rates of appearance of infective phage, decreasing final phage yields, and, in lysates from crosses of linked markers, increasing frequencies of recombination are observed.

It has usually been assumed that infective phage arise directly from vegetative phage. If 9AA blocked such direct maturation so that later removal of 9AA resulted simply in later sampling of the mating pool, it would be predicted that the recombination frequency would rise with the duration of 9AA treatment at only half the rate observed. Experiments in this laboratory are consistent with the following alternative model: vegetative phages replicate, recombine, and condense at normal rates in the presence of 9AA. The condensed phage formed are not matured into whole phage particles until the inhibitor is removed. Condensed phage formed in the presence of inhibitor give rise to noninfective fully mature phage; condensed phage formed after removal of the inhibitor give rise to infective mature phage. DNA condensation randomly withdraws phage from the vegetative pool; formation of fully mature phage randomly withdraws phage from the condensed phage pool.

This model of phage maturation differs from the one usually assumed in that there is postulated a pool of condensed phage as well as a pool of vegetative phage. Thus, the first phage withdrawn from the vegetative pool are not necessarily the first phage to appear as infective progeny. If this is true, the kinetics of recombination as measured among mature phage is a more sluggish measure of the kinetics of recombination in the vegetative pool than has previously been assumed.

**201. THE MASS PER UNIT LENGTH OF DNA**

Investigator: John R. Menninger  
Support: National Science Foundation  
General Funds

In order further to test the hypothesis for the replication of DNA suggested by Crick and Watson a measurement was attempted of the mass per length of the DNA molecules of bacterium E. coli. Meselson and Stahl were able to show that this DNA is composed of two complementary subunits, that during replication the
the two subunits are separated from one another and that new subunits are synthesized and combine with the old ones to form so-called hybrid molecules composed of one new and one old subunit. Rolfe showed that hybrid DNA when subjected to chain breaks by sonication still manifests the density of hybrid DNA, implying that the subunits are not joined end to end. By showing that the pass per length of DNA from E. coli is that predicted from the Watson-Crick model for the structure of DNA it was hoped to identify the subunits as the single polynucleotide strands of the molecule.

The measurement of the linear mass is accomplished by obtaining low-angle X-ray diffraction patterns from aqueous gels of DNA. In such concentrated gels the DNA molecules behave as relatively rigid rods and assume an hexagonally close-packed configuration. The diffraction spacings enable one to calculate the nearest neighbor distance of the molecules and thus the area of the unit cell. One also measures the macroscopic concentration of DNA in the gel and the macroscopic density of the gel. The former measurement is done by phosphorus determination and the latter by flotation of a sample of gel in a calibrated density gradient column of the type developed by Linderstrøm-Lang and utilizing toluene and carbon tetrachloride as liquid vehicles. It is then a simple matter to set up a relation involving the mass per length of the DNA, which equates the mass per volume of the gel with the same ratio referred to the unit cell. This relation is valid if the gel is homogeneous, which can be verified by performing the measurements on a series of gels of different DNA concentrations.

The measurements are now being carried out, but no conclusive results have yet been obtained.

202. THE SPECIFICITY OF RIBOSOMES

Investigators: S. Brenner, F. Jacob, M. Meselson
Support: National Foundation
National Institutes of Health, Public Health Service
Rockefeller Foundation

Ribosomes are thought to be the sites of protein synthesis. When E. coli is infected with phage T4, the production of bacterial protein halts and specific phage proteins are made instead. However, it is known that very few if any ribosomes are synthesized after phage infection. This suggests that ribosomes are not endowed with a fixed specificity for protein synthesis but instead may repeatedly receive their specificity. This might be accomplished by the repeated incorporation and subsequent removal of "messenger molecules" without turnover of the bulk of the ribosomes themselves.

Experiments have been performed to detect the incorporation of newly synthesized RNA into otherwise previously formed ribosomes. RNA was chosen as the object of this investigation because it is commonly supposed to function as a carrier of information from DNA to protein. Furthermore, a special RNA fraction is known to be produced during phage infection which turns over rapidly and which resembles phage DNA in its composition.

The experiments utilized heavy isotopic labeling and cesium chloride density gradient centrifugation of ribosomes to determine whether RNA, synthesized during phage infection, appears in ribosomes which themselves were synthesized before phage infection. Such an RNA fraction was indeed found and may therefore play the role of the presumed "messenger." It is not an indispensable component of ribosomes, for at low magnesium concentrations it dissociates from ribosomes which are not themselves destroyed in the process. Work is under way to clarify and extend these results.
203. STUDIES ON THE ULTRAVIOLET RADIOBIOLOGY OF PHAGE T4

Investigators: U. Winkler, H. E. Johns, E. Kellenberger
Support: North Atlantic Treaty Organization
Deutsche Forschungsgemeinschaft
National Foundation
Commonwealth Fund

Phage T4 was irradiated at a series of wavelengths from 220 mµ to 305 mµ, using a quartz monochrometer. With the exception of a small shoulder at low doses, killing curves were exponential at all wavelengths. The resulting action spectrum showed the expected peak at 265 mµ (corresponding to the absorption maximum of DNA) and a minimum at 235 mµ. Using much larger doses, there was also studied the effect of ultraviolet light on other phage properties.

Phage irradiated with up to 200 phage lethal hits (PLH) were examined for morphological damage in the electron microscope. At 235 mµ many particles were sufficiently damaged to release their DNA, yielding phage "ghosts." In addition, a number of different types of tail lesions were observed. Phage irradiated at 265 mµ showed no ghosts and no tail lesions.

One of the first visible effects after phage infection is the dispersal or "breakdown" of the bacterial nucleus. At 235 mµ and 265 mµ, the ability to cause nuclear breakdown was exponentially inactivated at a rate 1/15 of that for phage survival. Next to survival itself, this was the most sensitive property studied.

The inactivation of the bacterial-killing-ability (BKA) of phage T4 was studied at 9 wavelengths. The inactivation curves are not simple exponential functions. This might have been anticipated from the fact that phage ghosts prepared by a variety of methods are able to kill bacteria but with a lowered efficiency. From detailed studies of the inactivation curves, it is concluded that BKA can be inactivated in at least two distinct ways—-one corresponding to damages in DNA and the other to damages in protein.

When bacteria are mixedly infected with a UV-inactivated phage and genetically marked unirradiated phage, it is observed that markers from the "dead" phage appear in the progeny. This phenomenon, termed marker rescue, was studied at 9 wavelengths. For the same dose (expressed in PLH), the probability of a marker being rescued is highest in the range 255 to 265 mµ; at higher and lower wavelengths, this probability is very much less.

These studies show that phage can be damaged by ultraviolet light in many different ways, each of which may show a different wavelength dependence. The need for the use of a monochromatic source of radiation in future studies is thus indicated.
UV-inactivated KAPPA can be reactivated like other temperate phages by plating on UV-irradiated indicator cells. The frequency of c (clear plaque) mutants is increased by irradiation of extracellular KAPPA and plating on untreated indicator. This induction of mutants can be increased further about 2 to 4 times by using UV-irradiated indicator. The increase in the formation of c mutants under the latter conditions, with increasing UV dose given to the phage, was never a first-order reaction. The frequency of extracellular UV-induced c mutants plated on irradiated indicator is increased by the multiplicity of infection at multiplicities lower than 0.3, but decreases by increasing multiplicity above this value. Two c mutants studied in detail belong to two different cistrons as shown in a complementation test (map distance about 2.6%). One mutant was revertible to the phenotype c+ spontaneously and with a higher rate by UV. However, the "backmutants" do not have the original genotype; as shown in crossing experiments with the standard type, they originated from mutations in at least two different intragenic suppressor loci (map distances to the original c mutation were .32% and .07%). Plating of unirradiated wild type (or c mutants) on irradiated indicator (lowest survival fraction was $10^{-4}$) never increased the spontaneous mutation rate to c (or c+). Host range (h) mutants could not be induced by irradiation of the free phage, nor could mutants which can multiply in KAPPA-lysogenic cells. This indicates that the UV-induced high mutability of the c loci in KAPPA represents an exceptional case of behavior. Some h mutants could be isolated by plating irradiated KAPPA on irradiated indicator. Irradiation of a mixture of free ch and ++ phages increased by a factor of 5.5 the frequency of +h recombinants.
VISITING LECTURES

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

Dr. R. M. Gaze, University of Edinburgh: "The Eye and the Tectum: Experiments on Visual Recovery after Section of the Optic Nerve."

Dr. J. R. Laughnan, Department of Botany, University of Illinois: "Genetic Fine Structure and Mutation in Maize."

Dr. Hugh Huxley, Department of Biophysics, University College, London: "Staining of Nucleic Acids for Electron Microscopy."

Dr. N. A. Mitchison, Department of Genetics, School of Medicine, Stanford University: "The Kinetics of Immunological Tolerance."

Dr. I. R. Lehman, Department of Biochemistry, School of Medicine, Stanford University: "The Deoxyribonucleases of Escherichia coli."

Dr. Robert S. Bandurski, Department of Botany, Michigan State University: "Enzymatic Sulfate Reduction."

Dr. Robert Galambos, Department of Neurophysiology, Walter Reed Army Medical Center: "Some Experiments on the Auditory System."

Dr. Michael Feldman, Virus Laboratory, University of California, Berkeley: "Immunogenetic Studies on Radiation Chimeras."

Dr. V. Twitty, Biology Department, Stanford University: "Studies on Homing Orientation in Salamanders."

Prof. Janos Szentogothai, Department of Anatomy, University of Pecs, Hungary: "Neuronal Specificity and Plasticity Demonstrated in Functional Effects of Embryonic Disarrangement."

Dr. Richard Held, Department of Psychology, Brandeis University: "Analysis of Perceptual Integration in Man."


Dr. J. R. S. Fincham, John Innes Institute, England: "Biochemical and Genetics Studies on the Glutamic Dehydrogenase of Neurospora."

Dr. Charles Yanofsky, Department of Biological Sciences, Stanford University: "Genetic and Biochemical Studies with the A Protein of Tryptophan Synthetase in Escherichia coli."

Prof. George Melchers, Max-Planck-Institut für Biologie, Tübingen, Germany: "Genetic Aspects of Biological and Biochemical Studies of T. M. V."

Dr. Wolfhard Weidel, Max-Planck-Institut für Biologie, Tübingen, Germany: "The Cell Wall of a Famous Gram-negative."

Dr. D. G. Catcheside, Department of Microbiology, The University, Birmingham, England: "Some Aspects of Gene Function."
Dr. George Streisinger, University of Oregon: "Genetic Studies of Bacteriophage T4 Lysozyme."

Dr. M. N. Runner, National Science Foundation: "Experimentally Induced Congenital Deformities for Studying Mammalian Embryology."

Dr. P. S. Chen, Department of Zoology, University of Zürich: "Biochemical Studies on Sea Urchin and Amphibian Embryos."

GRADUATES

Ten students were awarded the B.S. or the Ph.D. degree in Biology in June, 1961. The names, degrees conferred, the titles of doctoral theses, and the plans of the recipients for future work are as follows:

Leland Harrison Hartwell, B.S. To Massachusetts Institute of Technology for graduate work in biology.

Joel Allen Michael, B.S. To Stanford University Medical School.

Douglas Keith Stewart, B.S. To Harvard University Medical School.

Harris Bernstein, Ph.D. Thesis: "I. Imadazole Compounds Accumulated by Purine Mutants of Neurospora crassa. II. Complementation Studies with Isoleucine-Valine Mutants of Neurospora crassa." He is now doing postdoctoral research in the Botany Department at Yale University.

Boyd Archer Hardesty, Ph.D. Thesis: "The Biochemistry of Cytochrome c in Relation to Maternally Inherited Phenotypes of Neurospora." He is now doing postdoctoral research in the Department of Biochemistry at Yale University.

Dennis Robert McCalla, Ph.D. Thesis: "I. Chemical Study of Necrotic Corn Mutants. II. The Metabolism of a Kinin. II. The Chemical Nature of an Insect Gall Growth Factor." He has been appointed Assistant Professor at McMaster University, Ontario, Canada.

Ann Roller, Ph.D. Thesis: "Studies on the Replication and Transfer to Progeny of the DNA of the Bacteriophage T4." She is now doing postdoctoral work at the California Institute of Technology.

Charles Myron Steinberg, Ph.D. Thesis: "I. Some Theoretical Problems Arising in the Genetics of Bacteriophage. II. A Critical Test of a Current Theory of Genetic Recombination in Bacteriophage." He will do postdoctoral work at the University of Cologne with Dr. Max Delbrück.

Ivan Jeanne Mayfield Weiler, Ph.D. Thesis: "Restoration of Visual Acuity after Optic Nerve Section and Regeneration in Astronotus ocellatus Agassiz."
HONORS AND AWARDS

E. G. Anderson has been appointed Visiting Professor at the University of Illinois.

Renato Dulbecco was elected a member of the National Academy of Sciences.

A. J. Haagen-Smit was appointed to the Motor Vehicle Pollution Control Board of the State of California; elected a trustee of the Los Angeles State and County Arboretum and of the American Chemical Society; appointed a member of the Advisory Council of the Association for the Advancement of Biomedical Careers.

Alan J. Hodge was appointed a member of the Council of the Biophysical Society; appointed a member of the Biophysical Sciences Training Committee of the National Institutes of Health.

Anton Lang was appointed a member of the Advisory Panel for Developmental Biology of the National Science Foundation.

Ray D. Owen was elected Vice President of the Genetics Society of America; appointed Chairman of the Genetics Study Section of the National Institutes of Health; appointed to the Governor's Cancer Advisory Council; appointed to the National Advisory Committee of the Learning Resources Institute; appointed a member of the Advisory Council of the Association for the Advancement of Biomedical Careers.

A. H. Sturtevant was Visiting Professor at the University of Washington from September to December, 1960.

Albert Tyler is President-Elect of the Society of General Physiologists.

C. A. G. Wiersma was appointed to the Physiology Training Committee of the National Institutes of Health.

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, as follows:

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<tr>
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<td>General biology support</td>
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<tr>
<td>A. C. Balch</td>
<td>General biology support</td>
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<tr>
<td>Mrs. Henry M. Robinson</td>
<td>Biological and chemical research, particularly as related to cancer</td>
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<tr>
<td>John H. Eagle</td>
<td>Biological and chemical research, particularly as related to disease</td>
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Frank P. Hixon  Neurophysiology, physiological psychology, and related research
E. S. Gosney  Gosney postdoctoral fellowships
Charles F. Holder  General biology support
Lucy Mason Clark  Plant physiology fellowships
Arthur McCallum  Biology fellowships

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<tr>
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<td>Alwilda Harvey</td>
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Tomato fruit set studies

Commonwealth Fund  
General support

Max C. Fleischmann Foundation  
Research on immunogenetics  
of Nevada

Herman Frasch Foundation  
Plant growth research

German Academic Exchange Service  
Bacterial virus studies

Lalor Foundation  
Fertilization studies

Lasker Foundation  
Fellowships, scholarships

Los Angeles County Air Pollution  
Smog research on plants
Control District

Mrs. Austin McManus for Desert  
Plant research in desert areas
Plant Research

Malcolm McNaghten  
Scholarships

National Foundation  
Bacterial and animal virus research,  
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National Science Foundation  
Studies in animal physiology, biochemistry,  
biophysics, genetics, plant and cell biology

Nutrition Foundation  
Research on biochemical genetics of  
Neurospora

Nutrition Foundation--McCallum  
Predoctoral fellowships
Foundation

Rockefeller Foundation  
General biology support; studies 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, terpenes

U. S. Department of Agriculture  
Plant physiology and corn cytogenetics  
research

U. S. Navy, Office of Naval Research  
Research on spinal reflexes in cords

U. S. Public Health Service  
Studies in basic experimental biology,  
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