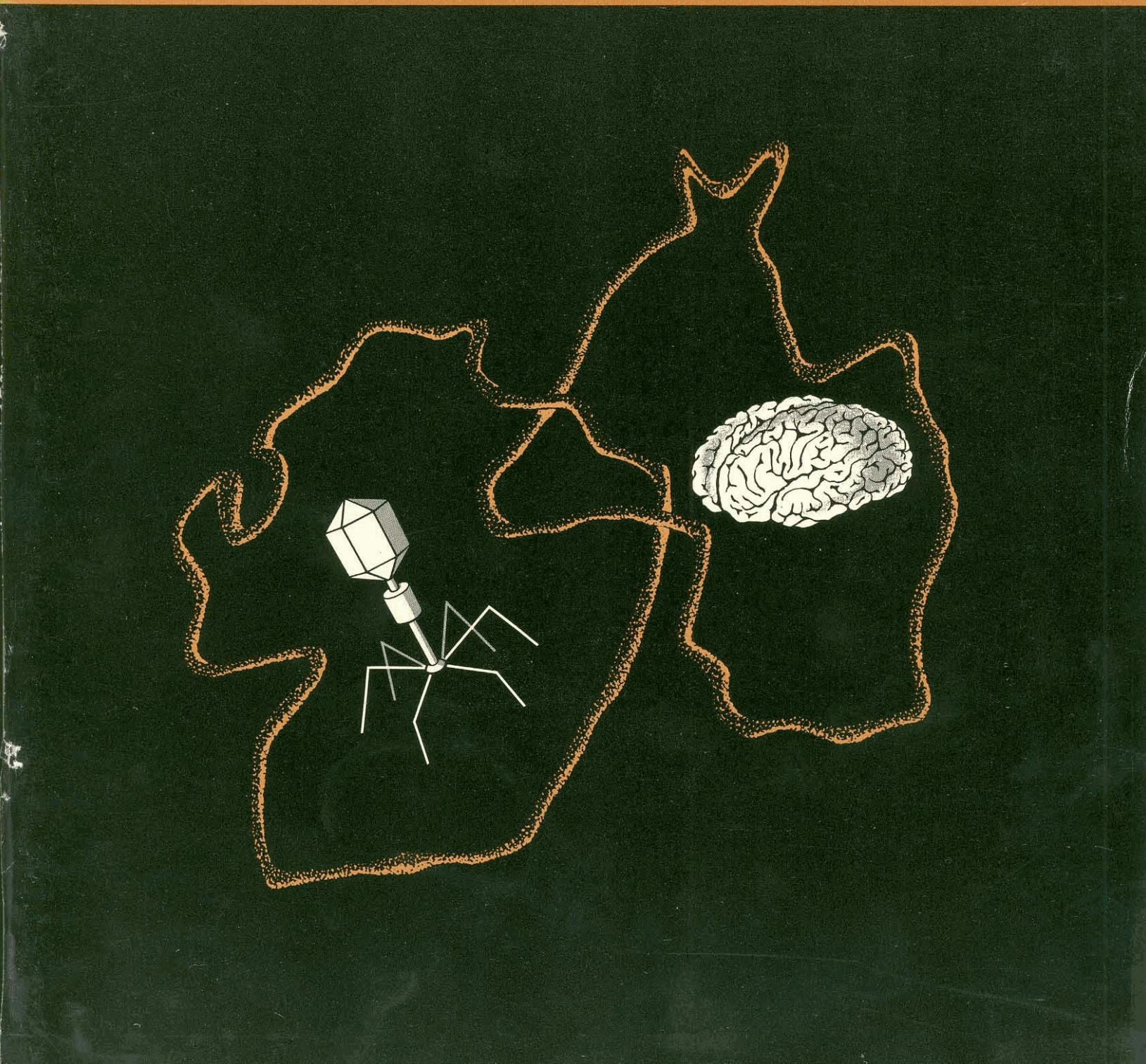
NOVEMBER 1968

E&S ENGINEERING AND SCIENCE

PUBLISHED AT THE CALIFORNIA INSTITUTE OF TECHNOLOGY



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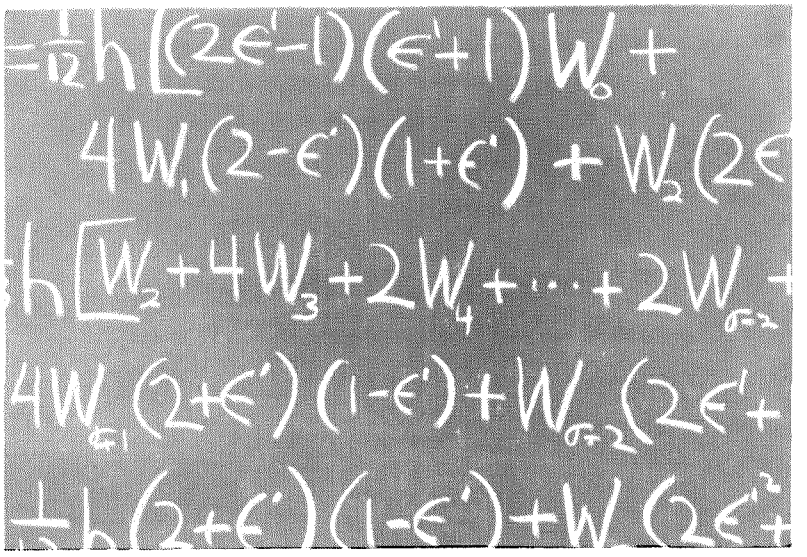
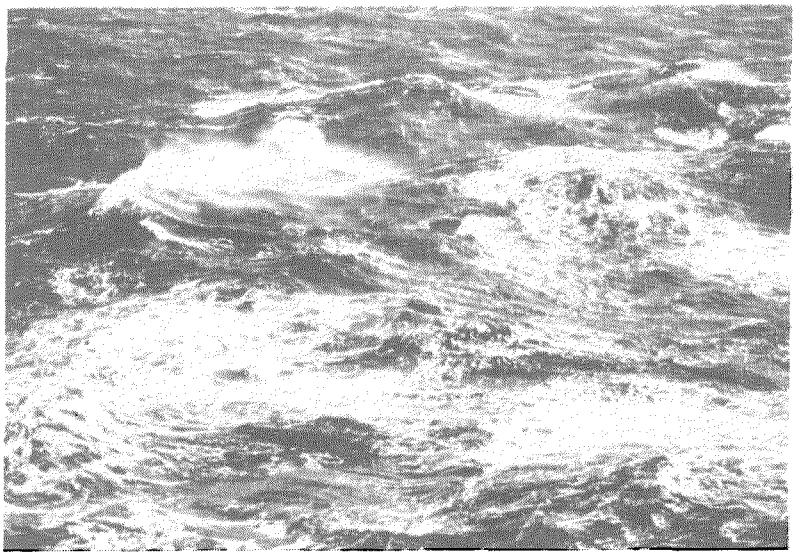


or knowledge of oceans.



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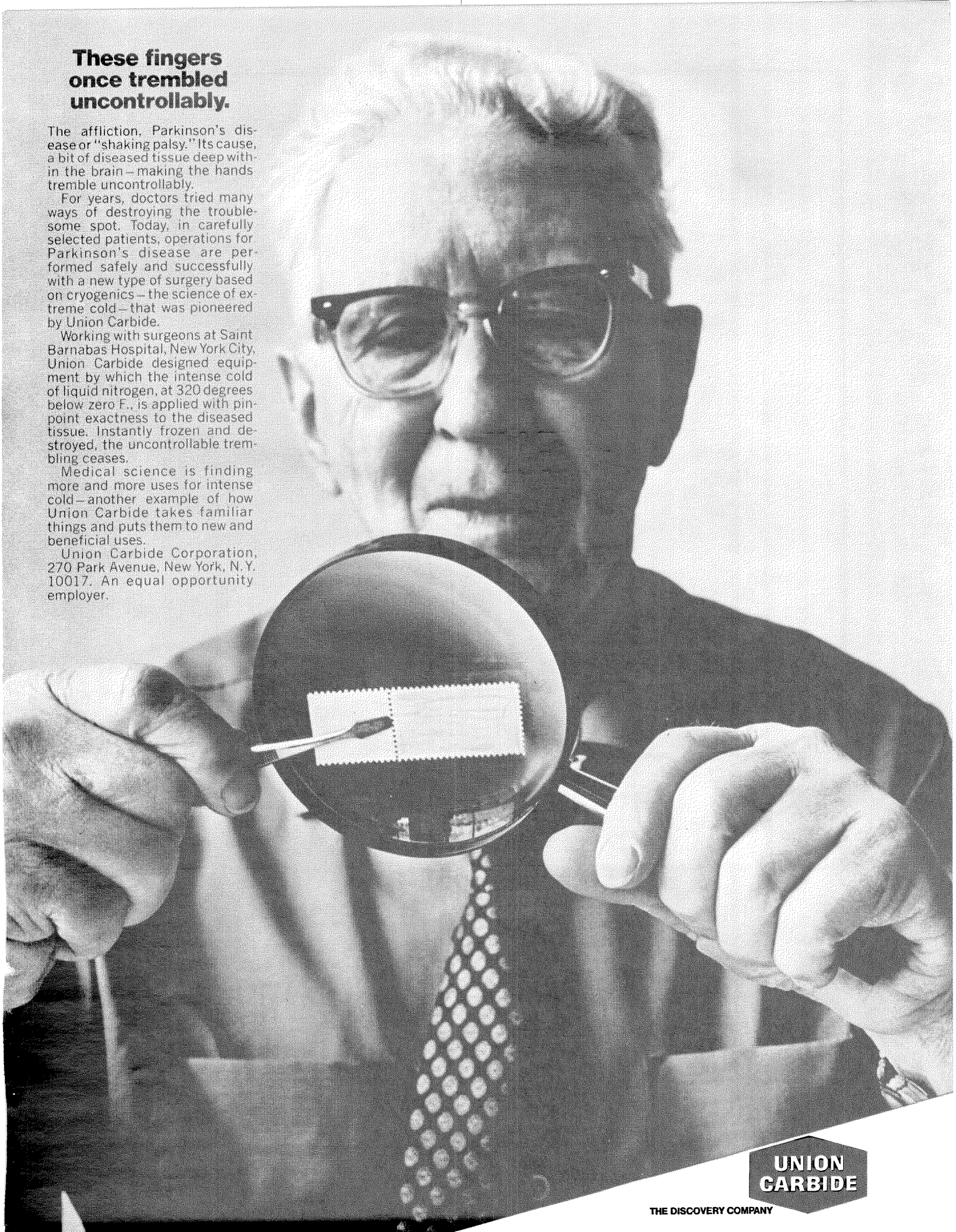

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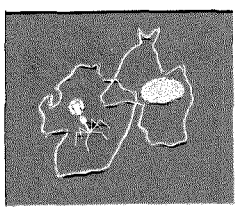
ENGINEERING AND SCIENCE

NOVEMBER 1968 / VOLUME XXXII / NUMBER 2

A SPECIAL ISSUE ON BIOLOGY AT CALTECH

Published monthly, October through June, at the California Institute of Technology, Pasadena, Calif. 91109. Annual subscription $4.50 domestic, $5.50 foreign, single copies 50 cents. Second class postage paid at Pasadena, Calif., under the Act of August 24, 1912.

A Special Issue



From simple bacteriophages to the complexities of the mammalian brain, all life forms spring from a common source—DNA, which has within it genetic information accumulated over billions of years of evolution. On our cover, two linked "circular" chains—each a DNA molecule—make a frame for a bacteriophage—a virus described as "life trimmed to its barest essentials"—and a brain, the most complex development in the development of life. These symbols of biology serve to introduce this special issue on biological research at Caltech. The issue marks the 40th anniversary of the establishment of the Division of Biology at the Institute.

Robert L. Sinsheimer, chairman of Caltech's biology division since April 1968, has been involved in several significant discoveries during his 12 years of study of the genetics of the virus *Phi X 174—the first single-stranded* DNA, the first ring-shaped DNA, and the first artificial synthesis of infective DNA. Now, as the division begins its 40th year, he turns his attention to the new alliance between the biologists and the behavioral scientists and their search for the biological bases of animal and human behavior.

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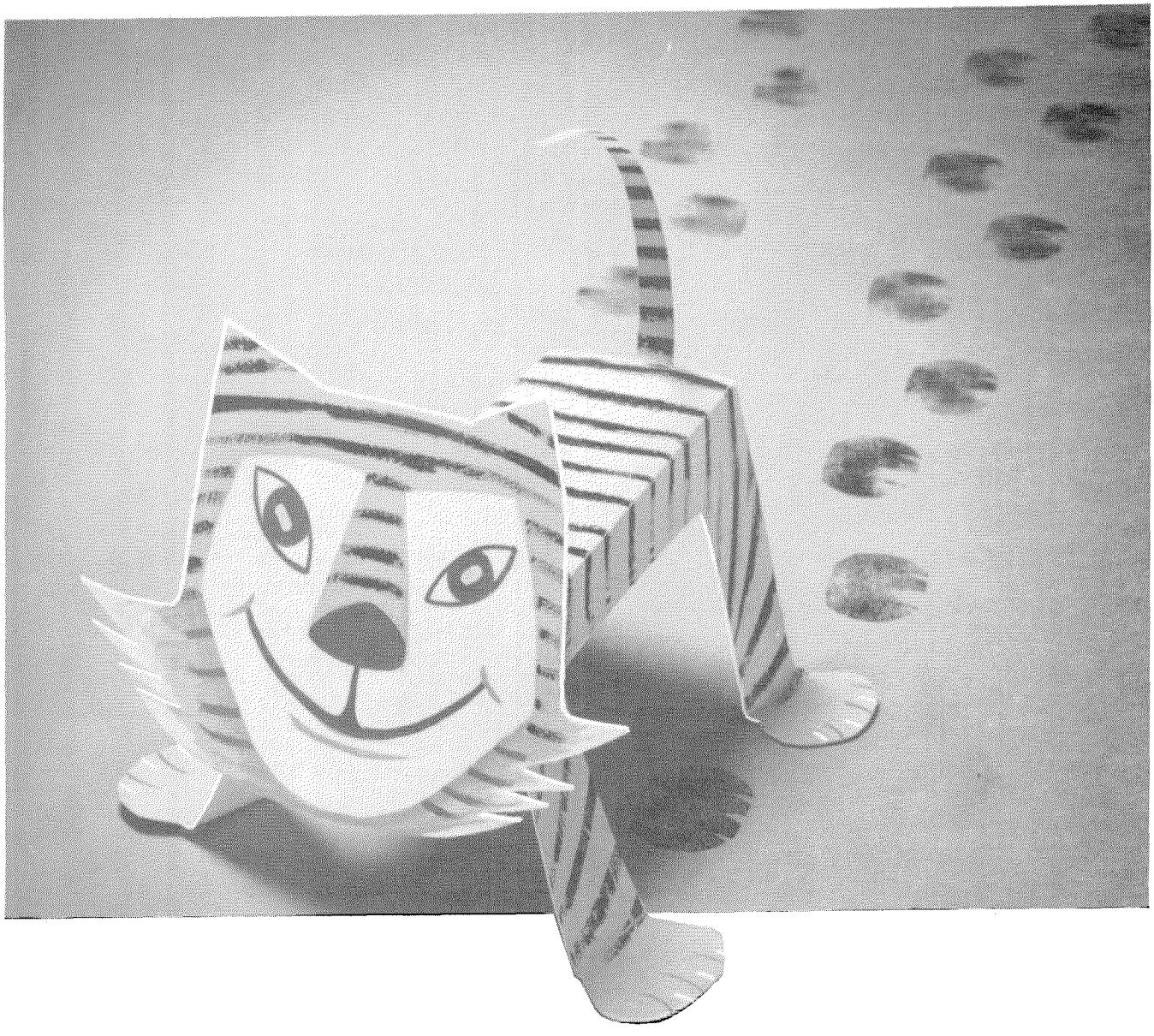

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Don earned a B.S.E.E. in 1965. Today, he's an Associate Engineer in systems design and evaluation at IBM.

Most of his work consists of determining modifications needed to make complex data processing systems fit the specialized requirements of IBM customers.

Small teams

Depending on the size of the project, Don works individually or in a small team. He's now working with three other engineers on part of an air traffic control system that will process radar information by computer.

Says Don: "There are only general guidelines. The assignment is simply to come up with the optimum system."

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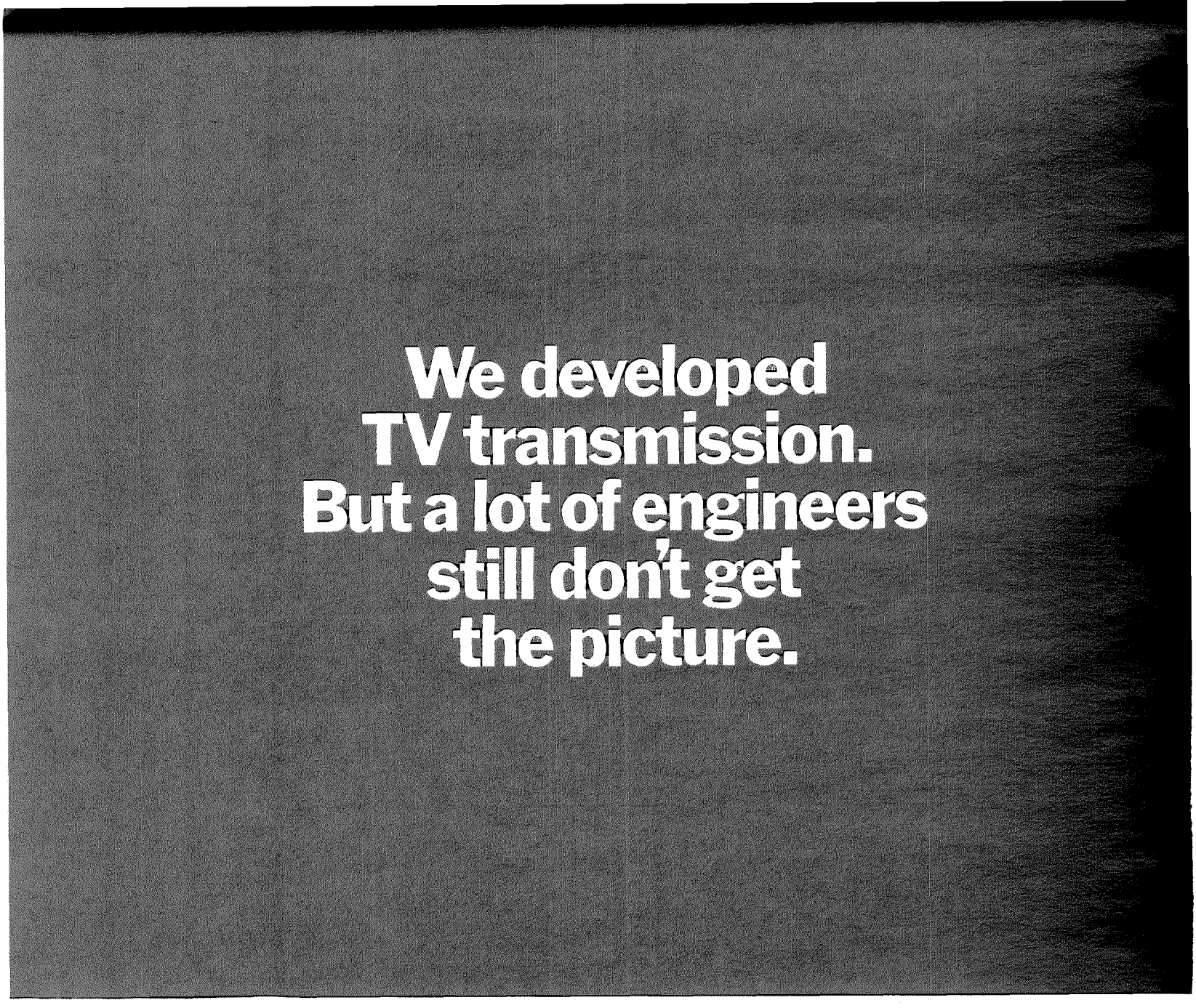

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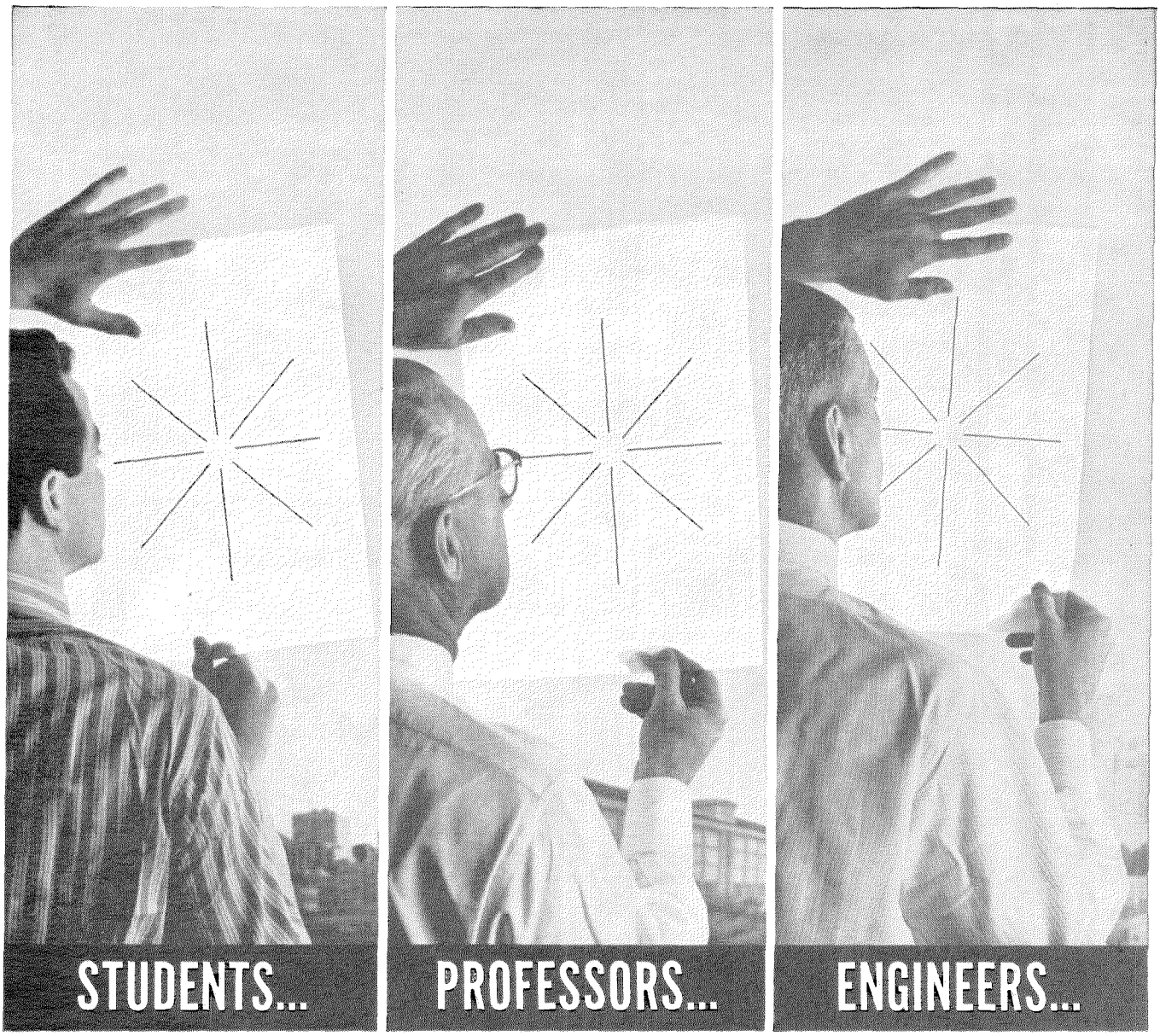

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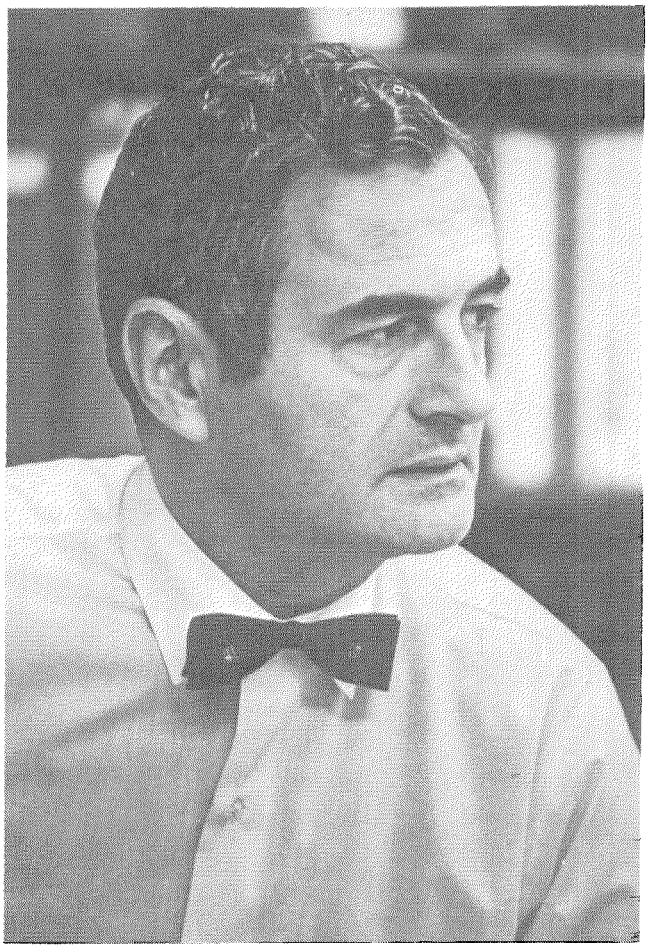


BIOLOGY—

Biology is the science of life—life constrained by, yet dependent upon, the laws of physics and chemistry; life emergent out of the elemental atoms and molecules of the primordial universe; life the persistent transformer of the surface of the planet; life the only means of which we know for that slow accumulation of structural complexity that has permitted the expression of the specialized capabilities inherent in increasingly organized forms of matter; and finally and most profoundly, life the bearer of logical intelligence and ordered consciousness, the originator of cumulative knowledge, the viewer of beauty, the maker of poetry, the dreamer of justice. With a concern that ranges from the special properties of carbon atoms to the inheritance of genius, from the viral origin of disease to the hormonal origin of aggression, from the quantum mechanics of photosynthesis to the conscious sensation of color, biology is the central science.

These are splendid years for biology—years in which long inaccessible mysteries are revealed one by one and even once-inexpressible quandaries seem to take

An Introduction by Robert L. Sinsheimer

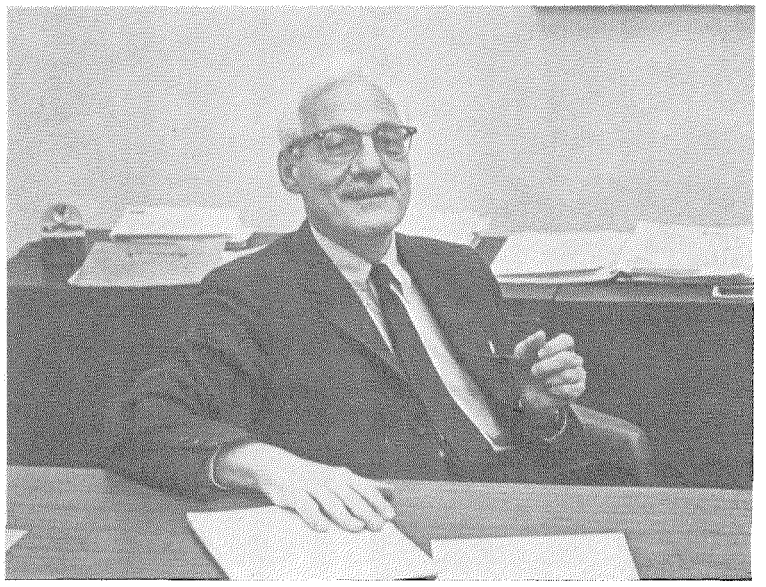
THE CENTRAL SCIENCE

form and offer foothold. This is so because *now* is the appropriate time in the history of science—the time in which physics and chemistry have achieved the maturity, the understanding of the nature of matter, to provide a secure base for biology; the time in which the patiently accumulated insights into evolutionary pattern and genetic law, into developmental principle and physiological function, suddenly fall into place on an underlying base of biochemistry and biophysics; the time in which cybernetic principle and computer analog begin to match neurophysiological pattern and thus provide beckoning leads to the analysis of mind.

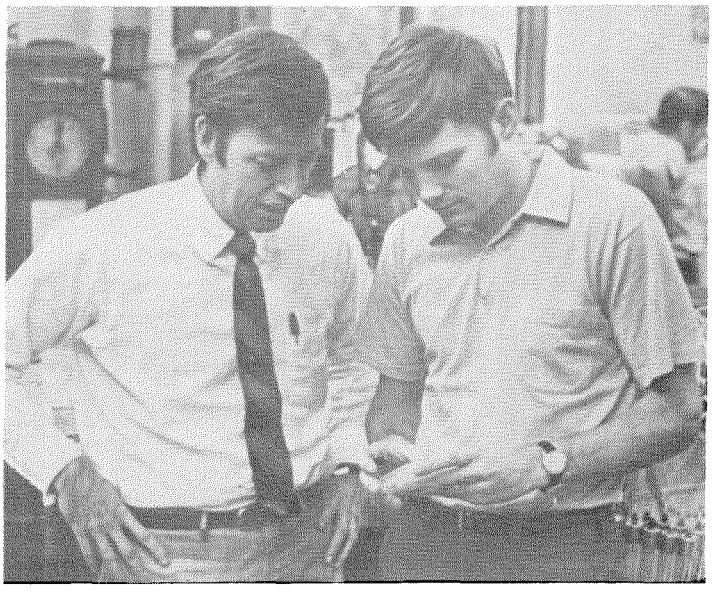
As you will learn *per exemplum* in the following pages, the biologists at Caltech are actively advancing their science—exploring these frontiers and training those who will continue this epoch of discovery. With its initial base in genetics Caltech biology has made good use of its intellectual proximity to vigorous divisions of physics and chemistry and has been a major contributor to the dramatic development of molecular biology. Today, molecular biologists are directing their newfound insights to classic problems of morphogenesis, of cellular biology, of the origin of developmental pattern and the physiology of sensory perception, and are probing tentatively yet sharply for the molecular bases of mental function and integrated behavior.

On other levels the neurophysiologist and the psychobiologist each in his own way are seeking and gaining understanding of the neuronal and organizational bases of mental function and integrated behavior. In the synergistic combination of these approaches—in the combination of the disciplines of the geneticist and the biochemist, the developmental biologist and the neurophysiologist, the psychobiologist and the ethologist, the experimental and the individual psychologist—focused together upon the analysis of mind and the biological determinants of behavior, the Division of Biology sees its future fulfillment: to be the central science in an Institute which couples profound scientific curiosity with intense human concern.

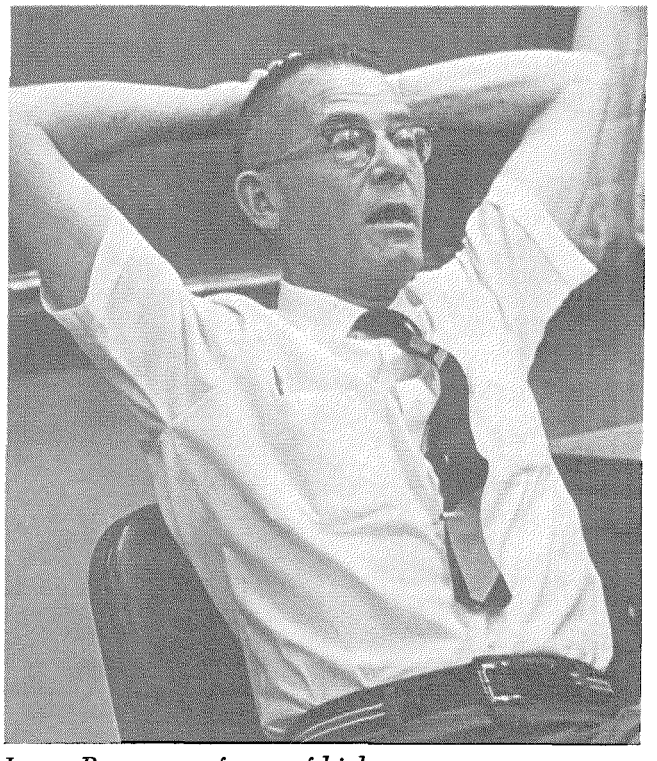
Contributors To The Biology Issue



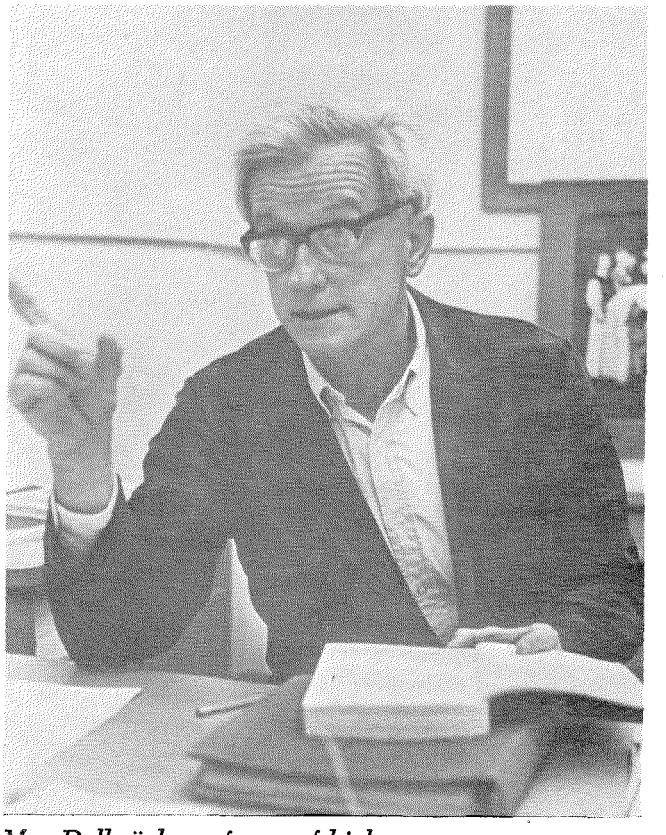
Jerome Vinograd, professor of chemistry and biology.



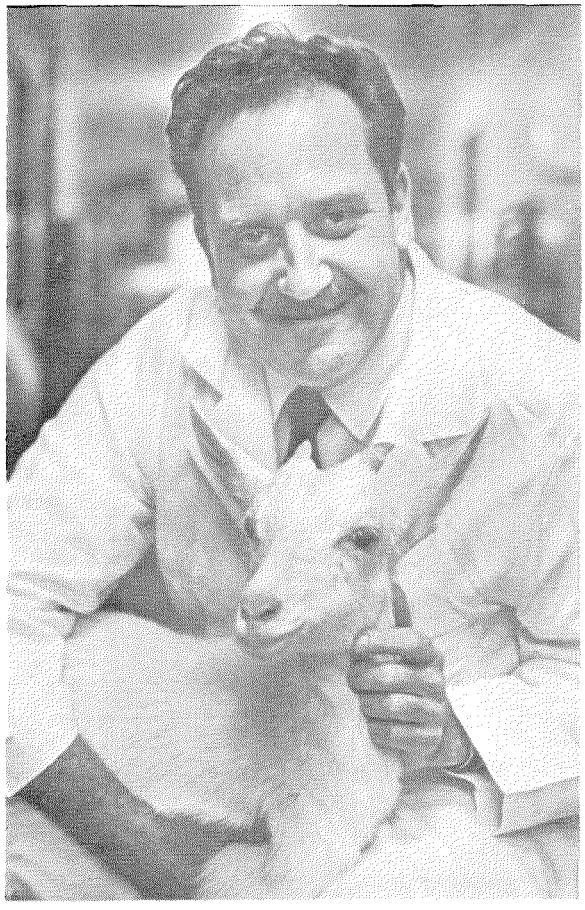
Robert S. Edgar, professor of biology, and William B. Wood, associate professor of biology.



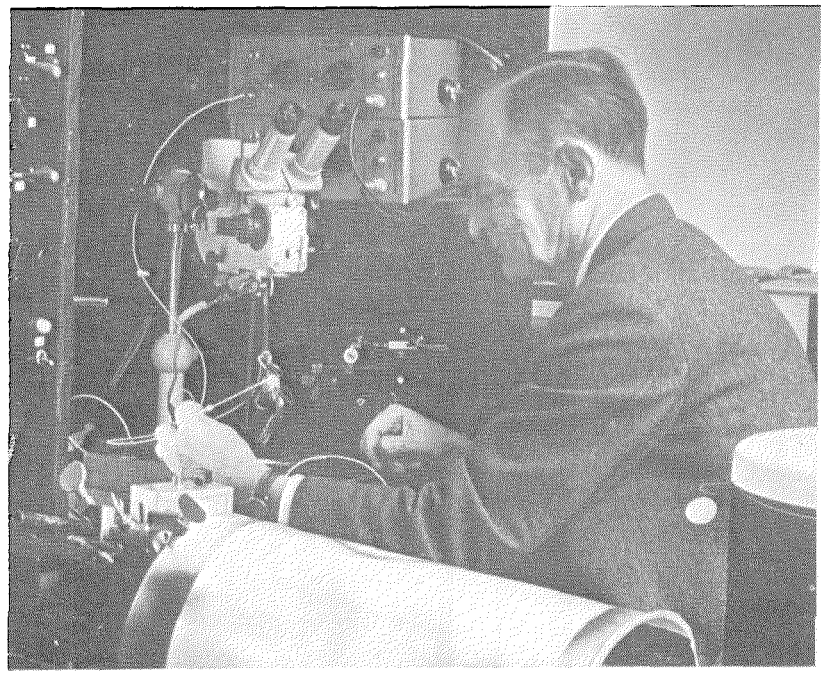
James Bonner, professor of biology.



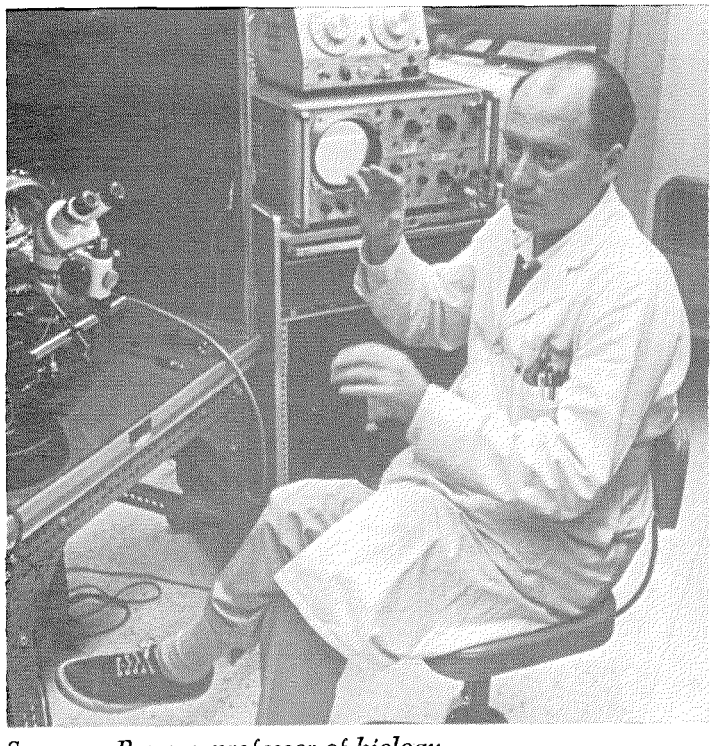
Max Delbrück, professor of biology.



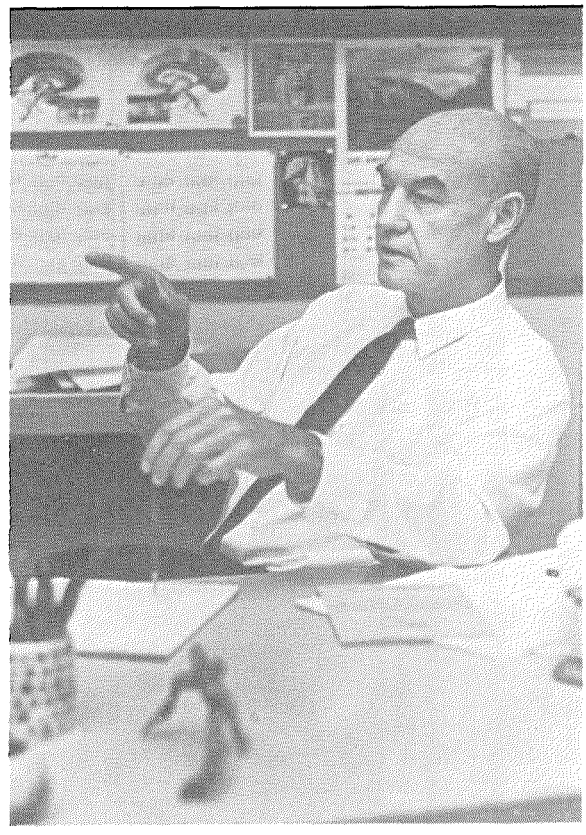
Ray Owen, professor of biology.



C. A. G. Wiersma, professor of biology.



Seymour Benzer, professor of biology.



Roger W. Sperry, Hixon Professor of Psychobiology.

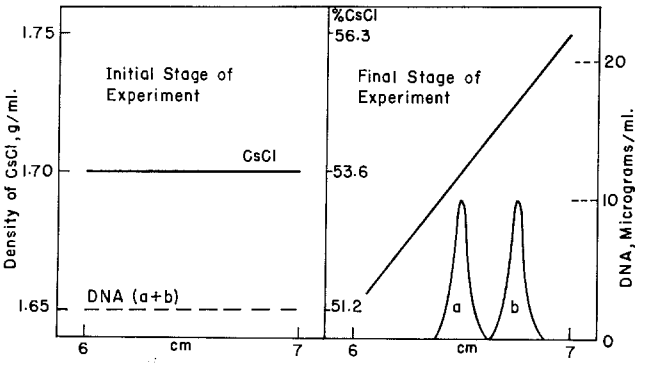
CENTRIFUGES, CIRCLES, AND CANCER

By JEROME VINOGRAD

Molecular biology has among its goals the understanding of the molecular events that occur in living cells. Centrifugation, circular DNA, and cancer have played major roles in this exciting new science.

Centrifugation is one of the principal tools of the molecular biologist. It is used to isolate and characterize cellular macromolecules and organelles. Circular DNA, because of its well-defined size and the absence of ends, sharply tests our knowledge of the cell's machinery for replicating DNA and expressing its information. Although the problems of cancer—the causes and cures—appear to be awaiting new discoveries in the molecular biology of normal cells, we already know that there are abnormalities in the chromosomes of patients having certain types of leukemia. We also know that when tumor viruses such as *polyoma* transform normal mouse cells into malignant cells, the originally circular viral DNA becomes an integral part of a long strand of nuclear DNA.

About ten years ago, three years after Watson and Crick described the duplex (double-stranded) structure of DNA, Matthew Meselson, then a graduate student in chemistry, approached me with a problem. Could light and heavy DNA molecules, differing in mass by 1 to 2 percent, be separated in a centrifugation experiment? If so, could a hybrid molecule of DNA containing one light progeny strand and one heavy parental strand also be distinguished? The presence of such hybrid molecules in the DNA of an organism initially grown in a medium containing heavy isotopes and then transferred for further growth to a medium containing normal isotopes would provide further direct evidence for the proposed duplex structure of DNA and for the Watson-Crick proposal that a DNA molecule replicates semi-conservatively (replicates, that is, to form two daughter molecules, each of which contains one of the original strands).



The results of a buoyant density experiment show how two DNA's (a and b), when centrifuged in a concentrated cesium chloride solution, move to regions of neutral buoyancy and form bands in the solution.

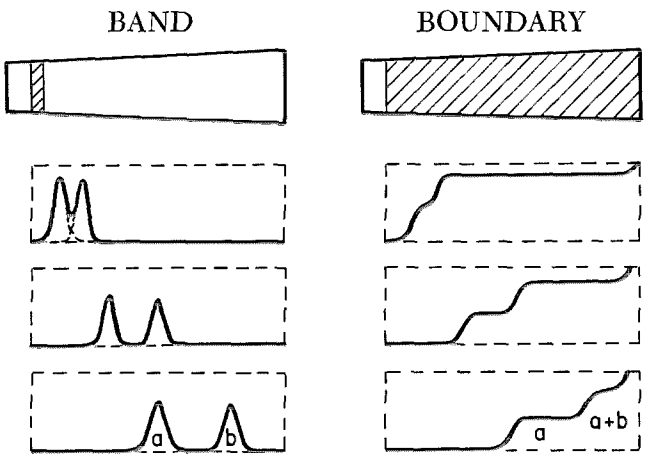
I replied that the resolving power of sedimentation velocity analyses as then practiced was inadequate, but that infinite resolution could be attained if one DNA species were held stationary while the other moved. I suggested that such a condition could be achieved by performing the sedimentation in concentrated salt solutions of high liquid density.

Each experiment begins with a change in DNA. With delicate specificity
the machinery of the cell amplifies and channels each change
until it reaches its functional expression and its harsh trial,
subject to the verdict of survival and reproduction or failure and extinction.
Thus in the cumulative laboratory of evolution has arisen the whole intricate pattern of life
which the mind of man now attempts to unravel.
Some probe the DNA itself.

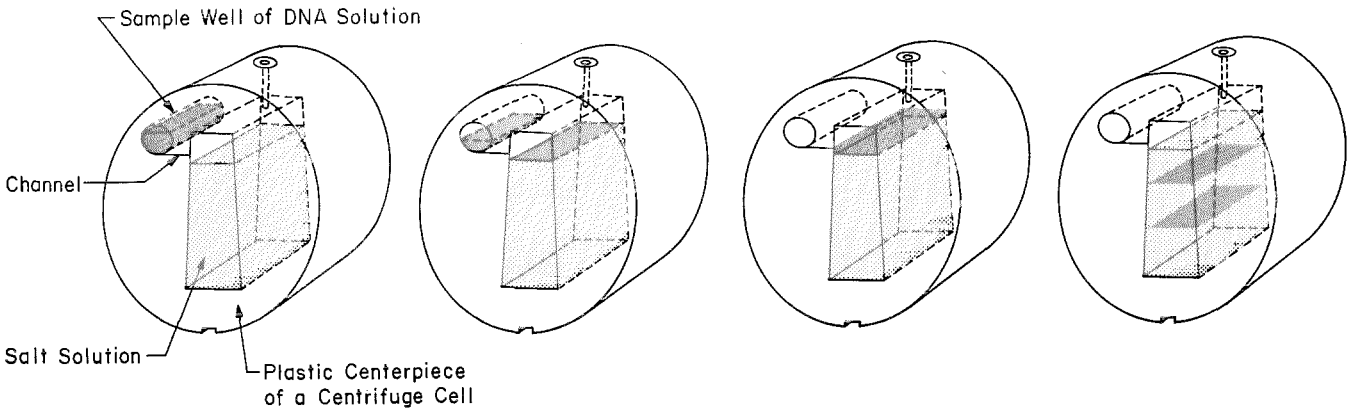
Our first experiment—in the autumn of 1956 in the subbasement of Church Laboratory—reminded us that the salt itself, redistributing in the centrifugal field, would form a significant density gradient. In density gradients of this sort, DNA species move to regions of neutral buoyancy and there form bands. Dense DNA's form bands in the denser salt solution, and "light" DNA's form bands in the less dense salt solution. The transfer experiment, now thought of in terms of separating dense and less dense species as opposed to heavy and light masses, could clearly be done and was carried out by Meselson and Franklin W. Stahl in their now classic experimental validation of the Watson-Crick hypothesis.

The theory and practice of buoyant density centrifugation has, since its inception, been vigorously investigated in the Caltech laboratories. The density of the macromolecular complex at band center, a quantity now known as the buoyant density, is numerically equal to the density of the solution at band center and is readily measured with high precision and accuracy. Proteins, carbohydrates, DNA, and RNA exhibit widely different buoyant densities and are therefore very easily separated by this procedure. Nucleoproteins such as viruses normally form bands at densities that correspond to the weight fraction of the nucleic acid. DNA's of differing base composition have different buoyant densities. The buoyant method has become a favorite procedure for the analysis of the base composition of DNA. The *Handbook of Biochemistry* lists the buoyant densities of some 300 DNA's from various organisms and viruses.

Our interest in circular duplex DNA arose in 1963 when Roger Weil and I and Renato Dulbecco and Marguerite Vogt discovered that the DNA in the tumor-inducing virus *polyoma* occurred in the form of a new DNA structure—a closed circular duplex without ends. The experiments that led to this con-



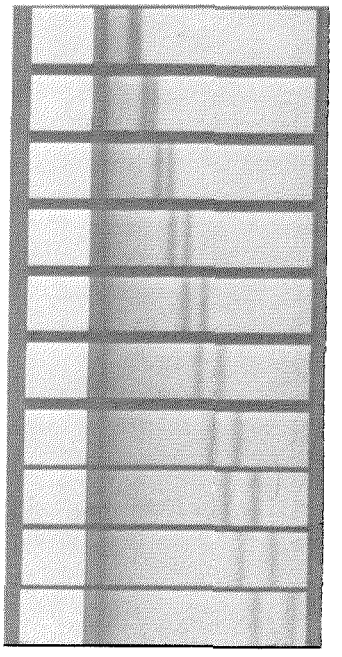
A comparison of the results of two methods of performing sedimentation velocity experiments shows the advantages of Caltech's new band method (left) over the boundary method (right). A smaller amount of DNA (indicated by striped lines) can be used. As sedimentation progresses (top to bottom frames), the concentration distributions of the DNA can be seen.



In a sedimentation velocity experiment a thin layer of a solution of DNA, stored in a sample well, flows out through a narrow channel under the influence of the centrifugal field and spreads out as a thin layer onto the surface of a salt solution. Diffusion of water from that layer into the slightly denser salt solution occurs rapidly and forms the density gradient necessary for stabilizing the band of DNA as it sediments through the salt solution. Both the DNA sedimentation and the necessary gradient formation occur almost simultaneously. The last drawing shows the position of two bands of DNA during the experiment.

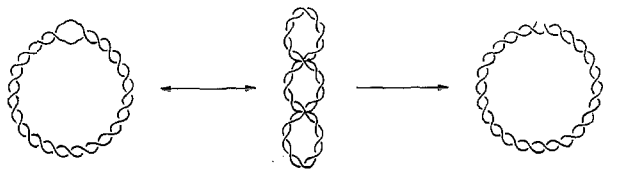
clusion were performed by a new method that we had just evolved for performing sedimentation velocity experiments with very small amounts of DNA. The new method, called *band sedimentation in self-generating density gradients,* had two significant advantages over the classical boundary sedimentation experiments. Smaller amounts of DNA could be used, and fairly large quantities of contaminants did not interfere. The procedures underlying this tricky method are illustrated above, and a typical set of results is shown below.

A band sedimentation experiment performed with a mixture of two variants of λ virus. Photographs of the rotating centrifuge cell taken at four-minute intervals show bands which contain about five billion viruses each.



Closed circular duplex DNA consists of two, covalently closed, single-stranded DNA molecules that are interwound. The two strands share no atoms in primary bonding, yet they cannot be separated from each other without breaking a covalent bond. Such systems are said to contain a *topological bond.* Molecules containing interlocked circular submolecules are called catenanes. Organic chemists have enjoyed contemplating the various possibilities for stereoisomerism in catenanes. One catenane containing two rings of 30 carbons has been prepared and isolated. The DNA from *polyoma* virus contains approximately 5,000 nucleotides in each ring. The covalent backbone chains are interwound about 500 times to assume the normal DNA structure.

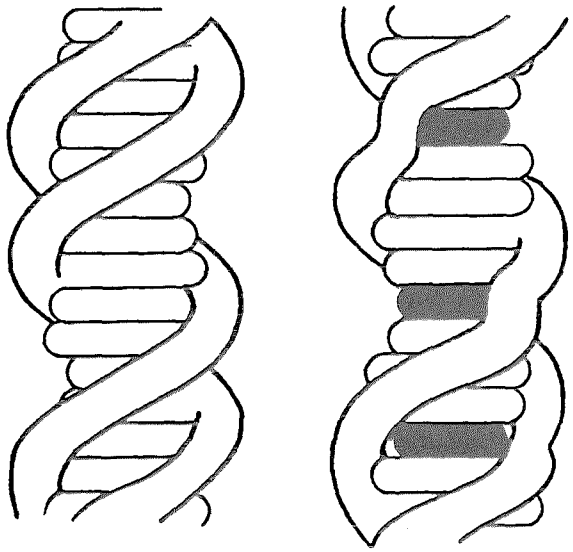
When closed duplex DNA is dissolved in common solvents, it assumes a structure in which the potential energy of the ensemble of atoms is at a minimum. The duplex winding number (the number of times one strand crosses over the other) in *polyoma* DNA, as in all other naturally occurring closed circular DNA's, turns out to be somewhat larger than the topological winding number obtained by counting the number of crossings when the helix axis lies in a plane. Although it is impossible to change the duplex winding number in a planar system, it can be changed if the helix axis is itself allowed to become helical so as to compensate exactly for the change in the duplex winding number. Closed circular DNA forms interwound superhelices, twisted molecules, in common solvents. The



In these three forms of circular duplex DNA, the lines represent single strands of DNA. Closed circular DNA forms interwound superhelices (twisted molecules) in common solvents. The handedness of the superhelix allows us to conclude that the duplex was slightly underwound in the cell at the moment the last bond was formed.

handedness (the direction, clockwise or counterclockwise, in which the superhelix turns) of the superhelix allows us to conclude that the duplex was slightly underwound in the cell at the moment the last bond was formed (above).

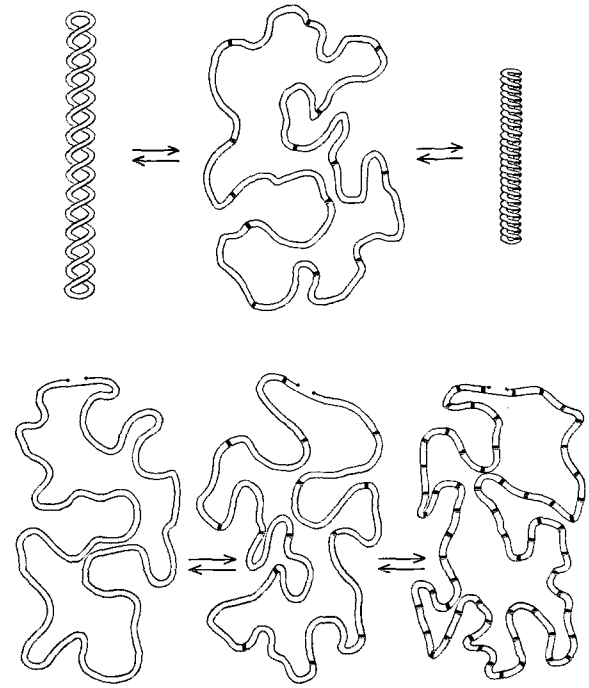
Twisting of the molecule obviously requires an expenditure of energy which is partly stored in the molecule. The twisted molecule is like a spring, ready to unwind if the restraining forces are released. Untwisting occurs spontaneously if any one of 10,000 phosphate ester bonds in *polyoma* DNA is hydrolyzed. The duplex then spins around the rotatable chemical bonds in the intact strand opposite the single-strand break (the nick). The untwisted or relaxed molecule has a very different conformation. It moves through a solvent in band sedimentation experiments at a much slower rate



In this representation of two DNA molecules, the molecule on the right shows how drug (dye) molecules (black bands) are intercalated in the DNA molecule.

than the superhelical molecule. This effect is a useful tool in the study of the cell's machinery for "nicking" DNA—a process which must obviously occur if a closed circular molecule is to be replicated semiconservatively.

The duplex DNA can be unwound in a controlled way by using any one of a set of intercalating dyes —dyes that slip in between base pairs of DNA. These intercalators force the base pairs of DNA apart and unwind the duplex slightly. The effects of the controlled addition of the drug ethidium bromide are exactly as predicted from our understand-



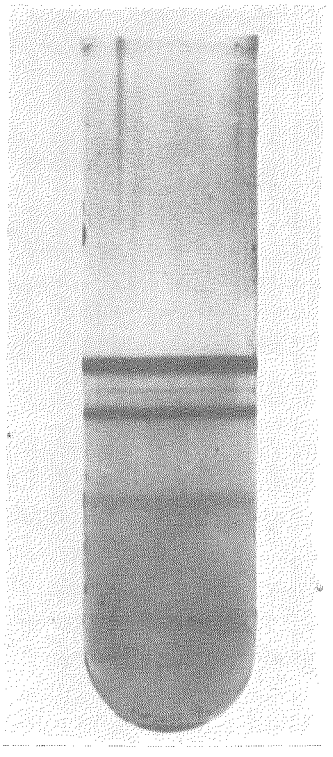
The effects of intercalating the drug ethidium bromide (black bands) into closed circular DNA are shown in the upper half of the diagram. First the drug relaxes the DNA, then it winds it up tighter. Binding the drug to nicked (open) circular DNA (lower part of the diagram) causes a rotation of the duplex at the site of the nick (the gap in the line). Each line represents a single strand of DNA.

ing of the topological properties of closed, double-stranded molecules. William Bauer, a Caltech graduate student in chemistry, recently showed that small amounts of this compound unwind and relax the intact molecule; larger amounts wind in superhelical turns that are left-handed instead of right-handed as in the native molecule. However, the nicked molecule merely spins at its rotation site, called a swivel, while binding the ethidium. It will

We are now faced with a large number of unsolved problems. Centrifugation, the chemistry of circular DNA, electron microscopy, hard work, and inspiration will all be needed to obtain solutions.

become saturated with the drug when one drug molecule is bound for every two base pairs. The relaxed DNA-ethidium complex will then have the appearance of a stack of sandwiches strung together in a circle, whereas closed molecules will wind up into a very tight spring with the free energy of spring formation opposing the binding of the ethidium. In consequence, less ethidium is bound by the closed molecules than by the nicked molecules.

The combination of the drug-binding by closed circular DNA and the buoyant density centrifugation method provides a very easy means to fish closed circular DNA out of mixtures which contain much larger amounts of linear DNA. The drug molecules are light and act like balloons attached to the denser DNA molecule. They cause the complex to move up in the gradient column to a region of lower density. The closed molecule, which takes up less drug, then has a higher buoyant density than do the nicked or linear molecules. The bands shown in the test tube (below left) are easily separated into containers for further study.



Three species of HeLa mitochondrial DNA are shown resolved in a cesium chloride-ethidium bromide density gradient. The dark upper band contains nicked (open) DNA, the narrow middle band contains singly nicked catenanes, the bottom band, closed DNA. The pale band in the lower part of the tube contains carbohydrate.

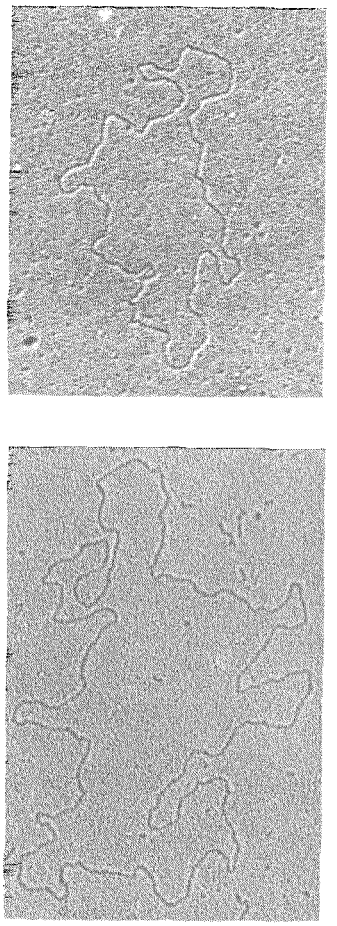
This simple preparative method made it possible for us to investigate the occurrence and the properties of a type of closed circular DNA contained in almost all animal cells, beginning with protozoa and continuing to man. This particular DNA is contained in a cytoplasmic organelle called the mitochondrion. Mitochondria are responsible for the transfer of energy in oxidizable compounds to ATP (adenosine triphosphate), a key source of metabolic energy in the cell. The mitochondria are therefore often referred to as the cell's power plants. We do not yet understand why mitochondria have their own DNA genetic systems, spatially separated from the chromosomal DNA in the nucleus. Nor do we know the identity of the proteins specified by the information in the mitochondrial DNA. It is known, however, that certain genetic traits in yeast and molds are inherited through mitochondria.

When the mitochondrial DNA from the lower band in the test tube was examined in the electron microscope, Roger Radloff, formerly a Caltech graduate student in biology, made a surprising observation. Not only were there circles five microns in contour length (as had been reported by other researchers earlier), but there were also double, triple, and quadruple length circles in small amounts. Careful measurement of photographs of these molecules revealed crosspoints which divided the multiple length molecules into five micron subunits. Bruce Hudson, a graduate student in chemistry, showed that the double length molecules were catenated or interlocked pairs of closed circular duplexes. Such molecules are properly called catenated catenanes. If one submolecule is nicked and the other is closed, the restriction on drug-binding will be only half as large as in a simple closed molecule. The middle band in the test tube was highly enriched in singly nicked catenated dimers.

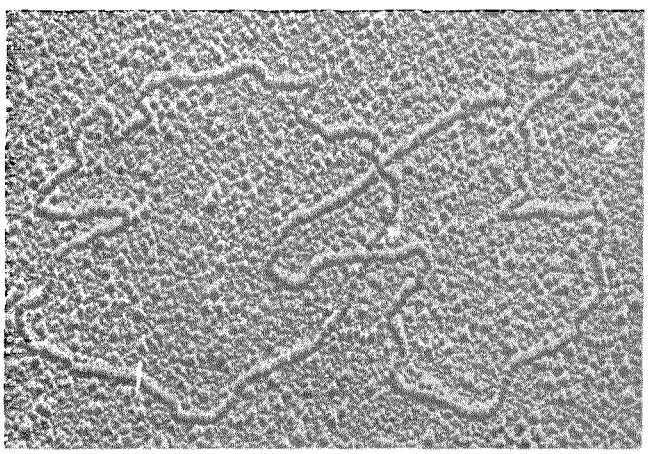
These structural studies were performed with

mitochondrial DNA obtained from HeLa cells, a line of human cancer cells that have been grown in tissue culture for more than 25 years. The biological implications of the interlocking of two or more sets of mitochondrial genes were puzzling. It was obvious, however, that we should try to find whether these structures were unique to HeLa cells or whether they occurred also in the mitochondria of normal and malignant tissues that had not been grown in tissue culture.

The first phase of this population survey is now almost complete and has taken a surprising turn. David Clayton, a graduate student in biology, has found that a large fraction of the mitochondrial DNA in the circulating white cells of patients with granulocytic leukemia are in the form of double length molecules that appear to have exactly twice the contour length of monomeric mitochondrial DNA (right). We call this kind of molecule a circular dimer, and tentatively we think of it as a villain. Chemotherapy appears to reduce the frequency of the circular dimer by a factor of about five. My collaborators, David Clayton, John Jordan, Charles A. Smith, Marlyn Teplitz, and Eric Wickstrom, have searched diligently but without success for a circular dimer among thousands of mitochondrial DNA molecules from various organs of healthy rabbits, guinea pigs, and rats. The circular dimer is also absent in mitochondrial DNA from immature, circulating white cells of patients with nonmalignant maladies that give rise to high white blood cell counts. In the course of this search we have, however, found catenanes in varying frequencies from 3 to 9 percent in every one of the mitochondrial DNA samples. It may safely be said that catenanes are normal constituents of our cells.



Electron micrographs of mitochondrial DNA from human leukemic white cells show (upper) a circular monomer five microns in contour length and (lower) a ten-micron circular dimer.



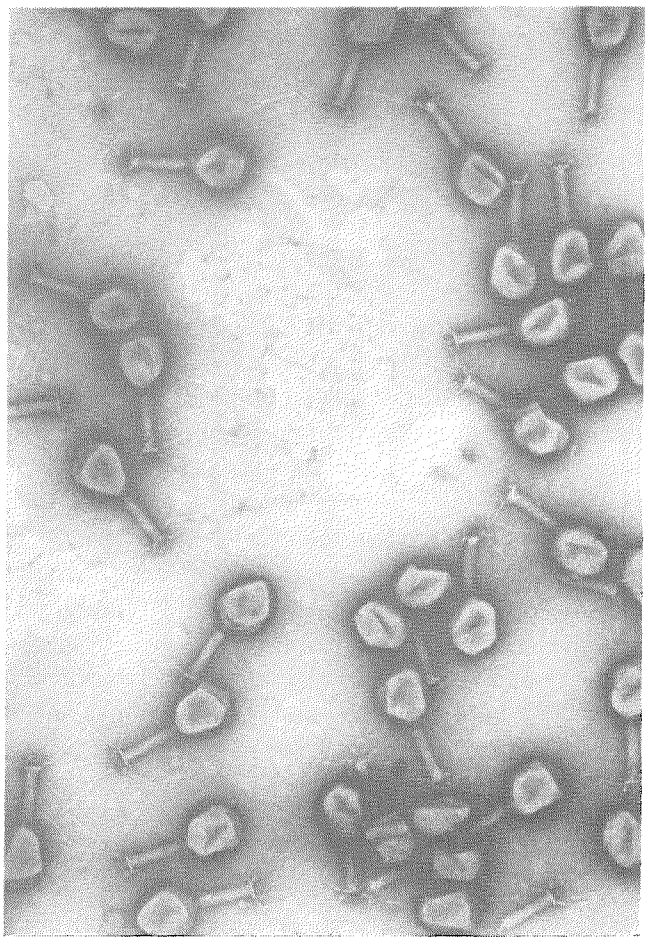
This electron micrograph shows a catenated mitochondrial DNA molecule from a human cell. The submolecules are connected like closed links in a chain.

We are now faced with a large number of unsolved problems. What is the mitochondrial DNA distribution in other kinds of leukemia and in solid tumors? How are the catenanes and circular dimers formed in the cell? How does the cell control their frequency? Do these molecules represent precise duplications of the mitochondrial DNA genes? If so, are excess gene products (proteins) formed? Are abnormal gene products formed? Finally, are we any closer to understanding the cancer problem? Is the correlation that we have so far observed between the occurrence of the circular dimer and granulocytic leukemia trivial or meaningful? Is the change in the size of the molecule an early event in an undifferentiated cell which gives rise after many cell divisions to white cells that do not mature properly and do not go about their job in an orderly and controlled way? Centrifugation, the chemistry of circular DNA, electron microscopy, hard work, and inspiration will all be needed to obtain answers to these questions. □

Genetics and Development
at the Threshold of Life—

The Caltech Phage Group

By ROBERT S. EDGAR and WILLIAM B. WOOD

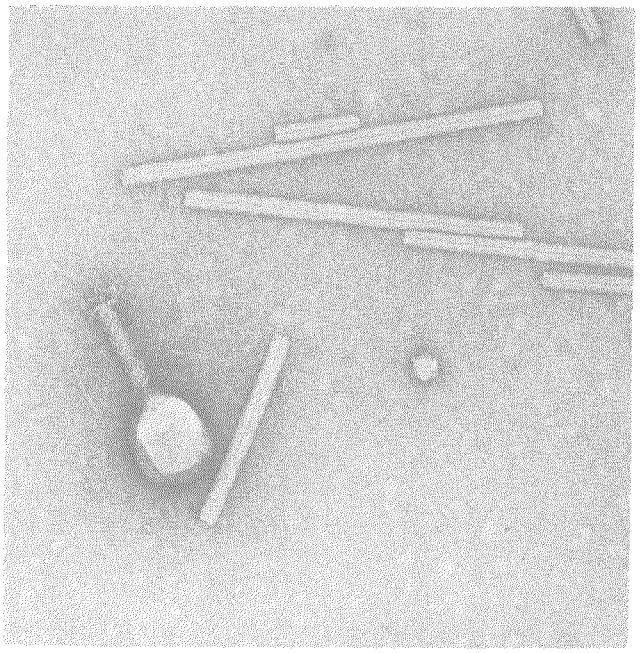


Heads, tails, and tail fibers of T4 bacteriophages can be distinguished in this electron micrograph taken by Ronald Luftig. Magnification is about 150,000 diameters.

Although bacteriophages—viruses which attack bacteria—have been known for over 60 years, the detailed study of their reproductive cycle began only in the early 1940's. At that time Max Delbrück, a former physicist and visiting research fellow at Caltech who had become interested in the mechanism of heredity, recognized in the phage an ideal experimental material for exploring the nature of the gene. In the succeeding years, many of today's leading contributors to the field now known as molecular biology passed through Dr. Delbrück's "Caltech Phage Group" as graduate students and postdoctoral fellows, and research with bacteriophages led directly or indirectly to much of the recent explosive progress in this new science.

Bacteriophages, as Delbrück suspected, proved ideally suited to studies of the molecular basis of heredity. They represent the simplest genetic systems we know—life trimmed to its barest essentials. The bacterial hosts on which they grow are themselves simple (as cellular organisms go), generally harmless, and easy to culture and manipulate in the laboratory. Despite their extreme simplicity, however, bacteriophages transmit and utilize their genetic information by the same basic mechanisms that are common throughout the biological world, with the consequence that phage research has been of value in understanding not only the process of virus multiplication but also problems of heredity and development in higher animals.

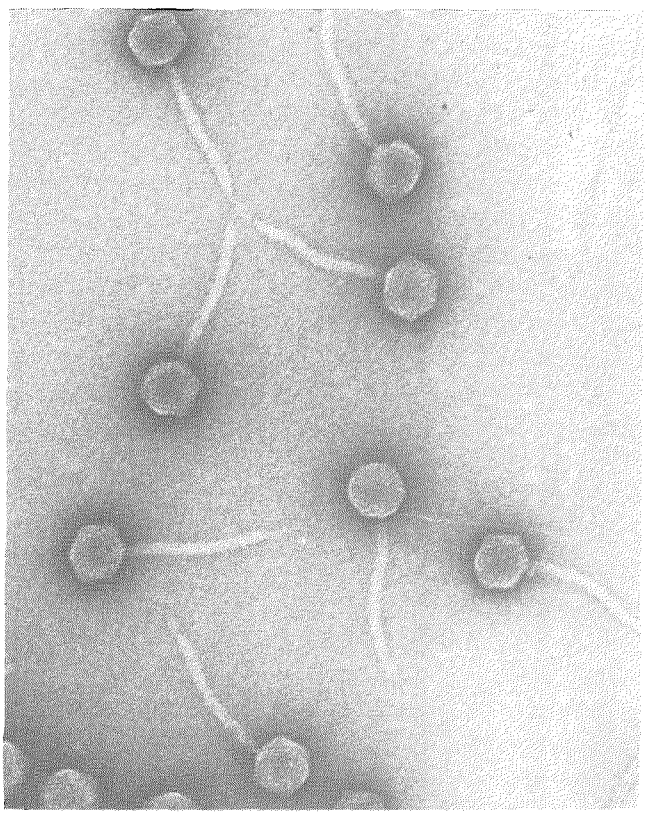
Some forms of life are little more than DNA and a protective coat. But even here countless generations of adaptation and survival have bred a complexity of structure and synthesis.



This micrograph, taken by Fred Eiserling of UCLA, clearly shows the large size and complexity of T4 relative to two of the simplest viruses. The long structures are particles of TMV, a virus that infects tobacco plants, and the small regular polyhedral object is a particle of Phi X 174, which infects E. coli.

Some of the fascination which bacteriophages hold for those who have worked with them can perhaps be understood through a more detailed description of two of these viruses and their life cycles. By accident initially, but increasingly by choice, the most intensely studied phage has been T4, a virulent virus that invades the common colon bacillus, *Escherichia coli* (*E. coli*). T4 is large for a virus. Its architecture is complex, and its effects on the host cell after infection are profound. T4 attaches to the wall of the host cells by means of six slender fibers located at the end of the tail. The syringe-like tail structure then contracts to plunge the end of the central tube of the tail through the wall of the host. This provides a passageway through which the viral DNA, located in the phage head, passes into the interior of the cell.

This DNA, a single molecule 54 microns in length, or about 80 times the length of the virus, is the viral genetic program. It includes about 150 genes, each carrying the information for the synthesis of a different enzyme or structural protein. Almost immediately after infection, the bacterial DNA is destroyed, and the synthetic machinery of the cell is reprogrammed to turn out the components necessary for phage reproduction under the control of the viral genes. The cell is "under new management." The infected bacterium can be thought of as a new organism, with a new set of genes and a drastically altered purpose in life. Its previous goal,



Phage λ is a simpler phage than T4, having a smaller, more regular head, a less intricate tail, and no tail fibers. This electron micrograph was taken by Fred Eiserling at a magnification of about 200,000 diameters.

Bacteriophages represent the simplest genetic systems known—life trimmed to its barest essentials.

self-duplication, has been completely abandoned in favor of virus production, accompanied by the eventual death of the cell.

Following this coup d'état, viral development proceeds by an orderly sequence of precisely timed events. Among the first proteins to be synthesized are enzymes for catalyzing the production of additional copies of the viral DNA. At 8 minutes after infection DNA replication begins. About 5 minutes later synthesis of the early enzymes ceases, and the machinery begins to turn out a new class of proteins which will eventually comprise the progeny virus particles. Packaging of the new DNA molecules and assembly of the viral structural components proceeds until at 23 minutes the first phage particle is complete. By 35 minutes, more than a hundred finished virus particles have accumulated. At this point the infected cell bursts or lyses, and the newly formed phage enter the world in search of new host cells to invade.

The inevitable consequence of T4 infection is death of the host cell. Many bacterial viruses, termed temperate phages, are less pugnacious. Phage λ, which also infects *E. coli*, is such a temperate virus. Depending on conditions at the time of infection, λ can either carry out a coup d'état of the host similar to that of T4, or it can establish a nondestructive long-term association with the host, by a process called lysogenization. Under conditions that favor lysogenization, the viral DNA, following its entry into the host cell, becomes inserted into the larger DNA molecule of the host bacterium. The host then resumes its growth and reproduction, unaware that hidden away in its own genetic material there is now a subversive plan to convert the cell to the manufacture of virus! The viral DNA, which is not distinguished as foreign by the host's replicating machinery, is faithfully reproduced at each generation and passed on to the daughter cells. When suitable conditions arise, perhaps generations later, the viral DNA takes command, and the bacterial cell is reprogrammed to produce the virus.

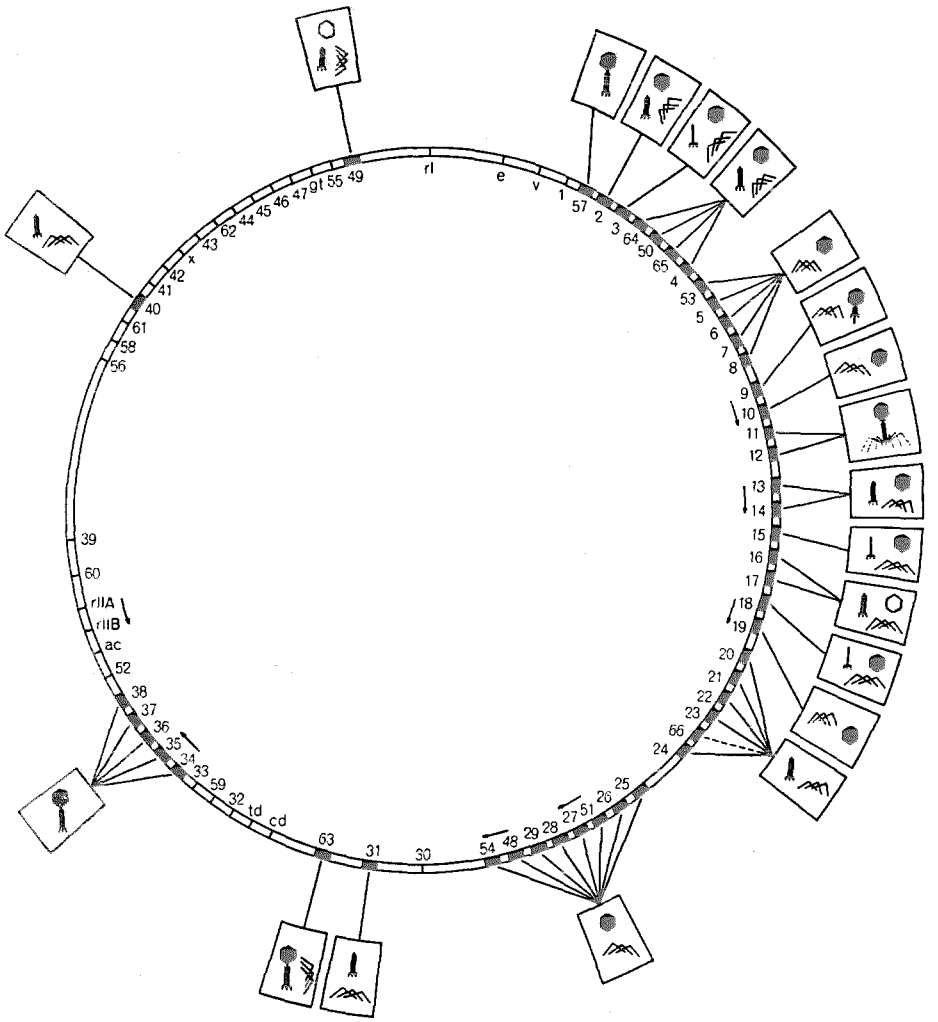
Over the years phages T4 and λ remained the principal objects of study of the Delbrück phage group and its descendants. T4 and its close relatives T2 and T6 were the phages which Delbrück and his co-workers originally selected for study. The principal source of inspiration for studies of phage λ at Caltech has been Jean Weigle, who joined the phage group as a research associate in 1948. Like Delbrück, Dr. Weigle was a fugitive from physics, having been at one time professor of physics at the University of Geneva. Weigle and his co-workers have successively exploited λ for studies on the general mechanism of genetic recombination, and in particular for the integration of λ DNA into the DNA of the host, a problem which promises to have some practical significance in view of the recent findings that some tumor viruses exhibit life cycles which closely resemble that of λ.

In the late 1950's Delbrück's interest in phage waned as his interest in phototropism in fungi waxed, but the phage group kept its continuity under the new leadership of Robert Edgar, now professor of biology. During the Delbrück years, the work on T4 consisted largely of always brilliant and often successful attempts to apply formal genetics—the transfer of hereditary traits of the virus to its offspring—to the problems of phage reproduction

The inevitable consequence of T4 infection is death of the host cell, although many bacterial viruses are less pugnacious.

A new direction was introduced in 1960 by the discovery and exploitation of conditionally lethal mutations by Richard Epstein, then a postdoctoral fellow in the group, and Dr. Edgar. Until then, the mutations available in T4 and λ were not of much use for providing insight into the functions of specific viral genes, since they had only slight effects on viral growth. Mutations causing defects in essential genes, being lethal to the virus, were inaccessible to study since mutants carrying them could not be propagated for genetic experiments.

The conditionally lethal mutations provided the first opportunity to study such genes, since they produced lesions whose effects could be controlled by the experimenter. Under one set of conditions, called permissive, these mutations have little effect on the function of the mutant gene, and the virus can be propagated normally. However, under a



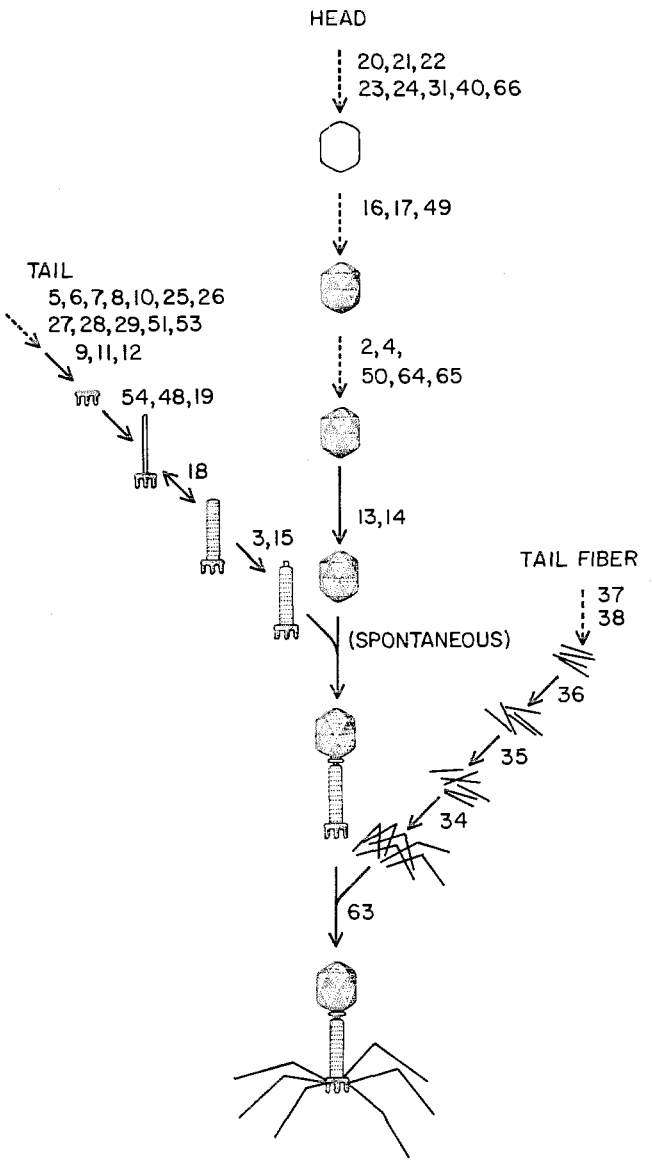
A Genetic Map of T4 Virus

In a genetic map of T4, genes identified by conditionally lethal mutations are numbered for identification. The genes represented by narrow black lines control replication of the viral DNA and other events occurring early after infection. The genes represented by heavier black lines control the synthesis of phage components and their assembly into virus. The aspect of the assembly process controlled by the various genes can be inferred from the symbols in the accompanying boxes, which represent the major phage components found in cells infected with a mutant defective in the corresponding gene.

second set of conditions, termed restrictive, the mutation blocks gene function completely. If the mutant gene is an essential one, viral development under restrictive conditions can proceed only to the point where that gene's function is required for continuation of the program; there the process must stop.

By examining such abortively infected cells to determine where development is blocked, or which phage components are missing, it is possible to infer a good deal about the normal function of the mutant gene. For instance, cells infected under restrictive conditions with a mutant defective in a gene essential for the synthesis of viral DNA exhibit the early stages of normal viral development—the destruction of the host cell DNA and the appearance of new virus-specific enzymes. But no new viral DNA is synthesized, and the latter half of the program, for synthesis of phage parts, never gets under way. Cells infected with a mutant defective in a gene controlling the formation of the head of the virus, on the other hand, go through a normal growth cycle and lyse but produce only virus tails rather than active virus.

During the last eight years, much of the work of the phage group has centered around the study of the conditionally lethal mutations in attempts to identify and determine the function of as many genes as possible in T4. So far about 80, or approximately half of the estimated total number, have been mutationally identified. Through studies at Caltech and other laboratories, the detailed functions of many of these genes are now known, as indicated on the genetic map above. A good many genes were found to be clearly involved with the synthesis of the various enzymes required for main-



The process of T4 assembly as currently understood can be represented as an assembly line with three major branches. Solid arrows are used here to indicate steps which can be carried out in the test tube. The numbers indicate the gene control of the various steps.

tenance of the host cell and reproduction of the viral DNA. However, more than half of the genes identified appeared to control the synthesis of viral components and the assembly of the phage particle.

In 1964 William Wood, a biochemist with an interest in genetics, joined the faculty of the biology division. Shortly thereafter, he and Edgar decided to collaborate in an attempt to attack the problem of virus assembly more directly. Using disrupted preparations of cells which had been infected with appropriate mutants as sources of unassembled phage parts, they were able to find conditions under which these components could be put together in the test tube to form complete infectious virus. Edgar, Wood, and graduate students Jon King and Jeffrey Flatgaard characterized the incomplete components produced by various mutants to yield a progressively more detailed picture of the process of phage assembly (left).

Construction of the virus seems to take place by a stepwise assembly line process, in which each step is under the control of a different gene. The heads, tails, and tail fibers are assembled independently of one another, so that the assembly line is branched. These subcomponents are then put together, also in a fixed sequence; that is, the fibers are attached to the tails only after head and tail are united.

Virus assembly in the test tube is not restricted to T4. Weigle, also using mutants, has shown that isolated heads and tails of phage λ, when mixed together, can unite to form normal active virus. In an intriguing series of experiments, he has shown that the free tails of the virus can attach to bacterial host cells. If heads are now added, they attach themselves to the tails, inject their DNA through them, and the bacteria become infected.

The encapsulation of viral DNA within a protective coat, a process common to the assembly of all viruses, is now under investigation.

For Wood and his collaborators, Delbrück's early consideration of T4 as a purely genetic system has now shifted to an emphasis on the phage particle as a complex supramolecular structure which is built up under the control of the viral genetic program. Molecular biology has provided a clear understanding of how genes direct the synthesis of single protein molecules; however, little is known about the assembly of higher order structures such as cellular organelles or viruses like T4 which are made up of many different kinds of protein molecules, or about how genes control this assembly.

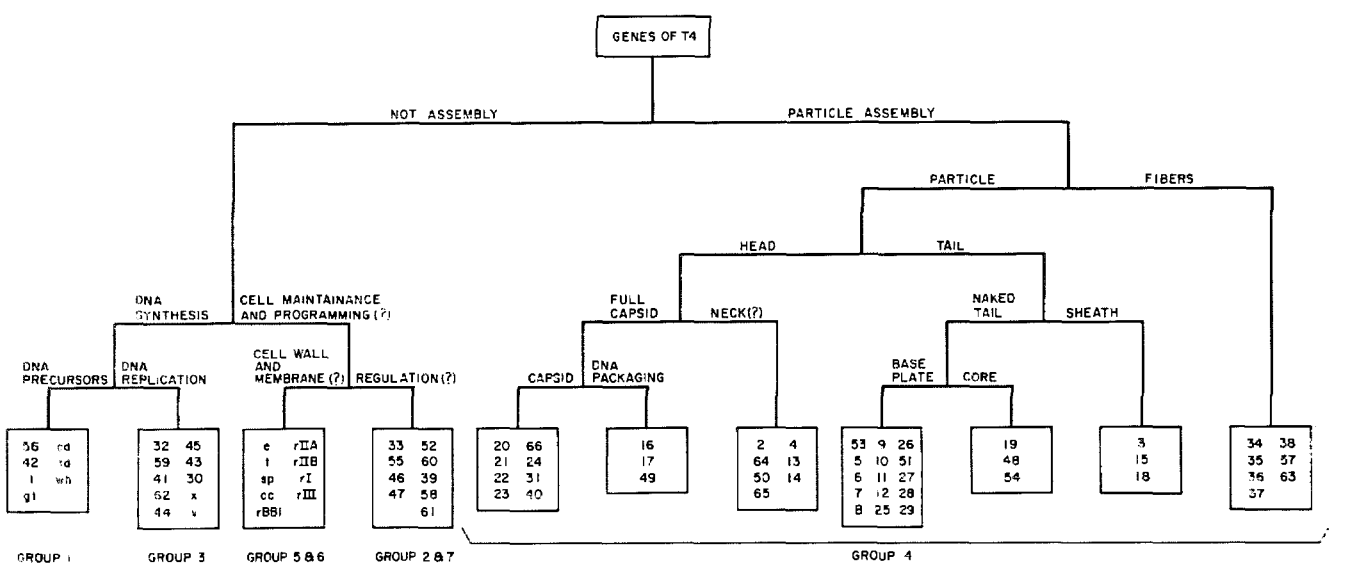
In the hope of better understanding such processes, Wood and his co-workers are continuing the study of T4 assembly in the test tube. The earlier work in collaboration with Edgar, Flatgaard, and King established a sequence of gene-controlled

T4 is just simple enough to foster the hope that continued research could lead to identification of all of the phage genes and a complete understanding of the program of viral development.

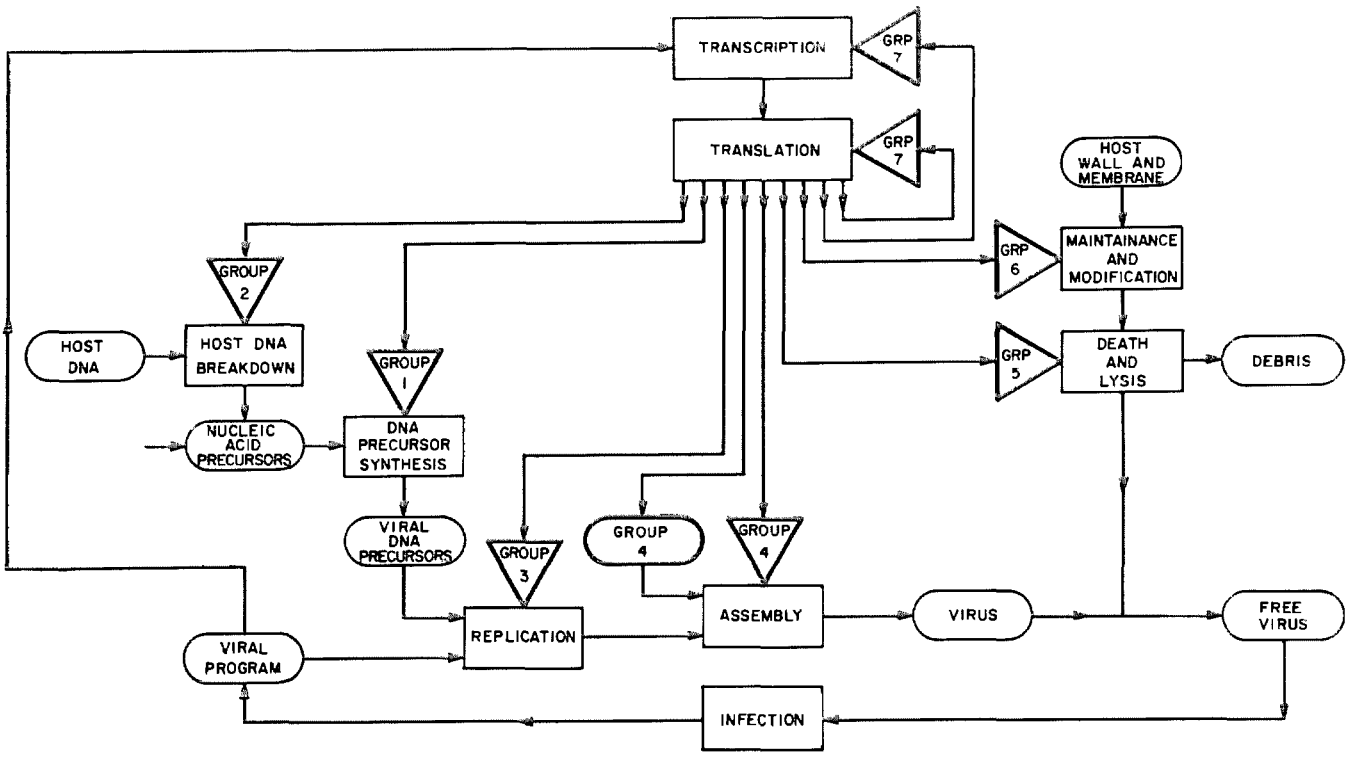
steps but provided little detailed information about their nature at the molecular level. The investigators demonstrated, however, that at least 15 steps could be carried out in the test tube and hence were at least in principle subject to detailed biochemical analysis. Characterization of selected steps is now under way. Graduate students Sam Ward and John Wilson have focused their attention on the sub-assembly pathway concerned with the tail fibers, and they have begun purification of the interacting components for detailed analysis. From work of Wood and research assistant Harriet Lyle on the final step in assembly, it now appears that a phage-induced enzyme catalyzes the attachment of the finished tail fibers to the otherwise completed particle. This is the first direct indication that assembly of complex multi-protein structures may involve enzyme-mediated reactions as well as simple interactions between the protein components themselves.

Also under investigation in the Wood group is a process common to the assembly of all viruses, the encapsulation of viral DNA within a protein coat. In T4, this step has so far not been accessible to study in the test tube. By radioactive labeling of cells infected with appropriate mutants, however, Ronald Luftig, a postdoctoral fellow working with Wood, has been able to show that in T4 development the protein membrane of the head is first made as an empty bag, and then subsequently somehow filled with the viral DNA. An elucidation of how this unlikely contortional feat is performed, which Luftig hopes to achieve, would be of importance in understanding the general process of viral development, and might conceivably also bear on the problem of how proteins and DNA interact to form the chromosomes of higher animals.

Edgar's interest has recently returned to the general nature of the T4 genetic program. Although highly complex as viruses go, T4 is just simple enough to foster the hope that continued research could lead to identification of all of the phage genes and a complete understanding of the program of viral development. Already, the known genes can be classified into a few groups on the basis of their functional roles (below). If each of these groups is



Using a genetic map of T4, all of the known phage genes can be classified according to their function in the infected cell.



If each of the classified groups of known T4 genes is thought of as a different kind of functional element, they can be incorporated into a tentative "circuit diagram" of T4 development. Transcription and translation refer to the principal stages in gene expression at the molecular level. In the first, the segment of DNA comprising a gene is transcribed to produce a "messenger" RNA molecule, carrying the same information. This information is then translated by the protein synthesizing machinery to produce the enzyme or structural protein controlled by the original gene. The remainder of the diagram indicates how these proteins participate in the various processes of intracellular phage development. Triangular boxes designate enzymes or catalytic proteins, while ovals represent structural components of the finished virus. Each group is numbered according to the classification of the corresponding genes.

taken to represent a different kind of controlling element, a tentative "circuit diagram" of phage development can be written (above). While on the one hand it helps to provide an overall picture of the nature of the genetic program, it also points up gaps in current knowledge of precise gene functions.

There is clearly another gap as well. The 80 genes of T4 so far identified account for only about one-half of the information stored in the DNA of the virus. What of the unknown half? Recent attempts to isolate conditionally lethal mutations in new genes have met with little success, although Richard Josslin, a graduate student, has discovered one new gene that appears to control the lysis of the infected cell. It appears likely that most if not all of the genes which are essential for viral growth, at least under laboratory conditions, have in fact been identified and that the functions of the remaining genes are nonessential. If so, mutations affecting them will be hard to detect.

For phage λ, one approach to this problem has been developed by graduate student John Parkinson, who has developed a simple method for isolating mutants of λ that have less DNA than the normal virus. Among these deletion mutants are strains which have lost up to 30 percent of their DNA and yet can still multiply in the host cell quite normally. This shows that at least 30 percent of the DNA of this virus is not concerned with functions required for reproduction. Parkinson has found, however, that most of these mutants are defective in the process of lysogenization. Since these mutants cannot integrate their DNA into the host DNA molecule, their only recourse is to multiply and kill the host cell.

As living creatures go, phages T4 and λ may be simple, but nevertheless the accumulated biological wisdom of two billion years of evolution is encoded in their DNA molecules. This is challenge enough to keep at least two genetic cryptographers intrigued for some time to come. □

From DNA To Development

By JAMES BONNER

In the higher organisms, cellular specialization of function is a key to efficiency. Specialization implies differential use of the inherited information available to every cell. How are the keys turned and the switches thrown?

Of all of the insights that the dramatic new developments in biology have given to us, none has more profound impact upon so many aspects of our thought and culture than our vision of the great sweep of evolution of living things. Today we know, as we did not even a few short years ago, that our planet and its elements were formed some 4.8 billion years ago and that the early earth possessed a reducing atmosphere quite different from today's—an atmosphere containing large quantities of methane, water vapor, and ammonia.

Our earth is nicely sized and appropriately situated with respect to the sun, so that it is neither too hot nor too cold, nor too big and therefore a sun, nor too small and therefore unable to hold its atmosphere. How fortunate! These facts made all else possible.

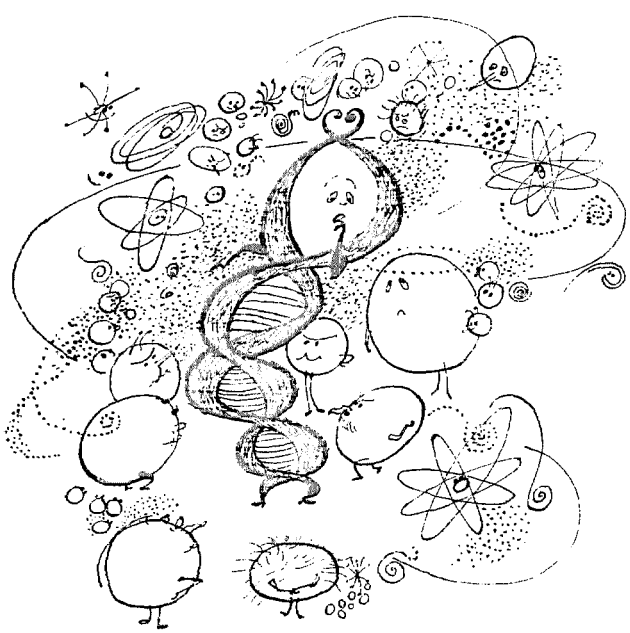
On the early earth, the rains rained, the lightning lightened, atoms were ionized, and chemistry took place—random chemistry, in which atoms were combined in all of the many permutations and combinations to form a vast variety of molecules, including, as we now know, those characteristic of today's living beings. And we know too that, as this random chemistry continued, organic molecules accumulated because there were no living creatures to eat them up as there are today. And then one day, as we see it now, a dramatic new event took place. The first DNA molecule—a long, linear molecule made of four kinds of building blocks stapled together, and thus capable of encoding information—was formed by just this kind of random chemistry. The appearance of the first lonely little DNA molecule introduced a whole new dimension into chemistry on earth; for the DNA molecule possessed what no molecule before it had possessed, the power to replicate itself, build copies identical to the original.

With the appearance of the first DNA molecule—a molecule because of its structure able to encode information, capable of self-replication, capable of mutation by occasional mistakes in replication—life as we see it now may be said to have begun. From that moment, one billion years after the creation of the elements, life on earth has been a continuum of mutation and selection, all based on survival of the fittest among the descendants of that first prototype DNA molecule.

By three billion years ago, living forms had evolved akin to those we know on earth today—creatures vastly more complex than the single DNA

molecule of early life. There were bacterial cells, and cells of blue-green algae, DNA molecules surrounded by semipermeable membranes, cells in which the information encoded in DNA served to generate—by transcription into messenger RNA—the many kinds of enzyme molecules which today's living creatures use to convert food to the building blocks for DNA replication and for the formation of other cellular structures. Indeed, the genetic code for translation from the 4-letter nucleic acid language to the 20-letter language of the enzymes was already established in its present form. Photosynthetic organisms, capable of transducing the energy of visible light into chemistry with the evolution of oxygen from water and the reduction of CO_2 to plant material, had appeared and were busily converting our atmosphere into an oxidizing one. Very possibly, by three billion years ago, the mechanisms responsible for interchange of DNA-encoded genetic information between bacterial cells had been developed, the kind of primitive sexuality which persists to this day in the bacteria.

Thus, in an exuberant burst of evolution, life on earth passed in a little over one billion years from simple, naked DNA molecules to complex single-celled organisms. At this stage, however, as we see it now, evolution took a long breathing spell—a breathing spell of almost two billion years. And then, quite suddenly, less than one billion years ago, a new kind of living organism appeared, an organism that we do not know today, but from which all of today's higher organisms are evolved. This new creature possessed cells much more complicated than those of bacteria. Its genetic material was composed of several DNA molecules, organized into chromosomes, which replicated and separated at each cell division by a complex molecular ballet, mitosis.



A dramatic event—the first lonely little DNA molecule.

The road to the complete understanding of the developmental process is at long last open before us.

These new organisms possessed regular programs for the periodic exchange of genetic information between organisms, the features of sexuality as we know them today. Their chromosomes were housed within an intracellular membrane, the whole structure forming the nucleus of each cell. Most surprisingly of all, these new creatures were not single creatures, but in fact societies of creatures. Each cell of the new organism contained within it, as symbionts, bacterial cells which happily evolved into today's mitochondria—bodies which the cells of all of today's higher creatures possess and which conduct the process of respiration. Others of the new kind of cells possessed not only mitochondria but also intracellular guests akin to the primitive blue-green algae. These have developed into the chloroplasts of today's green plants. Indeed, the cells of today's higher organisms are veritable barnyards of many kinds of self-duplicating entities housed within the cell of the organism. And it is from this new kind of cell that today's multicellular organisms have evolved. During the last billion years there has been a second explosion of evolutionary activity, and this burst has given rise to all of today's plants and animals.

How exciting it would be to find somewhere on earth a surviving prototype of that first aboriginal higher creature with its typical higher creature cellular structure.

Today's creatures, different as they are in form, habits, and life style, are basically very much alike, each composed of a great many cells of the new type. The human, for example, contains about a

thousand billion cells; yet each human being, and indeed each higher organism, starts his individual life as a single cell, the fertilized egg. The fertilized egg divides and divides, into two cells, four cells, eight cells—and in the course of time these cells start to become different from one another, to turn into the many kinds of specialized cells that characterize the adult organism. This is the process of development, and of today's problems of biology none is more exciting than that of exactly how development takes place—how it is that the individual cells of the body of the higher organism come to be different from one another, even though descended from a common ancestral cell, the fertilized egg. And it is to this problem that my colleagues and I have paid our full attention.

These last few years have been exciting ones in the study of development. Each day sees new excitement in the laboratory, new findings providing new insights into the logic of the organization and regulation of the genetic material of the higher organism. For development is a genetic problem; development too is controlled by the DNA of the chromosomes of the organism. Everybody knows about genes and how they control each and every one of our physical characteristics. Everybody knows that genes are contained in chromosomes, which are made of DNA. The form and features of each individual specialized cell of the higher organism are hereditary characteristics of that organism, and they are therefore encoded in that creature's DNA. How do genes work so as to control development? This is the question that our group asks.

It is a sobering thought:

We are only one thousand times more

complicated than a miserable bacterium.

Much has been found out about how genes work by the study of the simple organisms, viruses and bacteria, for they too have genes arranged in (albeit simple) chromosomes. They have, however, fewer genes than we do. Each cell of the colon bacterium, *E. coli*, for example, contains only one one-thousandth of the amount of DNA contained in a human cell. It is a sobering thought. We are really only one thousand times more complicated than a miserable bacterium. By the study of bacteria we have come to understand how the information contained in DNA is transcribed by the enzyme RNA polymerase to form informational copies, RNA molecules, each containing the message of one or a few genes. We have come to understand how the messenger RNA is translated by the ribosomal protein-synthesizing system to make the enzyme molecules, each assembled from the 20 amino acids in accordance with the instructions contained in the message of a single gene. But simple organisms, bacteria and viruses, do not grow into complex, multicellular creatures, with different kinds of specialized cells. The problems of development must be studied with the higher organisms.

Each day sees new excitement in the laboratory.

The first great truism of the study of development is that each and every cell of the higher organism, no matter what its external appearance, contains the same amount and kind of DNA. That each cell contains the same amount of DNA is measurable by chemistry; that each specialized cell contains all of the DNA required to make the whole organism is shown by such simple facts as that an individual specialized cell may be caused, under appropriate experimental conditions, to behave like a fertilized egg and regenerate the entire organism. In any single, particular kind of specialized cell, however, only a portion of the genes contained in the chromosomes of the nucleus of that cell are actively engaged in producing their messenger

RNA, and thence the enzyme molecules for which they contain information. That this is so is evident from such elementary considerations as the fact that humans contain, for example, genes for making hemoglobin molecules. These genes are turned on in those cells which give rise to the red blood cells, and they are not active—are turned off—in all other cells of the human body. The genes for making muscle proteins are turned on in muscle cells but turned off in nerve cells. And so it goes.

Of today's problems of biology

none is more exciting than that

of exactly how development takes place.

The study of the developmental process is, then, the study of how it is that the activity of genes is controlled in the chromosomes of the higher organism. What determines whether genes are turned off or turned on? What is the material nature of the repressor molecules that cause genes to be turned off? How are genes transformed from the turned-off to turned-on state, and vice versa? How does the systematic, orderly programming of gene activity work so as to bring about orderly development?

The study of the developmental process is, then, the study of the biology of chromosomes. We study chromosomes by isolating them from the cell in pure form, causing them to generate their messenger RNA in the test tube, and finding out what it is that makes only particular kinds of messenger RNA be formed by the isolated chromosome. The method we use for the preparation of isolated chromosomes is simple. We take some cells or tissues, grind them in a blender so as to rupture the cell membrane as well as the nuclear membrane, and then filter the material through Miracloth, silicone-treated paper which magically removes membranes. We then subject the resulting suspension of cell particles to centrifugation in a centrifugal field too slight to pellet mitochondria or enzyme molecules, a centrifugal field in which only the biggest and heaviest things in the cell homogenate are pelleted. And luckily enough, the biggest and heaviest things in the cell homogenate are the chromosomes, which we then recover in 95 percent or higher yield and which may be purified by sucrose density gradient centrifugation, a procedure in which chromatin is layered over sucrose solution ranging in concentration from 0 percent at the top to 1.8 molar at the bottom. Chromosomes can pellet to the bottom through 1.8 M sucrose because they are large and dense; they are made of DNA, which is heavy. Membranous and proteinaceous materials are lighter and float at that point in the gradient in which they find their position of neutral buoyancy. Our methods appear to be applicable to a vast variety of living creatures, plant and animal alike, and serve to provide chromosomes not only for the study of their biology, but also for the study of their physical biochemistry—studies of their shape, size, and configuration.

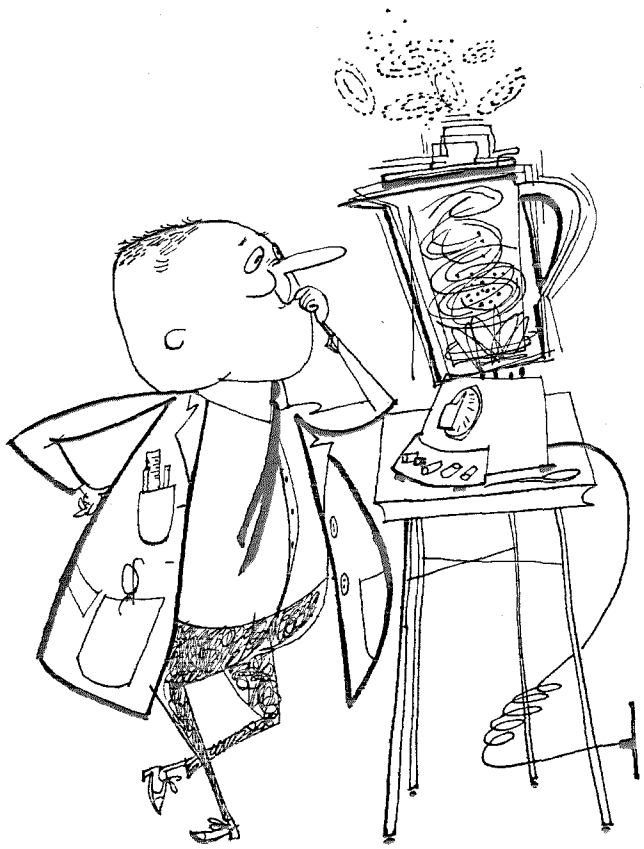
Isolated chromosomes possess a non-trivial property, the property of producing RNA if supplied with the four RNA building blocks, the four riboside triphosphates. Such messenger RNA is formed because chromosomes contain bound RNA polymerase, the enzyme that catalyzes the transcription of DNA to form messenger RNA. Since isolated chromosomes of higher organisms contain in general only a small amount of RNA polymerase, we can add more of the separately prepared enzyme. RNA polymerase may be purified for this purpose from microorganisms which live at a rapid pace and possess a higher ratio of RNA polymerase to DNA than do the cells of the more placid higher organisms. In this way, then, isolated chromosomes can be transcribed by RNA polymerase to produce RNA in vast amounts, amounts large enough to make it possible to study the kinds of molecules present and the messages they contain.

Our methods appear to be

applicable to a vast variety of living

creatures, plants and animals alike.

By this strategy it has been possible to show that the RNA molecules transcribed from chromosomes in the test tube are identical to the RNA molecules that are transcribed from the same chromosomes in life. No genes are repressed by the act of isolation; no genes are derepressed by the act of isolation.



We take some cells and grind them in a blender.

Isolated chromosomes are not artifactual; they represent the state of repression characteristic of life itself.

The DNA of isolated chromosomes is a poor template for the support of RNA synthesis as compared to deproteinized DNA made from the same chromosomes. Studies of the kinds of messenger RNA produced from chromosomes or from deproteinized DNA show that in isolated chromosomes only a small proportion, between 1 and 10 percent in general, of the DNA is available for transcription by RNA polymerase; the rest is repressed, turned off, and unavailable. This is, then, as it is in life. The great majority of the DNA of the chromosomes of any particular kind of specialized cell is repressed and does not produce RNA containing the messages encoded in the repressed DNA. What is the agent of repression?

To answer this question we have made a detour into the study of chromosome chemistry. Chromosomes of higher organisms contain, in addition to DNA, proteins of a particular class, the histones, which are found only in chromosomes and in association with DNA. Histones are basic proteins in which one amino acid in four is a cation, and histones are bound to DNA by interaction of these groups with the anionic phosphate groups of the DNA.

Chromosomes also contain a small amount of RNA, a portion of it messenger RNA which was in the act of being born when the biologist came along and isolated the chromosomes, and, in part, RNA molecules of a special class, chromosomal RNA. And chromosomes contain also a small proportion of nonhistone protein, ordinary proteins, of which RNA polymerase itself constitutes one portion. Removal of histones from DNA causes all of the DNA of that chromosome to become available for transcription by RNA polymerase. It is the histones which are the agents of repression of gene activity in the chromosomes of higher organisms. To put it simply, it is that portion of the DNA that is complexed with histone which is repressed—not available for transcription—while that portion of the DNA which is not so complexed is the portion that is turned on and is available for transcription.

To digress still further into chemistry, we have made a detailed study of the chemistry of the histones. It has been shown, in particular by biology graduate student Douglas Fambrough, that there are eight kinds of histones in the chromosomes of higher organisms, and that these same eight are present in organisms as different from one another as peas, cows, humans, rats, frogs, and protozoa.

Fambrough, in association with our colleagues at UCLA, Emil Smith and Robert DeLange, has studied the amino acid sequences which characterize the structure of one particular histone, comparing the structures of that histone in peas and in cows. They have found that the amino acid sequences of the homologous histone of these two organisms are essentially identical, differing only in two amino acid residues. The histones would appear to be the most conserved, as the biologist says, the most resistant to evolution of all of the protein molecules which have yet been studied—much more constant during the course of evolution than say, hemoglobin, or even the respiratory cytochrome enzyme molecule.

It would appear that the genes for making the histones, the regulators of gene activity, were established long ago in evolution—were established in that early creature which constitutes the ancestor of all of today's higher organisms—and that the genes for making histone molecules have been pre-

served essentially unaltered since that time, perhaps one billion years ago.

It is interesting to note too that the primitive and early organisms, the bacteria and blue-green algae, do not possess histones. What regulation of gene activity they possess appears to be conducted in an entirely different way, with a great variety of different kinds of proteins. Perhaps it is due to their cumbersome mode of genetic control that they are still lowly bacteria. The higher organisms, those

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How do genes that are repressed become derepressed, or vice versa? Our insight into this matter is growing.

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that have succeeded in becoming multicellular, and in exhibiting differentiation and production of specialized cells, all conduct their genetic repression operations with the same basic kit of eight kinds of histone molecules.

It is clear, therefore, that the repression of genes is accomplished by protein molecules of a special class, the histones. There are many genes to be repressed in any given kind of specialized cell. In the human, for example, whose genetic material contains about 2,500,000 genes, in any particular kind of specialized cell perhaps 99 percent of the genes are turned off, repressed. This leaves 25,000 turned on, to be sure, but there are still 2,475,000 different genes to be turned off. How is this accomplished with only eight kinds of histone molecules? This is the function of the chromosomal RNA. Chromosomal RNA molecules are short ones, 40 to 60 nucleotides in length in different organisms. In the chromosome, chromosomal RNA is bound to the DNA, apparently through the same complementarity rules that regulate DNA replication and transcription of RNA. Chromosomal RNA molecules are also bound at one end to a particular kind of chromosomal protein, which is in turn bound to the several different kinds of histone molecules.

We have shown, during the past two years, that it is the chromosomal RNA molecules which guide the histone molecules to the correct genes to be repressed. Histone molecules of themselves cannot read the information content of DNA. This is the function of the chromosomal RNA molecules, which do it by the regular procedures by which nucleic acid molecules recognize one another. In the world of nucleic acid, "It takes one to know one," biologists say. We have found that we can, for example, prepare chromosomal RNA and chromosomal proteins from the cells of one organ, bind these components to the purified DNA of a second and different organ, and reconstitute chromosomes identical to those of the organ which served as the donor of the chromosomal RNA. Our understanding and control over the process of repression is therefore considerable and growing fast.

How do genes that are repressed become derepressed, and vice versa? Our insight into this matter is growing too, and is based again upon information gained originally by those who work on simple creatures, the bacteria. In bacteria we know that small molecules enter the cell and turn on genes that were previously turned off. This mechanism serves primarily to cause the cell to not make enzyme molecules to utilize metabolites that are not present in the cell. The biologist says that the genes for making the particular enzyme molecules are inducible, and they are induced to form the enzyme only when the inducer substrate molecule is present. Similarly, certain classes of small molecules regulate gene activity in higher organisms. Classic examples are the hormones.

The higher organisms all conduct their genetic activity with the same basic kit of eight kinds of histone molecules.

Hormones are small molecules, a few dozen atoms at most, small enough for chemists to be interested in them; and they are produced in one organ and travel to other so-called target organs upon which they exert their effects. The effect of hormones in general, and perhaps in most, if not all, cases of hormone action, is to cause derepression of genes previously repressed and hence the production in the target organ cells of new kinds of enzyme molecules which those cells did not previously produce. Examples are the activity of cortisone on liver cells, which causes liver cells to produce enzymes needed

for glucose and amino acid metabolism; or the sex hormones which go to their target organ and cause the cellular activities which result in the secondary sex characteristics.

The way in which hormones work can also be studied with isolated chromosomes. We have found that when hormones enter the cell they bind first to specific hormone-binding proteins, a different species of protein for each different hormone. The complex thus formed is then capable of binding to the chromosome, finding the right gene in a way perhaps analogous to the way in which histone molecules find the right gene to repress. The new complex of chromatin, hormone, and hormone-binding protein is derepressed with respect to the particular genes which the hormone controls. Although the way in which this happens is not yet completely elucidated, it is nonetheless clear that the detailed study of the molecular basis of the interaction between hormone-binding protein and chromatin provides a key to the understanding of the molecular basis of derepression.

How then are we to understand the developmental process as a whole—the detailed programming of gene repression and derepression? We may imagine that each gene in the cell is reposing in a repressed state, waiting for the proper specific small molecule substance to come along and turn it on. Some such small molecule effector substances, as they are known, are certainly not only hormones but also everyday metabolites, dissolved gases, perhaps water, specific substances produced by neighboring cells, and so on. We can begin to visualize too how the derepression of one gene may lead to the production of material which causes derepression of further genes, and how this may in turn bring about long chains of genetic switching which can result in the developmental process.

Each gene waits to be turned on by a molecular substance.

The developmental process behaves as though it were a preprogrammed routine written down in the genetic DNA.

Consider, for example, the developmental process in the case of flowering. The vegetative bud of a plant goes along producing leaves and stems. Genes for making flowers and fruits are all turned off. Their activity is not required for vegetative growth. Suddenly, in the case of a short-day plant, for example, the leaf sees a short day (and a long night) and produces flowering hormone. The flowering hormone goes to the bud and to the meristematic (actively dividing embryonic) cells of the bud. There the flowering hormone says to the repressed genes for making flowers, "Genes for making flowers, I have seen a short day. It is time to become derepressed and start upon the pathway to flower development." Once started upon the floral pathway, development flows on, as it were, automatically. The developmental process behaves as though it were a preprogrammed routine written down in the genetic DNA.

How can we hope to study in detail the sequential genetic switching which results in a programmed developmental process? Certainly the task will be an enormous one. Today, however, we know how to approach the matter; we see that the problems of development must be resolved one by one to the level of the repression and derepression of individual genes by the interaction with the gene of its appropriate effector substance. The road to the complete understanding of the developmental process is at long last open before us. □

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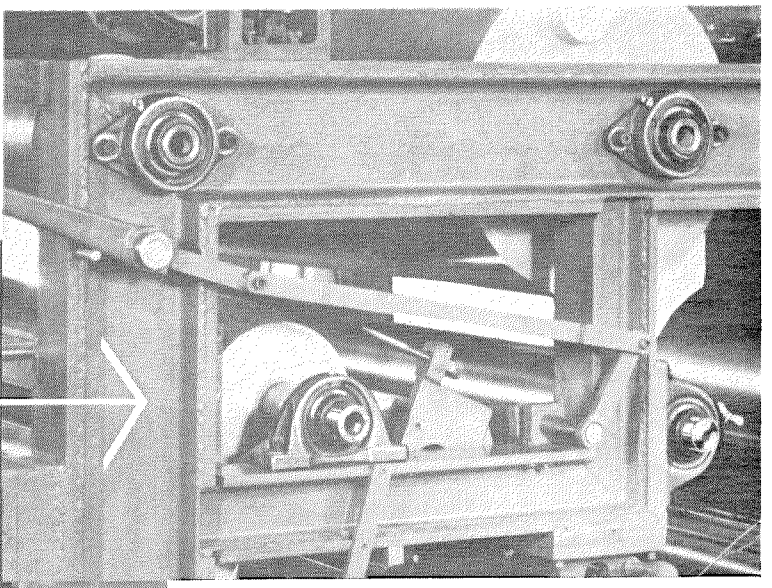

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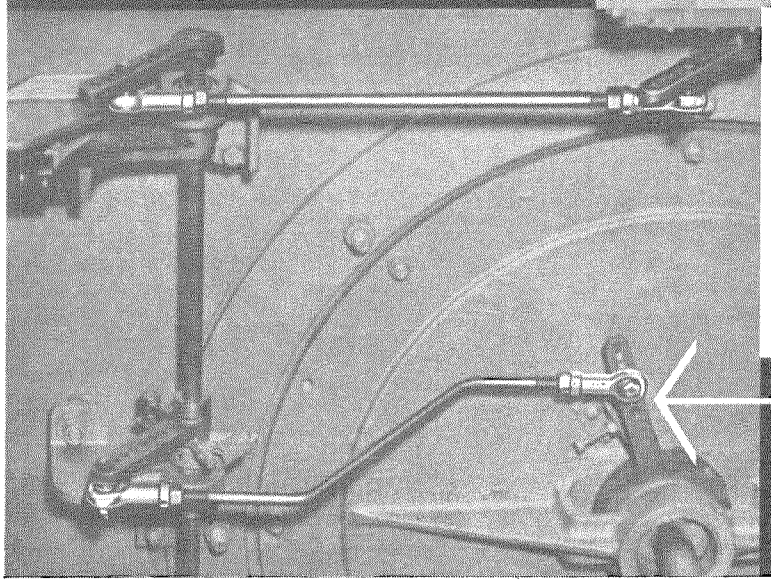

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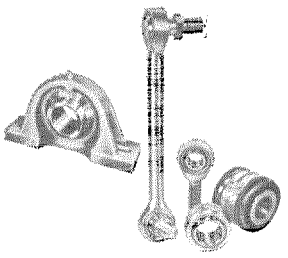

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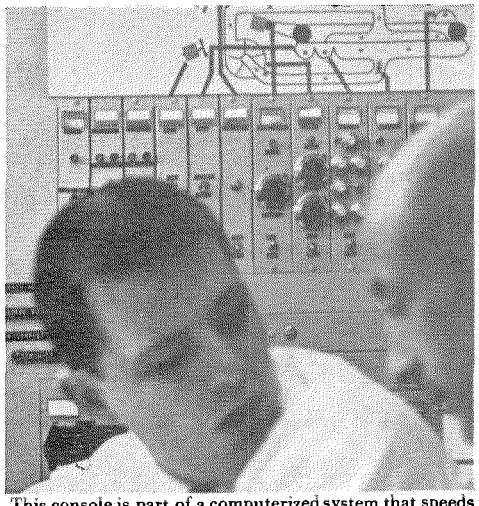

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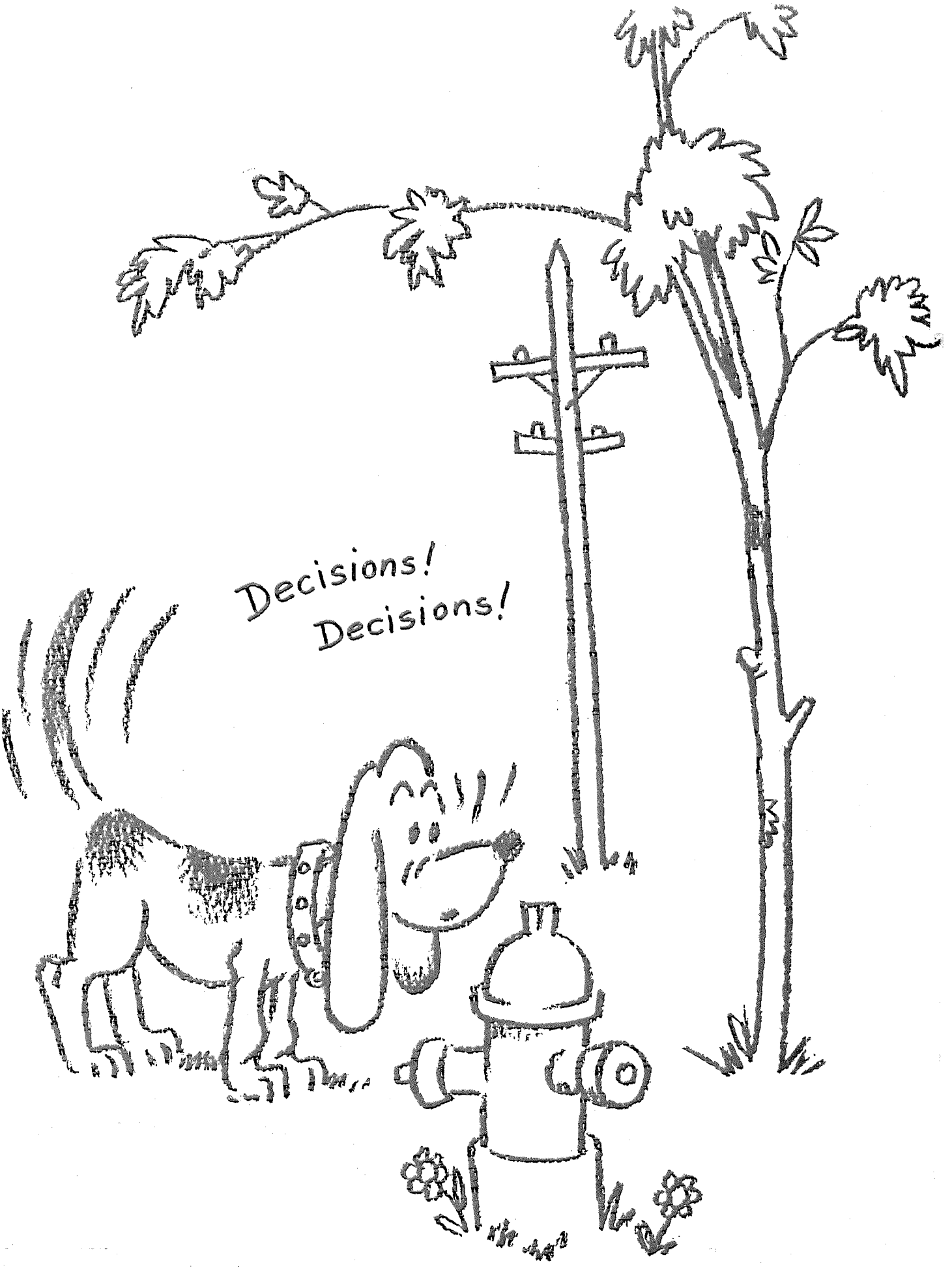
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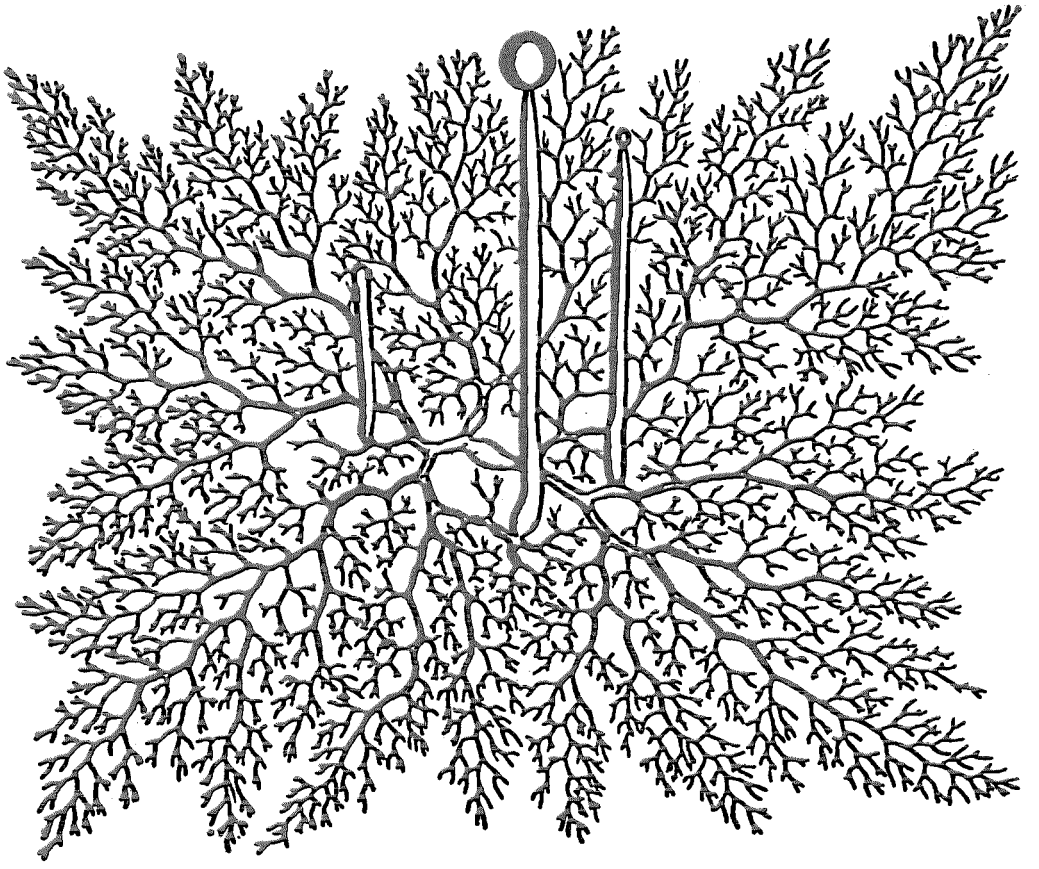


The humble fungus Phycomyces*—a valuable research tool for Caltech's molecular biologists.*

Molecular Biology—The Next Phase

By MAX DELBRÜCK

Some years ago R. P. Feynman gave a speech rather puzzlingly entitled "There's Plenty of Room at the Bottom." As it turned out, his comments were concerned with the revelations of molecular genetics showing that living nature had evolved degrees of miniaturization of devices for the storage, replication, and readout of information which far surpassed anything that engineering science has developed so far. As is now well known even to children in elementary school, the principal device employed here is the famous *double helix*. The molecular biology revolving around this helix might be classified as one-dimensional molecular biology, since, from the point of view of physics, the DNA molecule is a one-dimensional solid state object.

It stands to reason that nature, operating in three dimensions, long ago figured out that two-dimensional structures may also have their special virtues. Indeed, ultrastructural work done with the electron microscope during the last decades has amply revealed that the cells of all organisms employ two-dimensional structures not only on the outer boundary but also as parts of intracellular organelles. These structures are membranes with very characteristic general features—a thickness of about 70 angstroms and a composition always involving two classes of compounds, polar lipids and proteins. These membranes serve many functions. We find them functioning as parts of chemical factories—as floor space for the organized arrangement of systems of enzymes. We find them as phase separators, creating and maintaining volume phases of different chemical composition. We find them as surface structures of nerve fibers capable of transmit-

To subdivide space for concentration and seclusion, to localize function and partition structure, cells use membranes. Membranes are particularly conspicuous in many sense organs. What is their role therein?

ting signals along the length of the fiber.

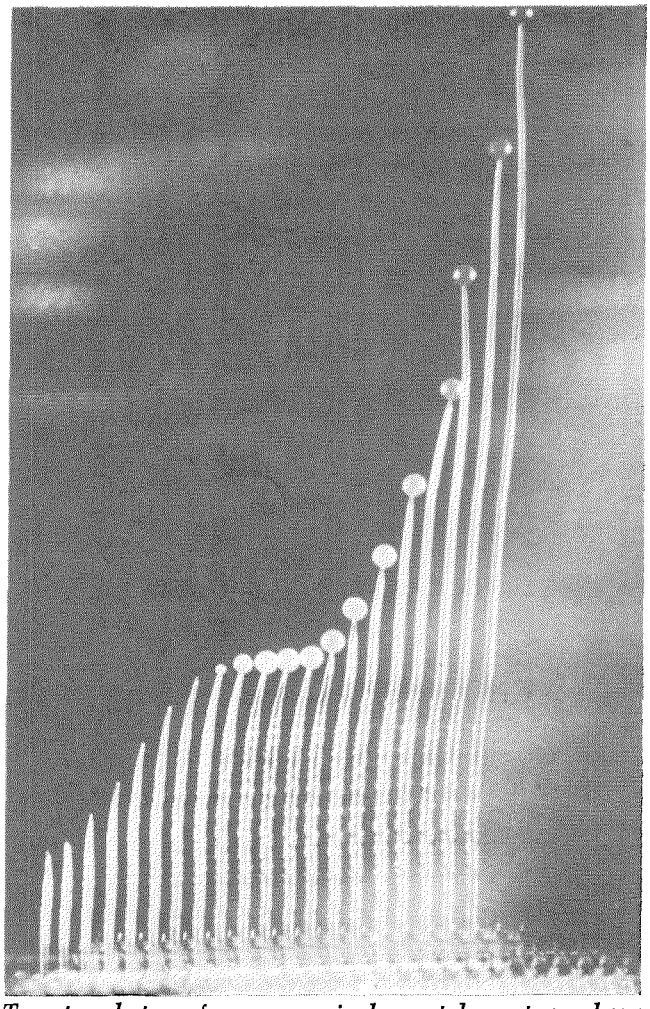
In all of these situations the functions of membranes are sensitively controlled by environmental physical and chemical factors. This control is driven to its ultimate degree of discrimination in the display of surface specificity involved in development, and reaches its ultimate degree of sensitivity in the devices used to process incoming signals such as light, touch, or smell—devices used to adjust the behavior of organisms to the external environment. Through modification of membranes the behavior of the whole cell is then profoundly influenced. On the molecular level these transducer mechanisms are not understood and will constitute the principal challenge for the next phase of molecular biology.

The depth of our ignorance in this area may be compared with the depth of our ignorance with respect to the molecular basis of genetics 30 or 40 years ago. We knew then that there were genes, and we knew that the genes were located in chromosomes, and we knew that they were arranged in a linear order. We also knew that the chromosomes contained proteins and nucleic acids, but for several decades we thought that the proteins represented information storage (specificity, as it was then called) and that the nucleic acids represented a structural backbone. We now know that the reverse is true. Similarly, with respect to membranes, there exists now an extraordinary degree of uncertainty as to the relative roles of protein and lipid: Which one determines structure and which one function?

It must not be thought that sense organs are a specialization limited to animals, though it is true that animals have developed a greater degree of organ specialization for the various sense qualities. But plants, too, respond very sensitively to light, touch, various gases, and gravity, as do microorganisms. It has been known for almost 100 years that some bacteria which use light as an energy source also adjust their motions so that, when they are exposed to a spectrum of light projected onto a microscopic slide, they will congregate at those wavelengths which they can utilize for photosynthesis. Bacteria will also move toward higher concentrations of oxygen, sugars, and amino acids. Wonderful work has been going on in the laboratory of Julius Adler at the University of Wisconsin in recent years, analyzing this sensitivity of bacteria to various stimuli. These studies are making use of the powerful methods of microbial genetics.

In vertebrates, the analysis of sensory transducer processes has made remarkably slow progress in spite of the sustained efforts of vast numbers of physiologists. The reason lies largely in the complexity of the sense organs, the smallness of the individual units, and in many cases in the inaccessibility of the sensory cells from the outside of the living organism. Situations exceptionally favorable for research are presented by the chemical senses of insects where the sense organs are often single cells, external and susceptible to a great deal of manipulation. A marvelous example is the silkworm moth. The male is attracted from great distances to the female by a sex lure emitted by the female in microgram quantities over several days. The receptor organs are hair cells, 10,000 of them, on the antennae of the male. Each of these hair cells is about 2 microns thick and 100 microns long. The cells are arranged in a basket form so as to create a sieve for the oncoming air to pass through. The dimensions are such that molecules of the sex lure are likely to strike one of these hairs while passing through the sieve. Each hair cell has a relatively thick cuticle quite impenetrable to the odorant, but this cuticle is perforated at distances of a few thousand angstroms by little pores about 150 angstroms in diameter. One must imagine that the molecules of the odorant—a C_{16} alcohol—when they strike the hair cell anywhere on its surface, move to these pores by surface diffusion and, having reached a pore, somehow trigger nerve impulses in the two or three fibers that innervate the hair all the way up the length of the hair cell.

The whole arrangement presents a system of



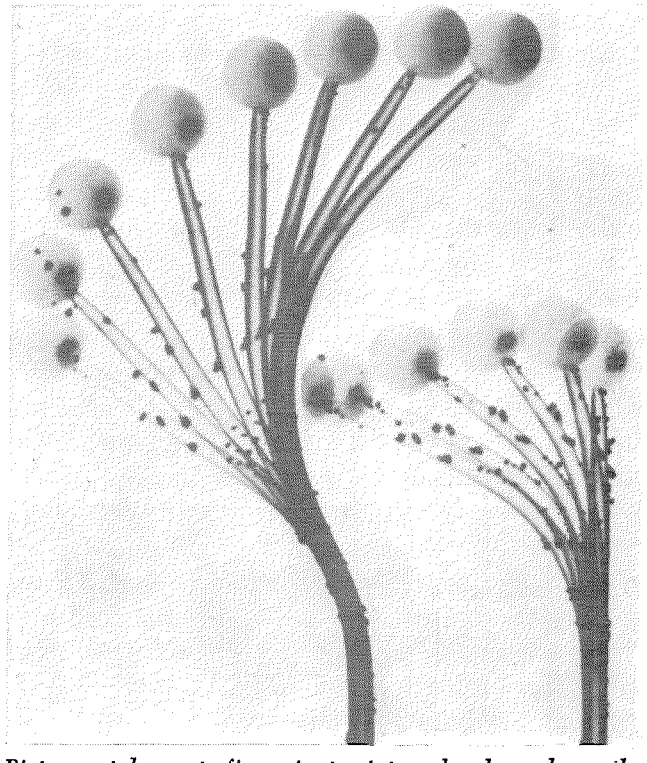
Twenty photos of a sporangiophore, taken at one-hour intervals, show how it grows from a simple cylindrical cell with a conical top to a height of one or two centimeters—at which point it stops growing and forms the sporangium. In a few hours, elongation resumes and quickly reaches a steady rate of about three millimeters an hour until the sporangiophore is about ten centimeters high.

molecular counters far beyond anything engineers have produced with respect to miniaturization, just as the DNA storage of information is far beyond current computer devices. The manner in which the molecules of the odorant trigger the nerve cells is unknown.

Our own work at Caltech has for a number of years been concerned with a microorganism, a fungus of a kind that the taxonomists place very low on the tree of evolution—lower than *Neurospora, Aspergillus,* or yeast, whose genetics have been studied so extensively. But experience with molecular genetics has shown that humbleness can be very profitable. Molecular genetics got its biggest boost from studies with bacteria and bacterial viruses, so why not stoop to a lowly fungus in the hope of here finding relatively simple answers to very deep questions? Let me hasten to add that we have *not* found the answers to these deep questions and that my presentation of what we are doing should be considered as an invitation to join an expedition rather than as a travelogue of adventures of the past.

Our creature, *Phycomyces,* forms a branching mycelium, which grows on almost anything. From this mycelium, sporangiophores, or stalks, are sent up into the air, carrying at their top a little ball—the sporangium—from which eventually 100,000 spores will be dispersed, each capable of initiating a new mycelium.

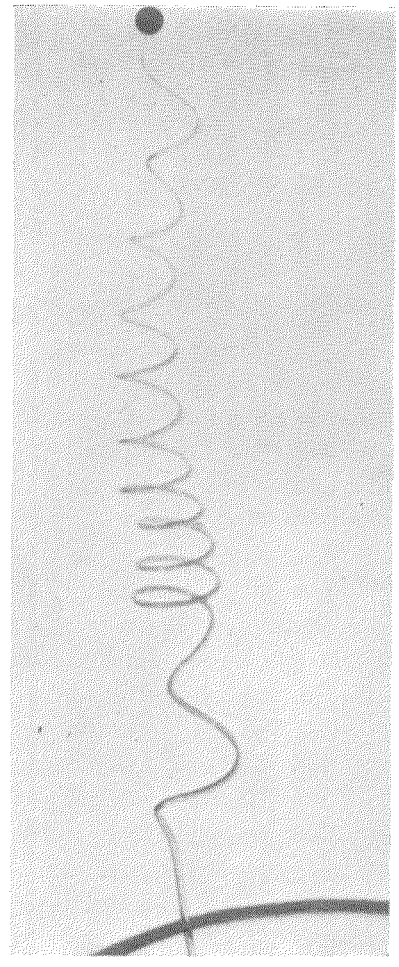
Of greatest interest to us is the bending response to light of the sporangiophore. How is this bending toward the light accomplished? It turns out that simple optics plays an important role. The receptive zone of the stalk, a portion about two millimeters long, is located immediately below the sporangium.



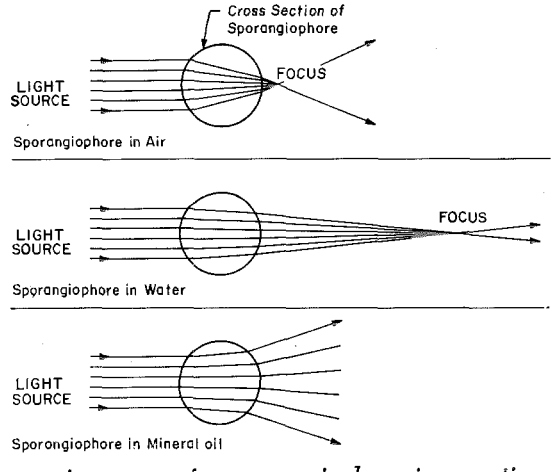
Pictures taken at five-minute intervals show how the sporangiophore (lower right), illuminated by a light source from the left, grows toward the light. When the light from the left is cut off and a light from the right turned on, the sporangiophore (left) quickly responds to the new light stimulus. The small dark specks are starch grains dusted onto the specimen. By following their position in successive pictures it can be seen where the sporangiophore stretches and how it twists during growth.

This is where elongation occurs, and this is also where the bending occurs. This stalk is transparent, and it acts like a cylindrical lens, focusing the impinging light near the distal side. We know that this focusing plays a determining role because if we immerse our specimen in mineral oil, which has a higher refractive index than the stalk, the converging lens becomes a diverging lens and the specimen, instead of growing towards the light, grows away from it (below). Counteracting this simple focusing effect, which in air favors the distal side, absorption and scattering subtract light on its passage through the stalk and favor the proximal side. These factors have been analyzed by finding the "balancing points," i.e., immersion fluids with refractive indices giving phototropic neutrality for a number of wavelengths and a number of mutants differing in their content of colored carotenes.

If our organism can respond to light, it must have a pigment absorbing the light, thereby initiating some mechanism controlling the growth rate. By measuring the relative effectiveness of various colors (the action spectrum), one can form an idea of the absorption spectrum of the receptor pigment. This action spectrum has some peaks close to those in the absorption spectrum of the principal pigment found in the organism. This pigment is β-carotene,



The phototropic reaction of a growing sporangiophore can be sustained for many hours if the sporangiophore is put on a turntable making one revolution every two hours, while being illuminated from only one side. Here, 11 full turns are made during 22 hours, and the growth is more than six centimeters.



The growing zone of a sporangiophore is a section two millimeters long, located just below the sporangium. This drawing of a cross section shows how the single cylindrical cell forms a converging lens which concentrates light on its distal side, resulting in a faster stretch on that side—thus a growth toward the light. In mineral oil the cylindrical lens is a diverging one, and the specimens bend away from the light. In water the lens is very weakly converging; the lens effect is overpowered by losses of light due to scattering and absorptions during passage through the sporangiophore so that a slow bending away from the light results.

and many people have guessed that β-carotene might be the receptor pigment. At Caltech, however, Martin Heisenberg recently obtained mutants which are practically free of colored carotenes or contain a different carotene—lycopene, the pigment which makes tomatoes red. It turns out that these carotene mutants respond to light just as well as the wild type, and we therefore do not think it likely that the receptor pigment is a carotene. Similarly, Gerhard Meissner, a research fellow in biology, was able to rule out retinal, the visual pigment of animals, found in trace amounts also in *Phycomyces*.

In the early stages before it has formed the sporangium, the stalk looks somewhat like a miniature centrifuge tube. In a sense it *is* a centrifuge tube. It is a single cell containing protoplasm and a vacuole and, in the protoplasm, a great variety of organelles. It can also be used as a centrifuge tube by floating it inside a capillary tube in a medium that buoys it up and then by putting the whole contraption into a high-speed centrifuge.

This technique was invented and worked out at

Some sound mathematical reasons exist for believing that nature has found it expedient to reduce all three-dimensional processes of transport to one- and two-dimensional ones.

Caltech by Marko Zalokar. It shows how the various organelles inside the cell are sorted out into layers. The nuclear layer, for instance, can be obtained in high purity, and the nuclei in this layer can be shown to be functional by injecting nuclei from one *mutant into the stalk of another mutant, thus form*ing functional heterokaryons. We were in great hopes that one of these layers would show the receptor pigment in such high concentration that we would be able to see it by direct microspectrophotometry. Indeed, a narrow yellow band was found, but closer study showed that this narrow band did not have an absorption spectrum in any way related to that of the receptor pigment. Electron microscopic studies by Dr. Zalokar showed that this layer contains *ferritin*, a protein enclosing a micelle of several thousand units of ferric hydroxide. It is a very interesting molecule in itself and has long been known to occur in animals and higher plants, but it is certainly not related to the light responses.

Another facet of our work concerns the fact that *Phycomyces* resembles the human eye in its ability to cope with an immense range of intensities covering a factor of about 10^9, from bright sunlight to the dimmest the human eye can perceive. The kinetics of this dark/light adaptation are quite similar in *Phycomyces* and in man. Our hope is that, however distant our relation to *Phycomyces* might seem, in this important respect nature may employ a similar device. We hope to analyze this device more deeply by utilizing another class of mutants, the "night-blind" ones, which seem to operate only in the upper range of light intensities.

There are other aspects which recommend the sporangiophore of *Phycomyces* as a model case for the study of sensory transducer processes. It turns out that the growing zone exhibits not only sensitivity to light but also to mechanical stretching, to gravity, and to smell. In regard to stretch sensitivity, which has been studied in some detail by David S. Dennison and Carol Roth at Dartmouth, the basic observation is this: A pull of about one milligram causes a transient slowdown of the growth rate, and a release of that pull causes a transient speedup. Now it must be understood that mechanical tension belongs to a class of stimuli fundamentally different from light or olfaction. In the latter cases the input is molecular. The product of a photochemical reaction or the olfactant molecule attacks the sensor at a defined point in space and time. In contrast, tension, like temperature or electric potential, is a continuous "variable of state," attacking a macroscopic structure as a whole. The type of instability leading to responses in the continuous and in the molecular case, respectively, may be quite different. We suspect, however, that in any of these cases the instability is an expression of highly cooperative phenomena, i.e., phenomena involving very large numbers of interacting subunits (thus giving rise to phase transitions, phase boundaries, and dislocations), and that the structures concerned are membranes of the type alluded to at the beginning of this article.

Characterizing the next phase of molecular biology as a step-up from one to two dimensions may create apprehension that 20 years from now we may be heralding a transition to three-dimensional molecular biology as the next phase. I consider this very unlikely. There exist sound mathematical reasons for believing that nature has found it expedient to reduce all three-dimensional processes of transport, such as tracking and control, to one- and two-dimensional ones. These reasons are related to an interesting mathematical discovery made in 1921 by George Polya. Polya showed that the attempt to reach a given destination by a random walk in *unbounded space is certain to meet with success* when it is carried out in one or two dimensions but not so in three dimensions. In bounded space the counterpart to this theorem is: If one wants a molecule to reach a specified site by diffusion in three-dimensional space, it is economical to embed the target site in a membrane to which the molecule in question will stick sufficiently tightly to stay adsorbed when it hits the membrane anywhere, and yet sufficiently loosely to enable it to perform two-dimensional diffusion on the membrane. It stands to reason that nature started exploiting the implications of this theorem a few billion years before Polya discovered it. ◫

IMMUNOGENETICS

By RAY OWEN

Through the immune system each individual knows its own molecules from alien forms: thus tolerance and thus rejection. What is the origin of this defense and how does it function?

Senator Walter Mondale of Minnesota, testifying before a Senate committee on a joint resolution for the establishment of a National Commission on Health, Science and Society on March 7, 1968, observed that "the scientific breakthroughs of the last few months, including the creation of an artificial viral core and the heart transplant operations, were current highlights in the dazzling half-century of truly unprecedented advance in the medical and biological sciences."

It is interesting that Senator Mondale's selection should have included two items from nearly opposite ends of the science-technology spectrum of modern biology—from the basic biochemistry of viral nucleic acids to the forefront of applied technology in human heart transplants—and that, in its way, Caltech has been importantly concerned with both of them. The reference to the "viral core" is, of course, the work in which R. L. Sinsheimer participated. We have been concerned with heart transplantation, in a much more indirect way, in immunogenetics. We have not transplanted any human hearts; in fact, about the closest we have come to that kind of surgery has been to exchange a great many skin grafts among mice. But we have worked for years in those fields of immunology and genetics related most closely to the advances that have made human organ transplantation a clinical reality (*E&S* – June 1959).

Transplantation research has been a very active and productive field during the past couple of decades, and a large number of workers, in many laboratories all over the world, have made important contributions to it. Rather than singling out our own contributions for parochial review, however, I will take this opportunity to outline, in a relatively nontechnical way, the current status of the organ transplantation field and some of its background.

As almost everyone knows nowadays (the knowledge is so common that it is difficult to recall how rare and inadequate it was just a few years ago), tissue or organ transplants between genetically different individuals are, under ordinary circumstances, unsuccessful. A graft from one brother to another, for example, at first "heals in" and appears to be doing all right. After only a few days there are signs of rejection; soon the graft dies.

The basis for rejection lies in the immunologic machinery of the recipient of the transplant—machinery that has been designed, through the long course of evolution, to recognize substances that are foreign to the organism and to respond by eliminating them. This machinery is of very considerable importance to us, because it leads to recovery from infectious disease and specific immunity to later attacks by the same disease. In clinical medicine it provides the basis for effective vaccination and, therefore, for the control of epidemic disease. But in the case of transplant rejection, as in some other kinds of immunologic malfunction such as allergies and autoimmune disease, the machinery operates to our disadvantage. It recognizes that a transplanted organ is foreign and destroys it.

The central problem of successful organ transplantation, therefore, is to understand enough about the machinery of immunity to devise ways of evading or controlling the immune response. As a significant side benefit, such an advance might well pay off also in the control of other unfortunate effects of immune systems. And the evasion or control of undesired immune responses should leave intact the desired responses, such as immunity to disease.

HISTOCOMPATIBILITY MATCHING

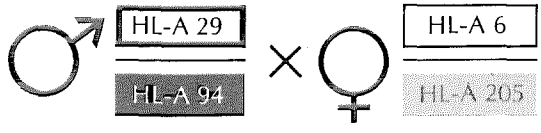
Given many different forms (alleles) of the main gene complex affecting graft compatibility (HL-A 1, 2, 3, ...N), and each allele individually rare, the two alleles present in a particular person (intended recipient) are likely to be different, e.g.:

HL-A 17
HL-A 126

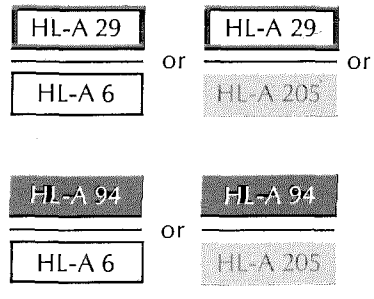
An unrelated prospective donor is very likely to be different from the intended recipient, e.g.:

HL-A 3
HL-A 241

But if two unrelated people marry, e.g.:



Each child receives one of the two alleles of the father, and one of the two alleles of the mother:



In contrast to the very low probability of a random match among unrelated people, therefore, pairs of children in the same family have about one chance in four of being perfect matches for this important characteristic, and three chances in four of being at least "half-matches," genetically.

In two very important ways the problems of evading or controlling immune responses to transplants lie as much in the field of genetics as they do in immunology. First, the basis of "foreignness" is genetic dissimilarity between graft and host. It is for this reason that grafts succeed between identical twins; being genetically alike, their relevant tissue and organ characteristics are identical. The inherited dissimilarities contributing to graft rejection, even among the members of a family, are in many respects very comparable to the blood-group differences that have been recognized for many years as important for blood transfusions or maternal-fetal compatibility. For the most part, however, they are not blood-group differences but a different set of individuality characteristics.

Until recently, practically all of our substantial information about the genetic similarities and differences involved in graft acceptance or rejection dealt with the mouse, because the mouse, in contrast to man, could be studied efficiently in the genetics laboratory. It became evident that many different genes are concerned with the kinds of individuality involved in graft rejection and that many different forms of some of these genes are present in laboratory mouse populations. Some of these genes could be identified with particular regions of particular mouse chromosomes. It also became evident that not all of these genes are of equal value for graft acceptance or rejection. In fact, only one complex of them, in the ninth linkage group of the mouse, provides for such strong tissue transplant barriers that differences between graft and recipient for them are very difficult to control.

We now know that the same facts hold for man; only one "major" tissue-compatibility gene complex has been found on one of the human chromosomes, though many "minor" genes are involved. Similarities or differences for the major complex of genes can be evaluated by tests of white blood cells in the laboratory. So great is human diversity for this complex of genes, however, that it is exceedingly rare to find two unrelated individuals who are alike for them. Within a family, the situation is different. Given an individual of any type, there is about one chance in four that his sister or brother will be just like him for this important gene complex (see chart left). This is undoubtedly the main reason why transplants of kidneys, for example, from living brothers or sisters have been more successful than have kidney transplants from

unrelated donors or cadavers. Of course, an important current hope in the field is that, through increased knowledge of the immunogenetics of the transplantation antigens, ways can be found of picking, from unrelated populations, relatively compatible donors so that cadaver sources of organs can be used more successfully. Adequate "matching" for transplantation, comparable to the system which has been so successful for blood transfusions, is currently the main hope of evading the destructive immune responses of graft rejection.

The other respect in which genetic approaches are basic to the transplant problem deals with the immune response itself. The main practical aim of these approaches is to control, rather than to evade, the response. Only within the past decade has it become generally recognized that the immune reactions are tailored by the genetic potentialities of the cell. The synthesis of a specific antibody, and the appearance of the immunocompetent cells that engage in graft destruction, are very probably dependent on the information available in the nuclei of the relevant cells, just as many other aspects of cellular differentiation and function depend ultimately on the cell's DNA. True understanding of the immunologic machinery, upon which reasoned efforts to control it must ultimately be based, is therefore in very large part a problem in developmental and molecular genetics.

Although a great many facts have been collected, we are still far short of the requisite understanding. Meanwhile, efforts to control graft rejection have proceeded in relatively arbitrary and empirical ways. They began, I suppose, with our 1945 observation of an experiment of nature—the fact that nonidentical twin calves, while they are embryos, accept and permanently tolerate blood-cell-forming grafts from each other. These efforts continued through the middle 1950's, with development of x-ray treatment to inactivate immune responses, particularly for the establishment of bone marrow transplants that saved the lives of heavily irradiated experimental animals. At the same time, rapid developments in experimental surgery paved the way for human organ transplants.

In the present decade, emphasis has been mainly on the chemical suppression of immune responses through the use of drugs that suppress particular steps in the series of reactions from DNA to protein synthesis. Chemosuppression, sometimes combined with irradiation, is now routinely used to promote

In human heart transplants

there is as yet no compelling evidence

that it is graft rejection that has led

to the death of so many recipients.

the establishment and function of tissue or organ transplants between genetically dissimilar individuals.

The methods currently available for chemosuppression have great disadvantages. The drugs themselves are damaging, and they inactivate immune response nonspecifically, leaving the treated individual vulnerable to infection. In the human heart transplants that have been done to date there is as yet no compelling evidence that it is graft rejection that has led to the death of so many recipients. On the contrary, some of the patients have died of the directly poisonous effects of the drugs that have had to be used. These people were already in extremely poor condition, because a heart transplant, which involves removing the heart from the recipient, would only be undertaken when a patient is already near death. Others have died of infections, often established in their bodies before treatment began, because these patients have had many complications from prolonged and serious heart disease. The immunosuppressive drugs cripple the patient's immune system severely and nonspecifically.

What is needed is a way of interfering much more specifically with graft rejection, with the particular combination of donor and recipient involved in any given transplant—but, at the same time, leaving the recipient's immunologic system intact for other kinds of bodily defense. Agents with this desirable effect may now be on the horizon, but none of them is as yet established for human medical practice. Progress is imminent, too, in tissue matching.

There is much to be learned in the interdisciplinary field of immunogenetics. It is a field in which challenges to basic understanding are most provocative and currently productive; it deals with a system that is in some respects a model for development and specific differentiation; it has important population as well as individual aspects; and it extends readily into a technology of undoubtable human significance. □

Action yields reaction; the humble crayfish provides an elegant model for studying the integration of all the available, varied forms of sensory information so as to fit each reaction to each action.

ACTIVITIES OF A NERVOUS SYSTEM

By C. A. G. WIERSMA

The functioning of the nervous system of the freshwater crayfish has been the primary object of investigations by the neurophysiology group at Caltech since 1934. The more we learn about different nervous systems, the more it appears that they all use the same kinds of tricks to deal with specific problems. But why do we concentrate on an animal that is so far off the main line of human evolution? The main reason, of course, is the limited number of nervous elements in the crayfish, which, though not as smart as many other animals, still shows a considerable variety of actions. Our experience has proved that it is possible to show in a number of cases that one certain nervous element, which can be readily identified in each specimen, is always involved in a given activity.

Our work started with studies of the motor axons innervating the muscles, and we found each muscle to be controlled by a very small and constant number of nerve fibers. Some muscles receive only a single motor fiber, most others two, and the legs a maximum of four. Not only are these numbers constant but each fiber also performs a special task that results in a specific type of contraction. We now know that these differences can depend on many factors—the differences in effect of the two motor fibers on a single muscle fiber being only one. But our original view, that each particular muscle acquires properties which make it especially adapted for its particular task, remains unchallenged. In many cases the additional presence of one or two peripheral inhibitory fibers—which can suppress to a greater or lesser extent the contractions initiated by the motor fibers—provides another way to vary the contraction according to circumstances.

When these observations were made, it was widely accepted that—as in vertebrate striated muscle fibers—any one muscle fiber was innervated at a single locus, the endplate, by a motor fiber branch. In the crustacean muscle, instead, each fiber receives many endings (multiterminal innervation), usually from more than one axon (polyneuronal innervation). Now it is known that these also occur in vertebrate smooth muscles, like those in the walls of the stomach, intestines, and blood vessels.

There are, of course, far fewer motor elements in the crayfish than in a frog or man, and this is part of the reason why the crayfish and all its relatives can manage with a greatly reduced number of neural elements in the central nervous system. It is estimated that the crayfish has several hundred thousand neurons, while man has about ten billion.

On the sensory side the crayfish is better provided than on the motor side. For instance, many hairs on the exoskeleton are innervated by one or two nerve fibers, whereas the compound eyes and the equilibrium organs (statocysts) respond with thousands of elements. In certain cases a great economy is evident. The function of the abdominal stretch receptors is especially impressive in this respect. There are only four receptors in each segment. The four are organized in two symmetrical pairs that, between them, signal the position and the speed of flexion of the abdominal segment behind the one they occupy. The ones that indicate position, the slow stretch receptors, have proved to be very useful for the study of many problems relating to the genesis of nerve impulses by mechanoreceptors. Because of the very small amount of tissue involved, these receptors are also favorable for a study of drug

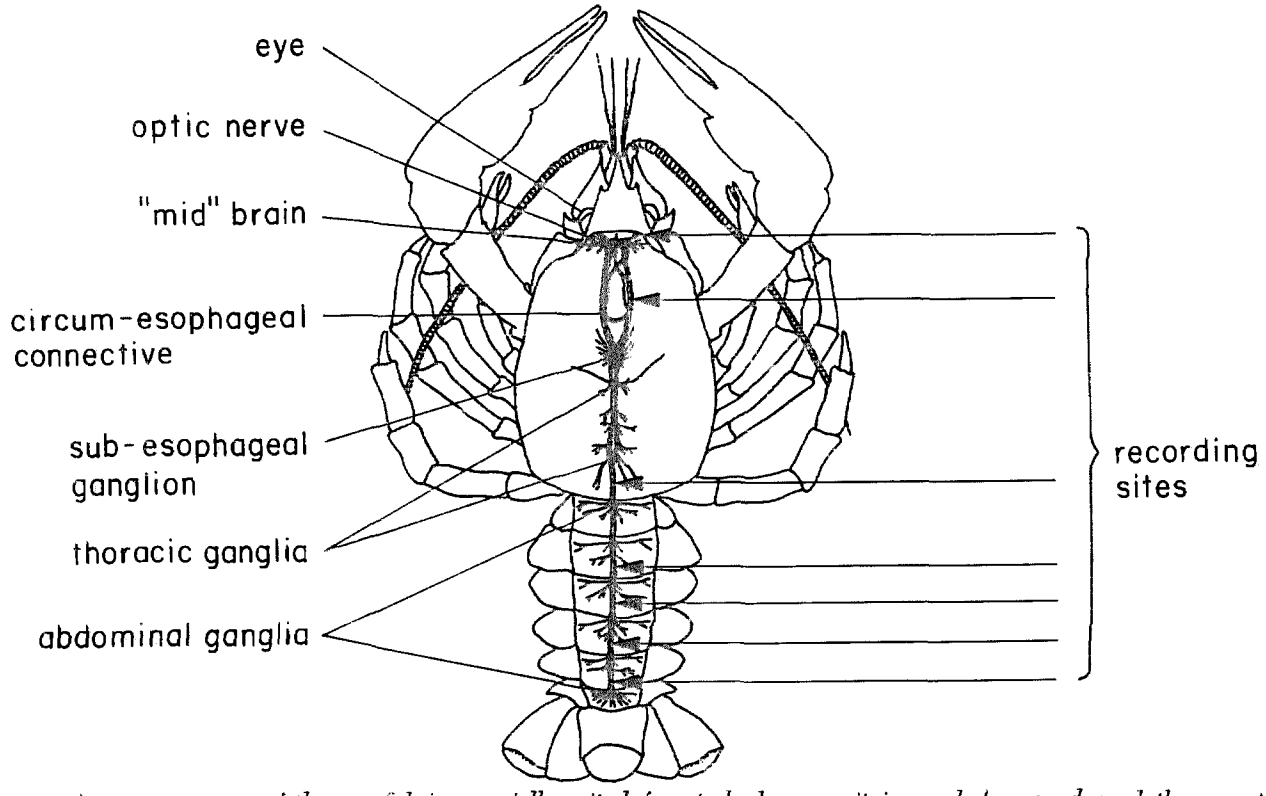
actions. In addition, they are among the few known sense cells that are innervated by an inhibitory fiber from the central nervous system that can "set" their sensitivity.

The axons of these sensory cells illustrate another aspect of the structure of the central nervous system. Upon entering it, the axons divide into two branches, one going forward to the brain and the other backward to the last ganglion. They thereby distribute their information throughout the whole system. Other sensory fibers, though more restricted, also have branches to neighboring ganglia. These extensions of the primary sensory fibers provide for integration of sensory events from more than one segment, which is of importance for the ultimate reaction to stimuli.

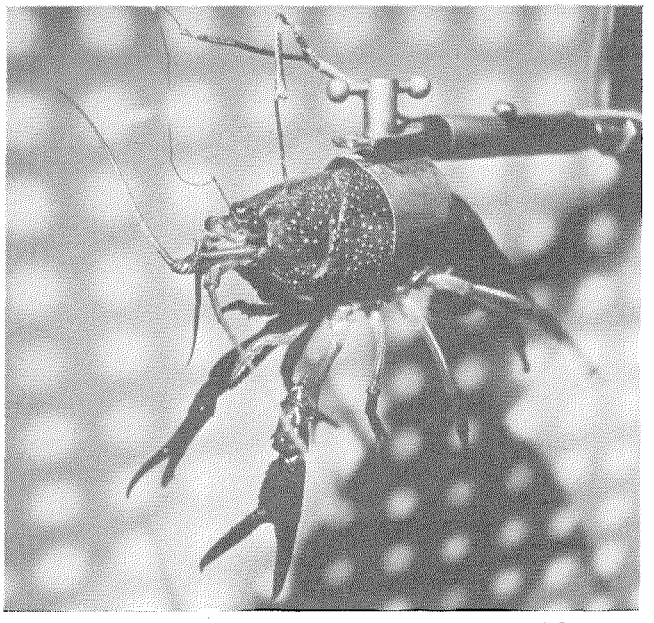
The integration of sensory events can be studied by recording the impulses caused by sensory stimuli in single interneurons—those elements of the nervous system that transform incoming impulses from sensory fibers to inputs for other interneurons or, more directly, for output fibers such as the muscle motor fibers.

The crayfish has proven to be particularly suitable for such studies. Its central nervous system (below) is much less condensed than in most other relatives, such as crabs, in which all thoracic and abdominal ganglia are fused. There are a number of levels in the crayfish where interneurons can be prepared for study. After removal of the sheath, the connectives can be split into small bundles containing one or a few reactive fibers. An interneuron will react, in contrast to most sensory fibers, to a relatively large body area—as, for example, to the touch of all hairs on one-half of a dorsal abdominal segment. Other interneurons have even larger fields, often consisting of equivalent areas on many neighboring body segments. For instance, one reacts to the hairs on the three peripheral parts of all five thoracic legs. This principle culminates in the sensory field size of an interneuron which reacts to touching the animal anywhere. As a consequence, stimulation of any one spot on the animal's surface will activate as many as 20 interneurons. The same information is thus channeled in many parallel pathways.

A plausible view of how this comes about would be that those interneurons with relatively small sensory fields provide the input for those with large fields—but this is generally not the case. Instead, the large field fibers receive input in many ganglia. This is shown by the fact that impulses in these fibers,



The central nervous system of the crayfish is especially suited for study because it is much less condensed than most of its relatives. The arrows indicate those levels of the system at which impulses have been recorded from interneurons.



Electrodes are implanted in the eye of the crayfish to record nerve impulses resulting from various stimuli.

passing a given location, travel forward when the back is stimulated and backward if the stimulated area is in front of the recording site.

Interneurons with other tasks are also present. There are, for instance, a number of interneurons that appear to have no sensory input, but that discharge continually by themselves before, as well as after, sensory isolation. Their functional significance is, as yet, problematic. "Activity" fibers discharge vigorously only when the animal struggles. "Command" fibers, when stimulated, cause coordinated body movements.

Our present investigations are mainly concerned with the optical apparatus. To obtain single-unit responses from the nerve tract between the brain and the four ganglia in the eyestalk, we push an insulated needle into the optic nerve (above). Its fine, bare tip will pick up impulse discharges of a single unit. A rather unexpected finding is that, in all species used, there are more fibers in the optic nerve which signal events from the brain to the ganglia than the other way around. Even when the very numerous primary sensory fibers, running in a tight bundle and activated by hair touch anywhere on the head region, are discounted, the statement still holds. This indicates strongly that the optic ganglia are the main location for the integration of visual and other inputs. The results of our research confirm this. First, there is no evidence for any fibers, primary or interneuronal, which have small visual fields. Though such negative evidence can never be considered completely conclusive, it is very likely that, during the many experiments that have been performed, such fibers would have been observed had they existed, since they obviously must be numerous. Second, all fibers reacting to visual input are also influenced by inputs coming from the brain, but in various ways.

One class of interneurons, consisting of 14 members, signals the light intensity falling on the eye. Each member responds to increases in light intensity over a very specific retinal area and is inhibited by illumination of all other areas. The fibers are all, therefore, influenced by any small area of the eye. However, since their excitatory fields overlap, light in any given spot will increase the discharges in several and decrease them in all others. This is another example of the principle of parallel computation.

The responses of all these "sustaining" fibers are influenced by the level of the "excited state." Thus the same light exposure causes fewer impulses when the animal is quiet than when it moves its appendages. But when an excited state is present in total darkness, it does not by itself cause these fibers to fire impulses.

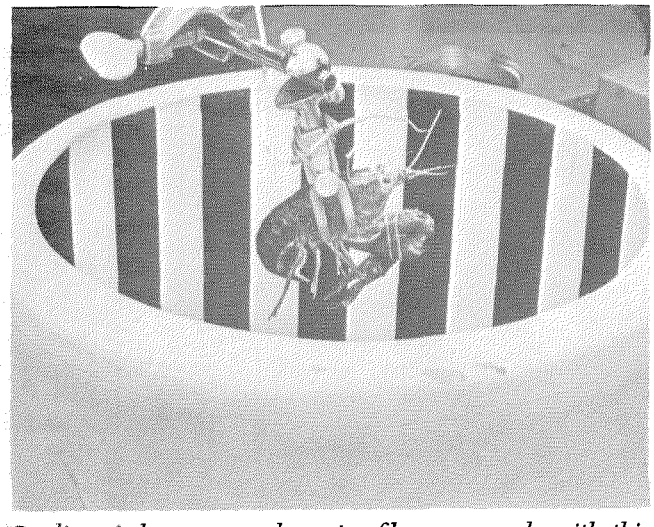
One is tempted to describe this process as being comparable to "paying more attention" to the visual stimulus. But this need not mean that the ultimate reaction to the stimulus is greater, for it may be, instead, a compensation for a lowered reactivity in the input-output chain (itself caused by the excited state) and thus a homeostatic mechanism.

The excited state has other effects. This is illustrated by the increase in impulses in the motor fibers to some of the muscles which move the outer eyecup. These muscles provide for the adjustment of the eye's position to changes in visual and gravitational conditions. Another muscle, otherwise quite similar but responsible for the eye withdrawal reflex (comparable to our blink reflex), is in no way influenced. The six muscles whose motor fibers do show the excited state are often called the eye retractors, since, when all are active, they retract the eyecup, bringing it closer to the body. This is exactly what happens when an excited state develops, causing the often considerable increase in the firing frequency of all their motor fibers. But in addition each muscle serves for much more subtle adjustments. These are being studied in detail since they present us with an excellent subsystem for

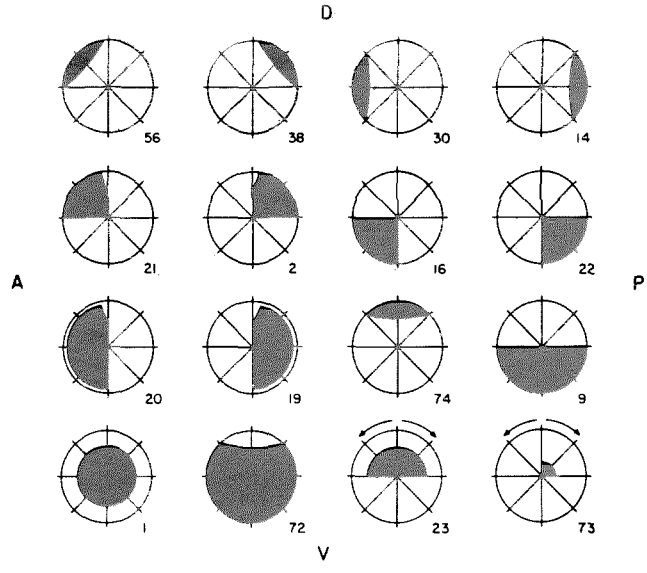
studying input-output relationships.

The six muscles are arranged in three pairs, each pair involved in a specific task, in a push-pull relationship. The first pair moves the eyecup forward and backward. The two sets of motor fibers show this antagonistic arrangement. When there is an increase in the frequency of impulses in the one, inhibition occurs in the other. The specific input for these fibers is wholly visual and derives from both eyes. Identical reactions are obtained both when the animal is rotated in a striped drum and when the stripes are rotated around the animal (below). Rotating the animal in the dark or in a totally white surround does not cause changes, proving that the equilibrium organs, or statocysts, have no influence on this pair of muscles. In order to affect the discharge rate of a given fiber in the same way, stripes must pass over one eye from front to back and over the other from back to front. There is, therefore, no change when the animal walks in a straight line, but only when he turns.

The second set of motor fibers regulates the position of the eye in the vertical plane. Here the effect of body rotation in darkness is at least as strong as under general illumination. The eye, which is turned downward by rotation of the animal, moves upward, and the impulses in the motor fiber to the responsible muscle increase in frequency until the eye looks straight down. There are two other ways by which a similar but smaller increase occurs on visual stimuli—turning stripes over the eye surface with a vertical, down-to-up component, or illuminating the other eye. When any of these factors is reversed, the same fiber is inhibited and its antagonist activated.



Studies of the eye muscle motor fibers are made with this experimental setup that allows rotation of either the animal or the striped drum in which it is suspended.



The excitatory visual fields of the 14 sustaining fibers in the crayfish eye are indicated by black areas. The fields of the two space-constant sustaining fibers, 0 23 and 0 73, are shown (lower right) for the animal's normal position.

The third set of motor fibers innervates muscles for eye rotations. These are influenced by way of the statocysts when the animal is turned head over heels, again in an antagonistic relationship. They also show the two types of visual input. Rotation of stripes produces an effect when the stripes are at right angles to the longitudinal axis of the body and are thus seen especially by the eye rims. Illumination causes an effect in only two areas of either eye, namely the front rim and back rim—the areas covered by the sustaining fibers 0 14 and 0 30 (above). Here the same areas of the two eyes work synergistically and against those on the opposite side. (For stripes turning in intermediate directions between the three described, all three systems are activated, and the resulting eye position is that place determined by the relative strength of contraction in all six muscles.)

The fact that it is possible to drive the eye muscles by moving stripes shows that there must be a way by which the crayfish can note them. Though this might be done by way of the sustaining fibers, the fact that the reactions are so independent of the intensity of illumination and the amount of contrast (as well as several other considerations) shows that such is not the case. We have found, in all spe-

cies investigated, a class of interneurons which reacts specifically to moving objects, and these fibers share, with similar fibers of the frog and rabbit, insensitivity to level of illumination and to amount of contrast. In the crayfish the most commonly observed movement fibers have been called "jittery," since they respond maximally to small dark objects moving in an irregular manner over their sensory fields. A large dark object such as a black cardboard is responded to only when its border enters the field of vision, unless the movement is stopped and then renewed.

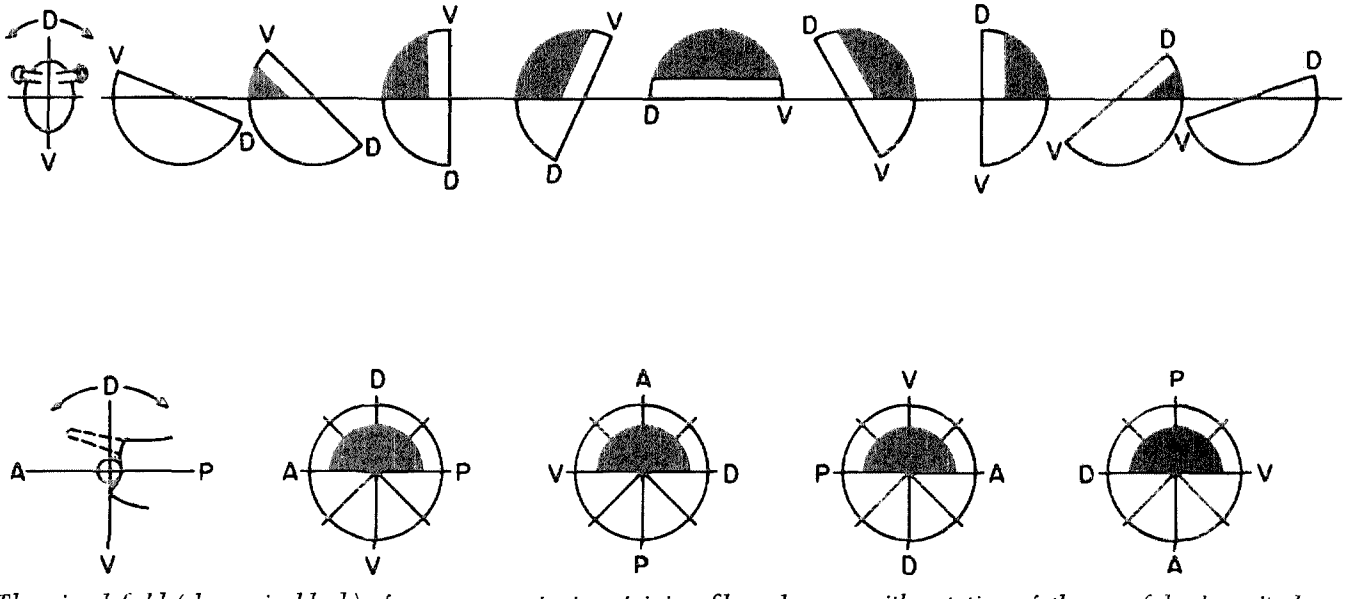
The movement fibers have fields similar to those of the sustaining fibers. This is especially apparent in the rock lobster, which has two additional movement fiber types. The 23 fields of the 23 members of the sustaining class of fibers are all represented by the 23 members of the jittery movement class, whereas for the other two classes respectively, 21 and 14 members with corresponding fields have been found. Therefore, visual events in one area can be signalled by four fiber classes. It is only in crabs that a type of movement fiber has been regularly obtained which reacts with a continuous discharge to a moving striped pattern. All other movement fibers have a more or less pronounced "habituation"—that is, sooner or later, they will stop reacting to a repeatedly presented stimulus.

In certain output channels the reaction to a few known interneurons can be inferred. In the case of the influence of illumination of the front and back eye rim areas, the participation of the four interneurons giving the sustaining discharges is likely. But since these same areas are also read by dimming fibers (a type that gives the inverse response from sustaining fibers), either might be the sole or the second source.

To decide about these matters, it would be necessary to exclude one or the other fiber type from reacting, which is technically impossible. It is therefore of interest that, on the basis of the known properties of still another class of optic interneurons, the space-constant fibers, such a relationship can be inferred with much greater confidence.

In the crayfish and rock lobster there are some fibers in each of the main fiber classes which are not considered real members of those classes because they have an additional feature. The visual field of these neurons is dependent upon the activity of the statocyst fibers. In the crayfish a space-constant sustaining fiber is present which, in the normal body and eye position, has a visual field that is limited to the inner upper half of the retina. The receptive field changes both its size and location, however, when the eye looks in a different vertical direction. The location of the field turns in a direction opposite to that of the animal when he is rotated head over heels (below).

When the animal is rotated around its long axis, however, it is the field size that changes from half-moon shape to full, quarter, and new moon, depending on how much of the circle of ommatidia is above



The visual field (shown in black) of a space-constant sustaining fiber changes with rotation of the crayfish along its longitudinal axis (top of diagram) and along its transverse axis (bottom).

and below the true horizon. In most other instances of space constancy, the maximum field, rather than being restricted to the middle part of the eye, covers nearly the total surface.

This relationship exists for two movement fibers found in the crayfish and for all four types of space-constant fibers found in the rock lobster. This means that the lobster's sustaining fiber, unlike that of the crayfish, does not become completely blind when the eye is turned to the ground. This is because the outer ommatidia in the eye rim are still about 5°-10° above the horizon.

In the crayfish the two space-constant movement fibers are both large and rather easily found. One has all the properties of a jittery movement fiber, whereas the other reacts strongly but with considerable habituation to quickly approaching objects. When a crayfish is highly responsive to optical input, the type of stimulus that triggers the jittery movement fibers also causes the so-called defense reflex. This reflex, which can be obtained by stimulation of a single interneuron in the commissure, consists of a raising of both claws and of the anterior part of the body, and is an expression of "aggressiveness." This fiber is a member of the group of command interneurons mentioned previously. When the animal is turned in space, this reflex is obtained only by stimulating the part of the eye within the receptive field of the space-constant jittery movement fiber. Therefore the latter is undoubtedly the most important, if not the exclusive, visual input channel for the reflex. Similarly, it appears that the fast space-constant movement fiber is the main trigger for activating the flight reflex, by which the animal "escapes" by swimming backward. This reflex is mediated by the two medial giant fibers, which originate in the brain. So long as the animal is in its normal position, the approach must be in the dorsal eye part. But when the crayfish sleeps (lying on one side), the reflex is also elicited by an object quickly approaching the lower eye half, which will now trigger the space-constant fiber.

Some years ago the prediction was made that if the functioning of any central nervous system is ever to be understood, that of the crayfish might well be the first. At present, though this goal has certainly not been reached, there is reason to remain optimistic. It may be significant that a viewpoint developed at Caltech over the years, as a result of our experimental findings, is closely akin to that of Konrad Lorenz's ideas about the relationships between different behavioral acts.

The central nervous system has a very democratic type of organization, in which "yes," "no," and "perhaps" votes are continuously cast.

Briefly, our viewpoint is that the central nervous system has a very democratic type of organization, in which at many places "yes" and "no" as well as "perhaps" votes are continuously cast. The results of these polls are expressed as an activation of certain command fibers, which will determine the actual performance. But in order that a coordinated act will follow, it is necessary that inhibition suppress all those effects which are incompatible. This can result from the additional activation of inhibitory central fibers which are known to be present. Peripheral mechanisms are also able to negate or change these central commands, up to the very last voting booths, by way of neuromuscular inhibitory nerve fibers. The one command which appears to be the least changeable at lower levels is the one for the flight reflex, which from a functional point of view is understandable. Associated with this supremacy is a "high" threshold, such that the occurrence of this command needs many more "yes" votes, and these in a tight temporal cluster, than do the more modifiable ones.

For the future it will be important to gather more and more information about the relationships between the different known subsystems and to complete as much as possible the survey of reactive neuron types. We can now implant electrodes which can record signals from a single known unit for days on end. This technique may materially contribute to our understanding of what is going on. So far it appears that for short-term experimentation the results obtained with these free-moving animals check well with those obtained previously. However, not only will we find out what changes in responsiveness take place, but it will also be easier to assign possible functional relationships between the activity of a given interneuron and the behavior of the animal. We have thus collected over the years a considerable number of the bits and pieces, which makes it possible in some instances to see how they fit together. But whether continuation along these lines will eventually solve all of the problems of how this central nervous system computes its many output reactions is unpredictable. □

Genes and Behavior

By SEYMOUR BENZER

Genes (DNA) that influence structure, genes that influence chemistry —and now we look for genes that influence behavior. Drosophila flies again!

Much of human personality is determined by heredity. For instance, recent studies have revealed that inmates of institutions for the criminally insane show an unusually high frequency of chromosomal abnormalities—suggesting that undue emphasis might have been placed on environmental factors in causing their behavior. To understand what lies between the gene and the personality is a great challenge for modern biology.

Since humans, especially the criminally insane, are not the most cooperative of subjects, one looks for a more amenable creature as a model system. Research has shown that the basic principles of genetics and molecular biology, whether for bacteriophage or the fly, have wide applicability to other organisms. The same may be true for the mechanisms underlying the wiring-up and functioning of the nervous system. The genes contain the information for the circuit diagram, but little is known about the relationship between this primary information and its conversion into the end result. During development, tags of specificity are parceled out among the neurons so that they connect in the proper network. How this is done is an open mystery. It is not even known what kinds of molecules carry the specificity that distinguishes one neuron from another. How does a neuron know where to go and how to recognize others so that only the appropriate connections are made?

Once assembled, the functioning nervous system embodies a complex of interacting electrical and biochemical events to generate behavior. The fine structure and interlacing of even the simplest nervous systems are such that to dissect them requires a very fine scalpel indeed. Gene mutation can provide such a microsurgical tool; with it one might hope to analyze the system in a manner analogous to the one which has proven so successful in unraveling biochemical pathways and control mechanisms at the molecular level.

The wealth of genetic knowledge of the fruit fly, *Drosophila,* and the availability of many mutants and special chromosomal arrangements make it an organism of choice for the genetic approach. The same features that favored *Drosophila* for genetics; namely its short generation time and the facility with which large populations can be grown in the laboratory, also make it advantageous to use for behavioral tests—which can be applied to populations rather than to individuals—and for the isolation of rare mutants by selective techniques.

There are two objections to choosing *Drosophila* for such studies. The first is that it is too big: Its brain contains around one million neurons, a rather complex system. The second is that it is too small: Many of the usual techniques of neurophysiology are not applicable with ease. In some ways, however, I feel (like Goldilocks) that it is just right. The number of neurons, being close to the geometric mean of a single neuron and the human brain, is sufficient to display many of the aspects of behavior associated with higher organisms. On the other hand, it is possible to focus one's interest upon a smaller, simpler part of the system. For instance, the

compound eye of the fly consists of about 800 ommatidia in a neat hexagonal array, each containing eight photoreceptor cells. The axons of these photoreceptor cells are distributed to a hexagonal lattice of interneurons in the first optic ganglion in a precise pattern that is reducible to repeated identical subunits, the morphological unit containing only eight photoreceptor axons and one interneuron. Thus, what at first glance appears to be a formidably complex structure can be reduced to a relatively simple system for studying neurospecificity.

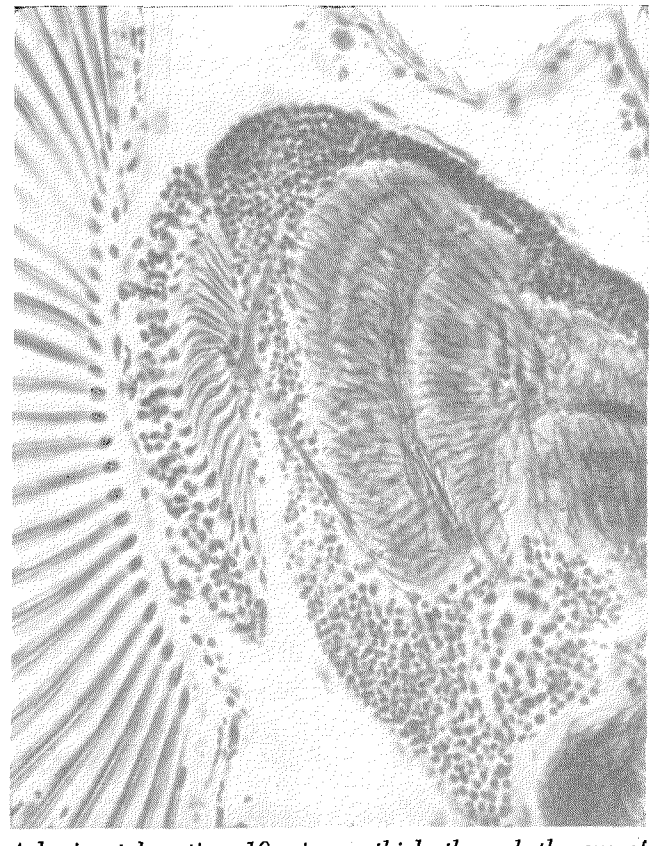
Most of the behavioral work with *Drosophila* in the past has been concerned with mating behavior because of its importance in evolution (and partly, perhaps, for secret reasons of the investigators). Flies do, in fact, engage in an elaborate courtship ritual that can be embarrassingly anthropomorphic. Intriguing as these experiments may be, they suffer from a serious drawback; namely, that it takes two to tango. One therefore must reckon with the interaction of the behavioral idiosyncrasies of both the male and the female. Things are bad enough with only one fly.

Take the response of a fly to light. To observe this, simply lift the lid of a garbage can. Activated by vibration, the fly moves in the direction of the light, thereby escaping. This behavioral reaction, phototaxis, obviously has positive survival value for the fly. Although relatively simple as behavioral phenomena go, it is nevertheless the result of a complex series of events in an intricate structure. There is absorption of light by photopigment to produce neural excitation, transmission at synaptic junctions, integration in the central nervous system with other inputs, and generation of appropriate motor signals to activate the muscles so that the fly moves in the correct direction.

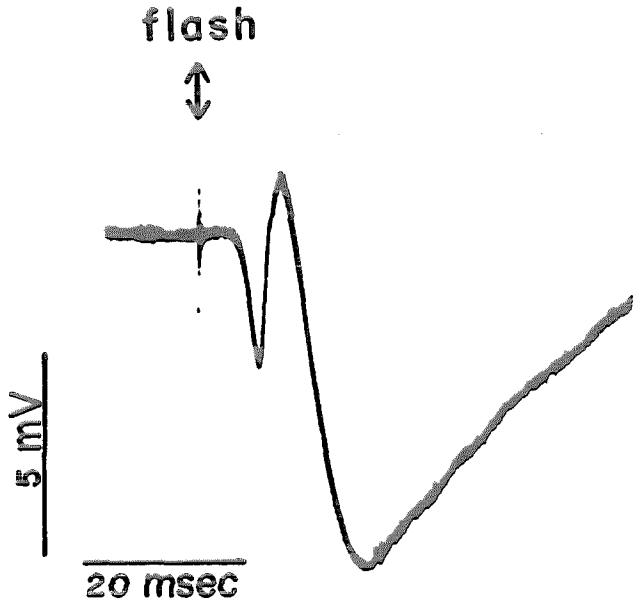
This system contains models of many of the basic neural mechanisms involved in all behavior. A defect in any one of the structures or processes involved can lead to modification or elimination of the response. Thus, there are mutants known that do not show any response to light. Among a collection of such non-phototactic mutants, one might expect to find defects affecting the various elements of the system.

To find these defects, one treats normal flies with a mutagenic agent and isolates mutants that do not show the normal phototactic response. Additional material is provided by the vast collection of previously isolated *Drosophila* mutants available at Caltech, some of which are non-phototactic. To localize the defect, the first step is, of course, anatomical examination of the fly (below). Some mutants simply have no eyes, so the lack of response is easily enough explained. It is interesting to note, however, that such eyeless flies are, in other respects, quite normal, active, and fertile. This points up an important feature of the visual system as a choice for these genetic studies: It is dispensable. Genetic defects—large or small—provided their effects are localized to the visual system, can be picked up without interfering with viability.

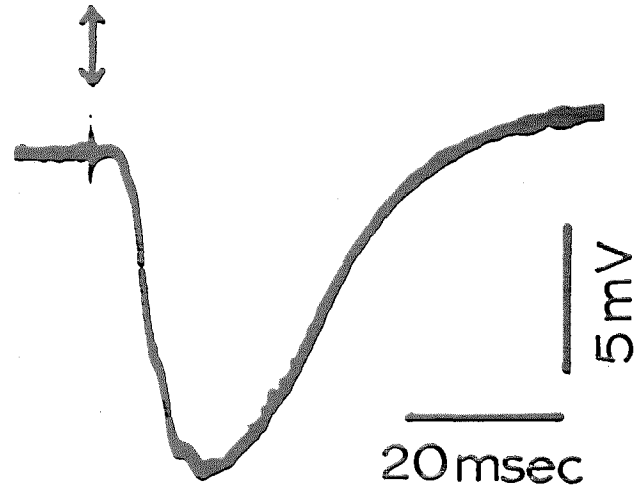
Other non-phototactic mutants can be seen to have defects such as absence of photoreceptor cells, gross distortion of the ommatidial array, or degeneration of the neural elements behind the eye. Still, there is also a class of mutants whose eyes appear normal by microscopic examination, yet the flies show no phototaxis. Yoshihi Hotta of Caltech has begun to examine these by neurophysiological techniques. Even by the most elementary method, the electroretinogram, it can be shown that some of



A horizontal section, 10 microns thick, through the eye of a normal Drosophila *is stained with silver to show the nerve fibers. At left are the photosensitive elements of the eye that connect with the various optic ganglia.*



An electroretinogram (ERG) of a fly records the nature of its response to light (phototaxis). The ERG above shows the potential of the cornea versus time in a normal fly after a 20-microsecond flash of light.



ERG of a non-phototactic fly with a genetic defect in the retinal function. The main negative move of the ERG shows that the photoreceptor cells are functioning properly. But in this mutant, the flash of light fails to induce the normal neural impulses indicated by the sharp positive peak in the ERG of the phototactic fly at left.

these mutants have defects in eye function (above). Thus, the photoreceptor elements appear to be functioning properly, yet the neural impulses normally generated in response to photoreceptor action are not produced. Whether this is due to a genetic alteration of excitability of the photoreceptor cell axon or due to failure to transmit excitation to the next interneuron has not yet been determined. An interesting feature of these mutants is that they also show changes in body pigmentation, which may be an important clue to an underlying biochemical mechanism. Finally, there remain mutants that have perfectly normal electroretinograms yet are not phototactic. Their defects must be sought at higher levels of the nervous system.

This search for defects in non-phototactic mutants describes the outline of a research program to attack the mechanisms underlying behavior by genetic methods. It is by no means limited to phototaxis, which is simply one model system. The problems of development of the nervous system, rhythms in behavior, and learning may yield to the same approach. The vast majority of work in neurophysiology in the past has been done with organisms that are impractical for genetics. These organisms may have inordinately long generation times, require difficult conditions for growth and breeding, or both. Conversely, geneticists, with only a few exceptions, have concerned themselves rather little with behavior, preferring to use easily identifiable morphological or biochemical characters as indicators for their genes. To join these two widely separated areas calls for a non-disciplinary outlook.

Actually, there is already a movement among molecular biologists to tackle behavior in various organisms. For example, Julius Adler of the University of Wisconsin is studying chemotaxis in bacteria. Max Delbrück of Caltech is working on phototropism in a mold, Sydney Brenner of Cambridge University has taken up the nervous system of the nematode, and Francois Jacob of the Pasteur Institute has now plunged in with the same animal.

Each of these organisms is, like *Drosophila*, too big and too small but offers certain advantages. The common denominator in all cases, however, is genetics, since molecular biologists are, from past experience, keenly aware of the importance of the genes in determining the development and structure of an organism and of the power of mutations as a dissecting tool. It is of interest to note that many of these people had already switched their fields once before—to go into molecular biology when it was a pioneering venture. But the rapid development of that field has made it, within two decades, a classical science. Whether these renegades can repeat their performance on new and more difficult problems remains to be seen. □

PSYCHOBIOLOGY AND VICE VERSA

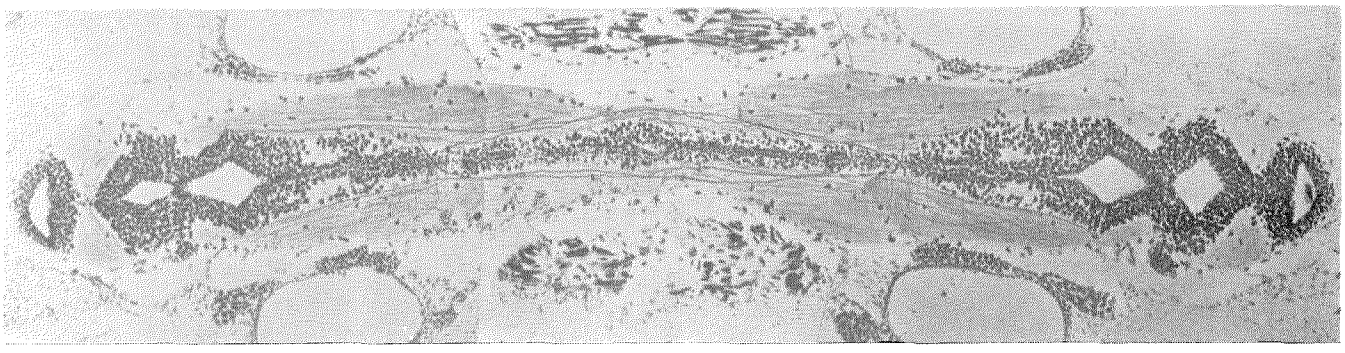
By ROGER W. SPERRY

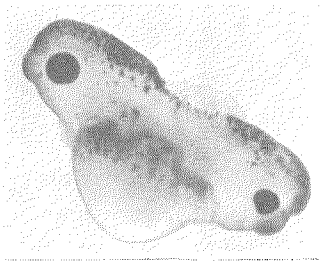
In which the human brain seeks within itself the structural bases, inherited and learned, of psychic function—for the essence of memory and sensation, speech and thought. Can we shape a mirror to the mind?

Psychobiology, like its synonyms biopsychology, psychophysiology, physiological psychology, neuropsychology, neurobiology, behavioral biology, behavioral science, neuroscience, etc., is a term that is rather loosely defined and means different things in different places. Regardless of the name of the game, our research strategy is to keep our biological sights trained on the higher functions of the nervous system—the mental, cerebral, or psychic activities for which brains are particularly noted. This concern for the higher or mental functions separates psychobiology somewhat from the more broadly defined "neuro" sciences. If some of our projects deal with subjects like "the cytochemical basis of morphogenetic gradients regulating selective interneuronal adhesivity," it is not because of any prime interest in the molecular phenomena as such, but because some general principle of cerebral integration is at issue. The direct bearing on questions of higher mental function makes the difference.

In modern biology few problem areas remain where one can point to phenomena that are still genuinely mysterious—phenomena for which science cannot as yet even conceive, in principle, a satisfactory explanation—but the higher functions of the brain clearly qualify in this category. We lack even a reasonable hypothesis for the way in which cerebral tissue generates conscious awareness or any form of mental experience. Mind/brain relations continue to offer a challenging frontier with plenty of room at both top and bottom for new discoveries and some major conceptual breakthroughs.

A general idea of where we stand in the field today may be inferred from a quick sampling of some of the changes that have taken place in psychobiol-





To test the influence of body gradients in nerve growth, frog embryos (left) are joined surgically at the point where the tails would normally develop. (The black spots are the eyes, the bulge is the yolk sac of the joined embryos.) The stained microscopic section of the same embryos (above) shows how the spinal fibers of one embryo (hairlike strands on either side of the spinal cord), that normally grow head-to-tail, cross the junction and then grow tail-to-head in the spinal cord of the other embryo.

ogy in the last several decades and of some of the kinds of problems currently under investigation. Only 30 years ago it was generally believed, largely on the basis of dozens of nerve transplantation studies, that the machinery of the brain was so designed that one could interchange its fiber connections—disarrange its basic wiring diagram—without causing more than a transitory disturbance in function. Having its wires crossed seemed to be no problem for a brain. It was believed that the functional plasticity in the learning process and the very nature of cerebral control could transcend the specifics of brain connections. Neurosurgeons were treating various forms of motor paralysis by cross-connecting shoulder nerves to face nerves, for example, or even arm nerves to leg nerves; while less distant nerve-muscle substitutions were being performed routinely, all on the assumption that somehow orderly function would prevail in the face of the disordered structure.

This concept of a wholesale dynamic plasticity in the organization of brain circuitry was contradicted in some of our early experiments. When more careful experimental controls were instituted, it was found that surgical disarrangements in nerve connections do in fact cause directly corresponding disturbances of sensory and motor function. The disfunction had been missed earlier because the remaining intact portion of the nervous system does everything possible to compensate for the local malfunction of the interchanged parts.

The outcome of the later experiments led shortly to a complete reversal of doctrine. It meant that we no longer had to imagine elusive dynamic properties of cerebral control that were independent of the brain's wiring diagram. Cerebral function can now be seen to be much more closely tied to brain structure and hence much more accessible to scientific investigation.

The idea that our behavior might be partly instinctive or inherited was considered quite intolerable by the majority of psychologists 30 years ago. The very word "instinct" could not be used in professional circles in psychology except in a derisive context. Experiments on nerve development seemed to show that the growth and connection of developing nerve fibers are entirely diffuse and nonselective. Thus, no conceivable means could be imagined by which an inherited behavior pattern could be directly grown into a brain. Extreme, and today laughable, efforts were resorted to in order to show that what seemed to be inherited in behavior was actually a result of experience, environment, and a series of conditioned reflexes and training extending back into fetal development.

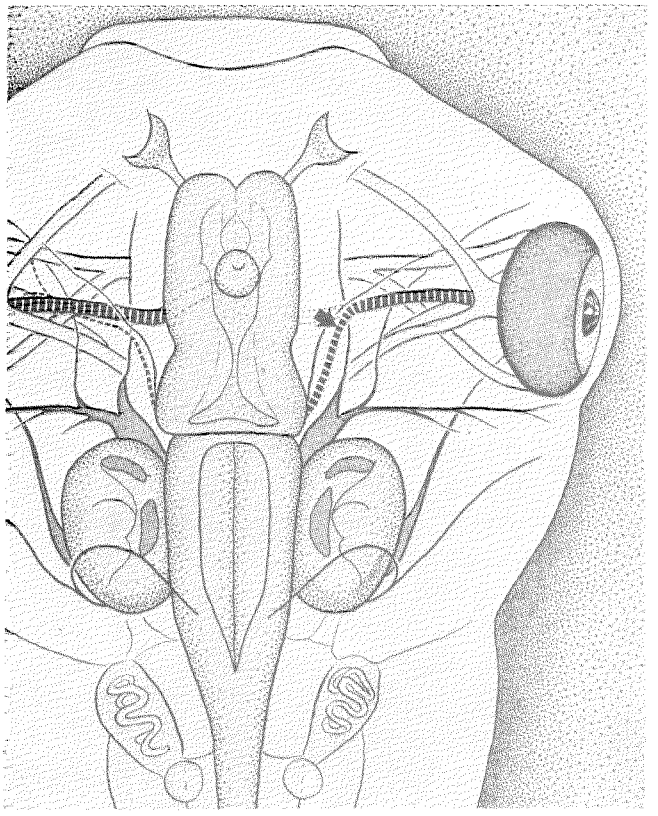
Subsequent experiments have now contradicted the earlier results to show that nerve fibers do indeed grow and connect with the utmost precision within the brain centers, in contrast to what had been seen earlier in the peripheral nervous system and in tissue-culture studies. It is now widely accepted, on the basis of our newer evidence, that

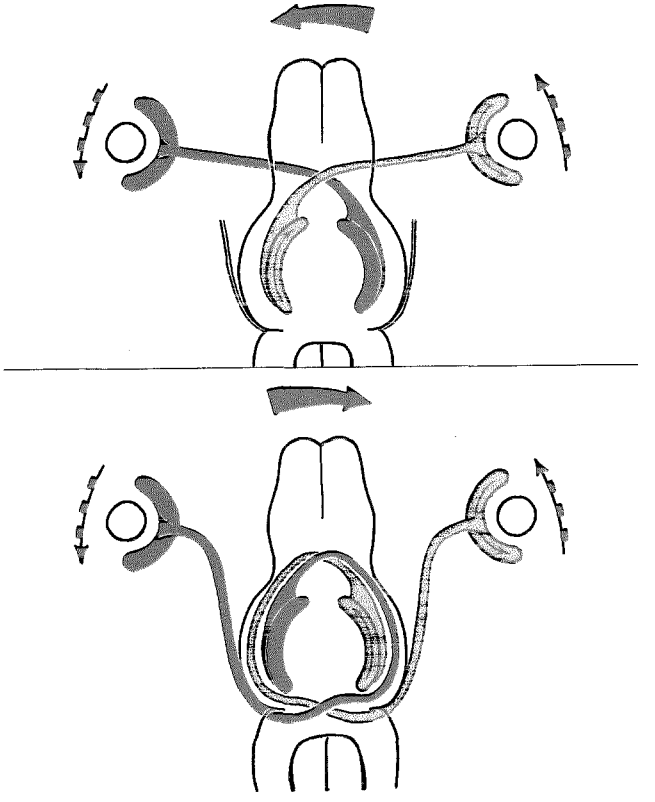
each brain cell is specifically tagged with identifying chemical labels bound to the surface of the cell and that an elaborate chemical guidance system operates in development to insure that each of the millions of brain fibers grows along the proper pathway to reach the proper relay centers to connect with just the proper nerve cells to form adaptive behavior patterns. Some of the most highly intricate and precisely organized connection systems of the brain, like the central pathways and connections that subserve vision, have been found to be laid down in the normal predetermined order by the growth process itself—without aid of function.

The balance of the evidence brings us far from the extreme environmentalist bias of 30 years ago to a position close to the general impressions that prevailed before 20th-century science discovered the "conditioned reflex," "behaviorism," and "nonselectivity in nerve growth." Modern biology now routinely accepts the position of ethology that an entire evolutionary tree can be built in terms of inherited behavior traits just as it can on morphological traits. Psychology and the social sciences, with a somewhat heavier investment in learning and environment, have been slower to relinquish the older environmentalist views.

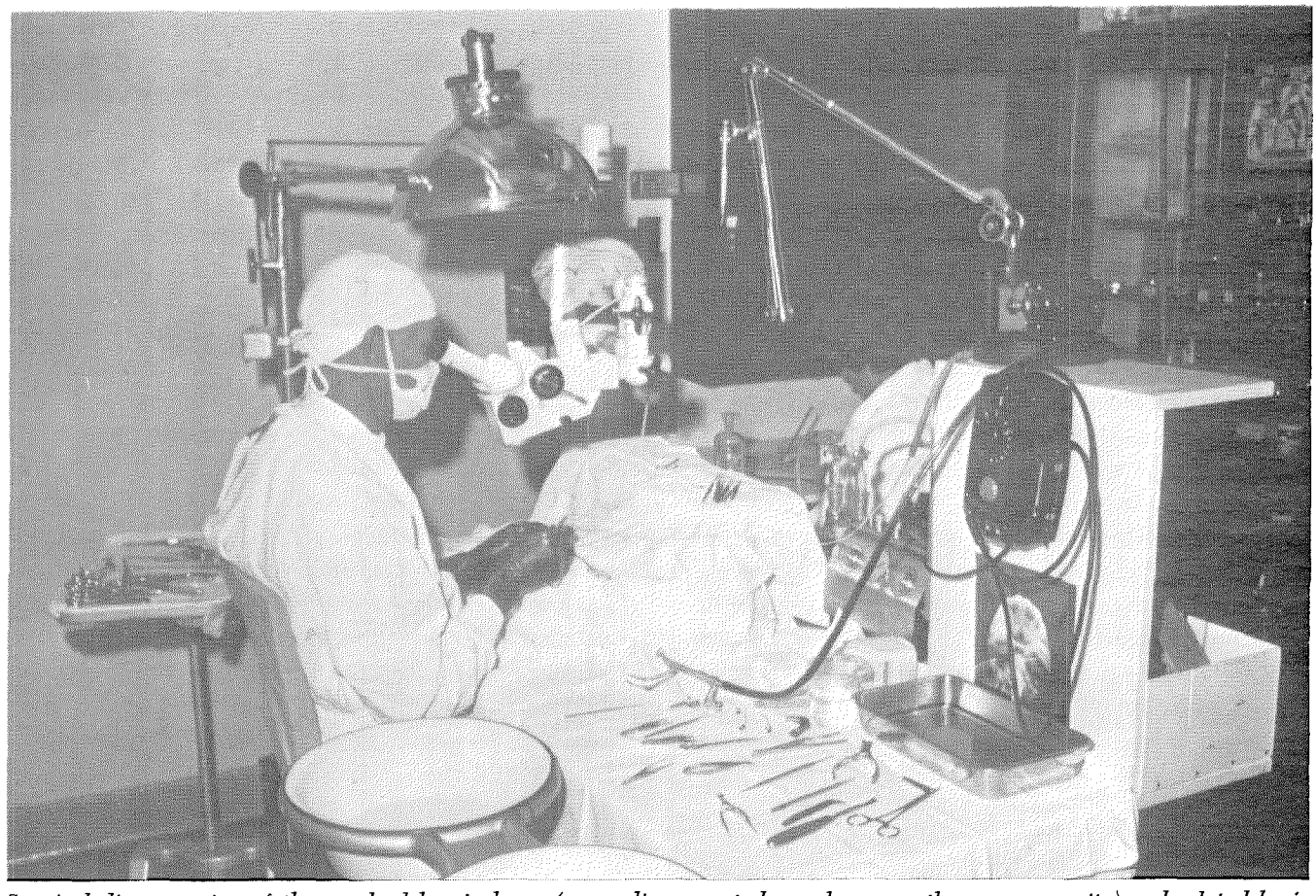
A substantial part of our research in psychobiology is still concerned today with further study of the nature of the developmental mechanisms by which the growing brain gets itself prewired for adaptive function. These studies center around the role of neurospecificity in growth and appear to be headed toward the biochemistry and specificity of macromolecules.

In efforts to account for some of the more challenging of the higher properties of brains, many hypotheses have been proposed regarding the basic nature of the underlying cerebral activity. Some examples are electric field theory to account for Gestalt phenomena in perception; specific nerve energies to account for different qualities in sensation; and reduplicated interference patterns to explain cortical equipotentiality in memory storage. Thus far, however, no single general theory that





Growth properties of the optic nerves are studied in the frog tadpole brain after optic nerves have been cut and deflected into foreign brain pathways (above). Deflected optic fibers methodically grow through roundabout routes to find their appropriate central connection zone in the optic lobe (above). The right and left sides of the brain are not chemically distinct; hence, the deflected nerves often form connections on the wrong side. Visual responses then show right-left reversal (large arrows) to outside movement (small arrows).



Surgical disconnection of the cerebral hemispheres (areas dis connected are shown on the page opposite) and related brain operations are performed on laboratory animals with special surgical procedures that involve use of a high-power stereomicroscope and special instruments, along with standard aseptic precautions.

would much simplify an understanding of the complexities of brain action has survived experimental test. When we attempted to check a proposed role of electric field forces in cortical organization by implantation into the brain of dielectric plates and metallic conductors, the results failed to indicate anything above and beyond the classical type of fiber conduction of nerve impulses. We seem to be forced more and more to think in terms of highly complex, intricate, and precisely organized communication circuits in which neural excitations are generated, rise and fall, and are passed along at high speed from cell to cell within the microscopic fiber networks of the brain.

Persisting holdouts for some kind of unknown controlling forces in the brain that operate independently of discrete fibers and their connections found support in a longstanding observation that the largest and most central cable of fiber connections in the mammalian brain, the *corpus callosum* (once designated as the seat of the soul), could be completely severed in surgery without disturbing mental or physical functions. The *corpus callosum,* containing in man some 200 million fibers, forms a rich system of reciprocal cross-connections between right and left halves of the brain and is the principal channel for communication between the cerebral hemispheres. Even so, people with congenital absence or complete surgical section of the *corpus callosum* were found, most surprisingly, to be quite free of any distinct functional deficits.

This enigma of the *corpus callosum* has been largely resolved in studies of the past ten years—first with animals and later with human patients—in which we have succeeded in demonstrating that a whole series of distinct functional symptoms are indeed produced by section of the *corpus callosum.*

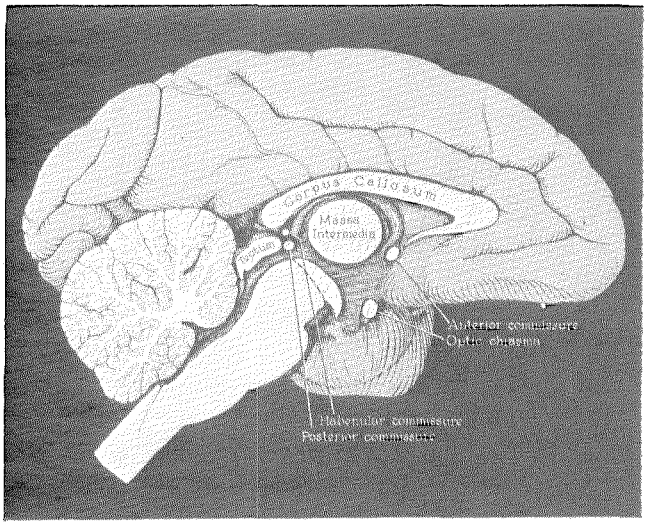
In brief, we find that, although the surgically separated hemispheres do indeed continue to function at high level for most ordinary behavior, they operate independently to a large degree with respect to the higher cerebral activities. In effect,

each hemisphere has a separate mind of its own. Each separated hemisphere, that is, can be shown to have its own private sensations, perceptions, feelings, ideas, memories, and related mental experiences, all of which are out of contact with the corresponding experiences of the other hemisphere. Breakdown in right-left integration, though not apparent in ordinary behavior nor in routine neurological tests, can be demonstrated for a host of sensory-motor performances with tests of appropriate design. Other types of deficits result from the loss of cooperation between the more specialized functions of right and left hemispheres, as in tasks involving language and spatial perception. With the important functional role of this strategic fiber system finally clarified, the close correlation between cerebral function and the underlying fiber pattern seems more firmly established than ever.

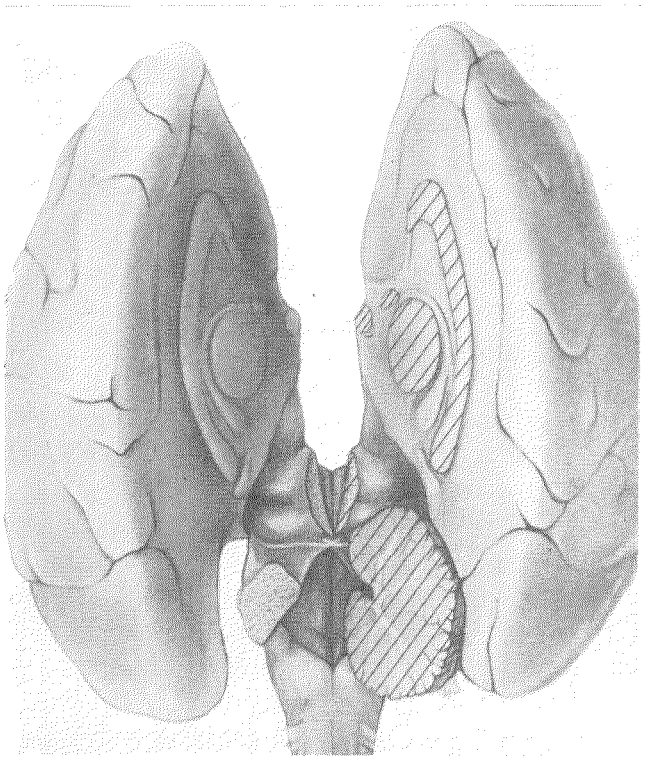
Much of the research of our Caltech laboratory has been centered in recent years around this intriguing twin-brain situation. The two half-brains are mirror images in their anatomy and largely reduplicate each other in activity as well. Each contains most of the main cerebral functions, and each is self-sufficient to a large extent. Accordingly, it becomes feasible, with the hemispheres disconnected, to carry out research with surgical and other analytic procedures in a single hemisphere, leaving the other hemisphere intact for the use of the animal. This approach offers a number of technical advantages and provides as well many new research possibilities. The work is supported by grants from the National Institutes of Health and by the Frank P. Hixon Fund.

In collaboration with Philip J. Vogel of the Neurological Medical Group and Joseph E. Bogen of the Ross-Loos Medical Group in Los Angeles we have been able to study a number of human patients with related neurosurgical operations. These studies lead into complexities involving cerebral dominance and the lateral specialization of function in the human hemispheres.

Recently Ronald Saul of the USC School of Medicine and I did a study on a woman patient in whom the *corpus callosum* was discovered to be totally missing as an anomaly of development. This patient was a 19-year-old college sophomore at the time, with an average scholastic record of B's and C's. Prior to the x-ray diagnosis of her condition she had been considered to be entirely normal. It seemed quite possible that in this person, as in the surgical patients earlier, the symptoms of hemisphere deconnection might have actually been present but had gone undetected. Accordingly, for a year we put her through our entire battery of tests using the same procedures as with the surgical patients. Her



Right and left hemispheres of the brain, largely separate in the natural state, may be completely disconnected by strategic surgical sections through the structures labeled above. The extent of the anatomical separation achieved as seen in the monkey brain is illustrated schematically at the right.



Newly hatched chicks are given electroshock treatment to test for memory consolidation. A mild subconvulsive current applied for only 0.25 seconds will disrupt memory for preceding events up to 30 seconds. After that the memory traces become sufficiently "consolidated" in brain tissue to survive.

performance throughout was quite comparable to that of normal subjects and showed none of the cross-communication deficits that are still pronounced five years after operation in the surgical cases.

The striking difference between the effects of congenital and of surgical separation of the hemispheres is attributed to the special functional plasticity of the developing brain. During that long period from birth to adolescence while the brain is still growing and at the same time learning and remembering, the human brain in particular possesses an enhanced plasticity and a special potency for the shaping of cerebral organization and behavioral patterns that is no longer present after maturation.

The underlying cellular mechanisms responsible for this special plasticity of the still-growing brain remain unknown. On the one hand they would appear to be related to the processes of learning and memory and on the other to those of growth and neural maturation—the two processes overlapping and interacting in some unknown fashion. It is of critical importance to learn more about these underlying mechanisms and their potentialities and limitations because of the direct bearing on problems concerning the effects of early experience on adult behavior—the plasticity of human nature, Head Start programs, and early enrichment of experience.

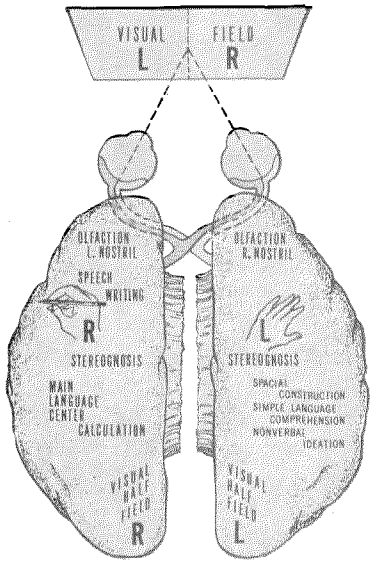
The age-old problem of the memory trace still

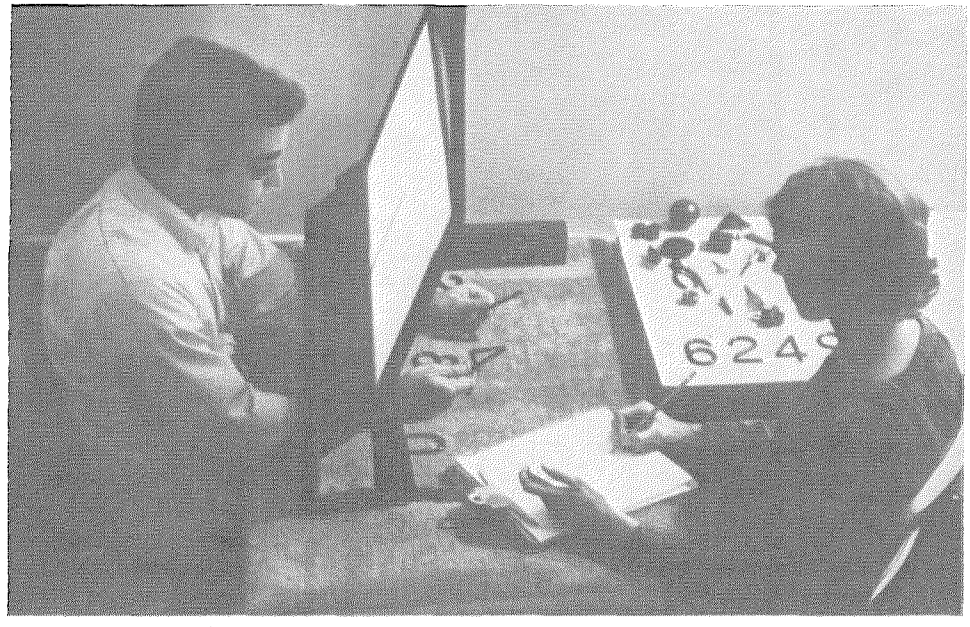
remains unsolved. In recent years there has been a great flurry of studies aimed at the "chemistry of the memory" and the "memory molecule." The answer to the question of the nature of the molecular changes that underlie long-term memory should not be far away. It is important to recognize, however, that any molecular answer promises to leave unexplained many of the phenomena of memory as we think of memory at the behavioral level. It would be like discovering the chemistry of the ink in which a coded message or secret map is inscribed. Many of the more remarkable and intriguing features of memory, such as the orderly filing, the selective retrieval, the information content, etc., seem likely to require an understanding of the broader and more involved complexities of cerebral organization.

Considerable publicity has been directed recently to reports that chemical extracts of trained brains injected into the blood stream, body cavity, or brain ventricles of a naive animal may carry the mnemonic information directly to the naive brain in molecular form where it may serve in place of actual training to guide behavior. Transfer of memory in chemical form has been reported to occur also via cannibalism in flatworm studies. None of these experiments, however, has been confirmed to a point where one can feel any assurance about the phenomenon itself, much less the interpretation.

Clues to the nature of the underlying process by which memory traces are formed in the central nervous system can be obtained by measuring the kinetics for the consolidation of the memory trace. Electroconvulsive shock, brain concussion, or other trauma seem to wipe the brain clean of memories for the immediately preceding experiences, leaving only the more firmly established, better consolidated traces of long-term memory. When electroconvulsive shock is applied at increasingly long intervals after a learning experience, one can determine how long it takes for the particular memory trace to become sufficiently consolidated to resist erasure. This "consolidation time" has recently been measured with new precision by Evelyn Lee-Teng at Caltech. Applying a statistical approach with large numbers of newly hatched chicks she finds the critical time for the chick brain to be about 30 seconds.

As we look ahead in psychobiology, it is difficult to imagine any quick solution to an understanding of the cerebral events that underlie even the simplest mental experience. An understanding of the



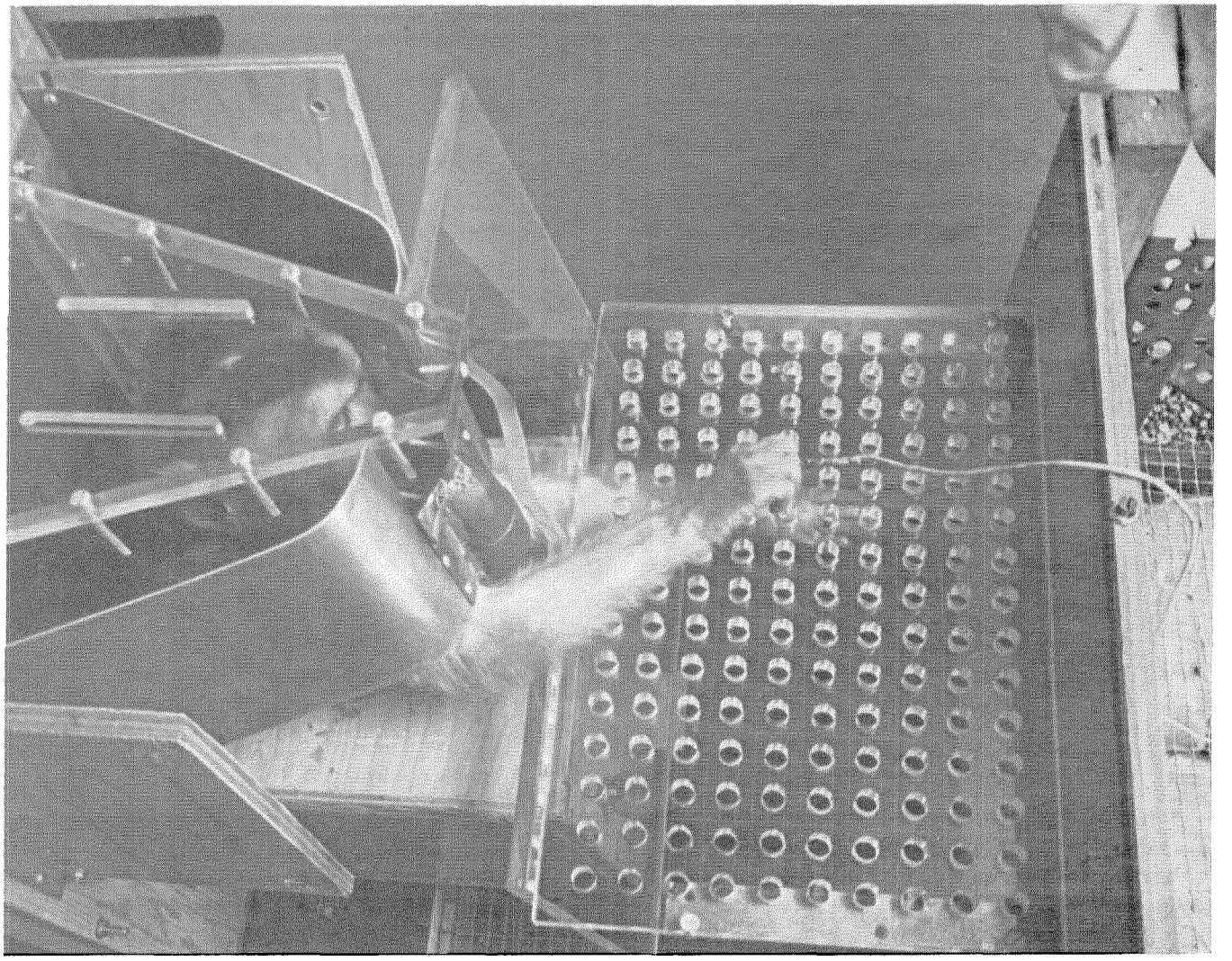


Tests for differential function of right and left hemispheres in human subjects are carried out with strict controls for lateralized sensory input. An upright screen serves for back-projection of visual material and keeps the subject from seeing the test items, his own hands, and the examiner. Diagram at left indicates some of the lateral specialization in cerebral function found in patients whose brain hemispheres have been surgically disconnected (for control of intractable epilepsy). Each hemisphere is simultaneously aware of activities in its own hemisphere but lacks conscious contact with the other hemisphere. Only the left hemisphere can talk or write in most right-handers. Thus the patient can describe what he sees in the right half of his visual field, but not what he sees in the left.

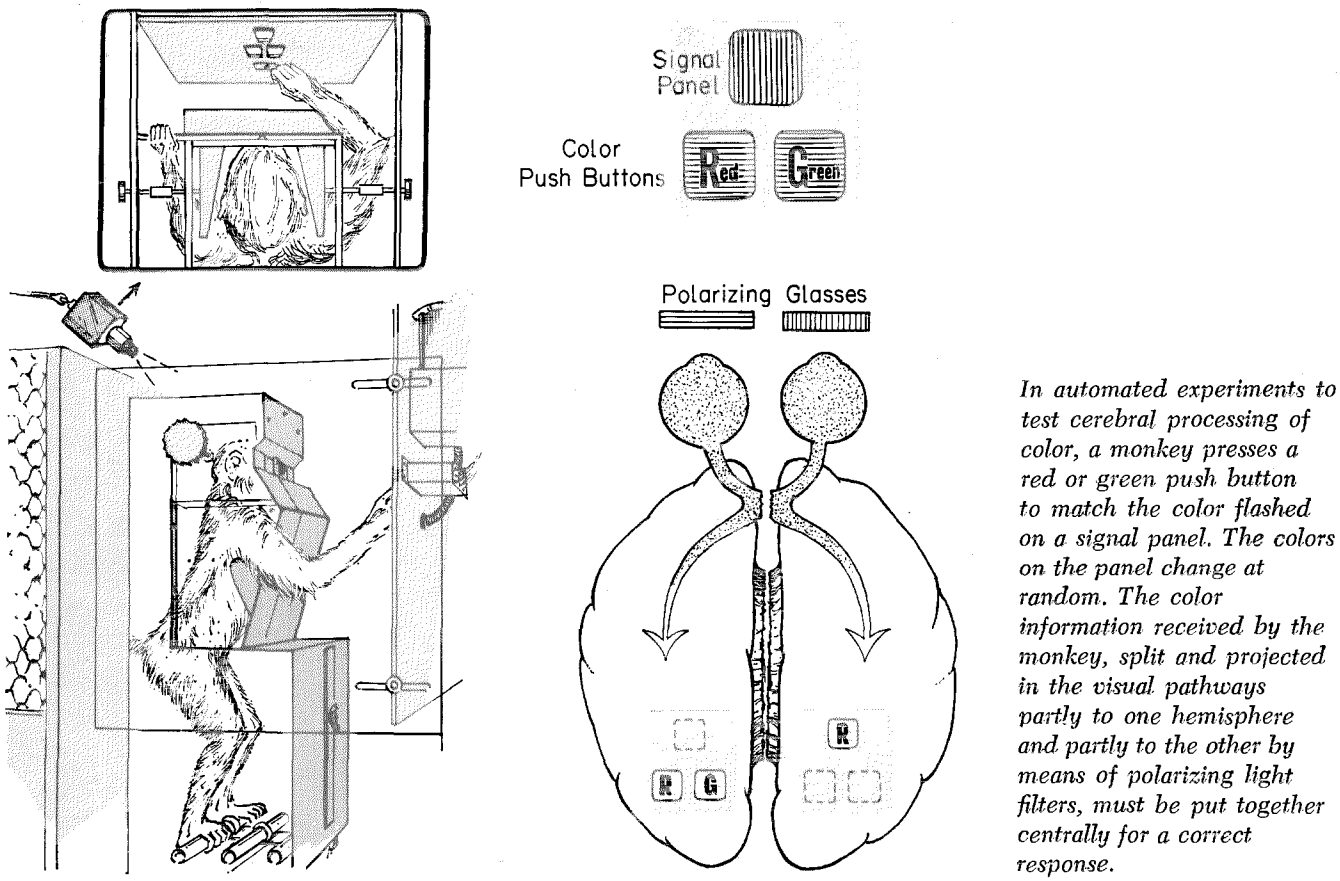
central mechanisms responsible for the subjective perception of color, for example, would seem to require an extensive analysis of highly complex and intricately organized circuitry of microscopic dimensions—attainable, with present methods, only one slow, small step at a time. Even the basic principle by which these circuits produce conscious color sensations remains far out of sight.

Aside from their overwhelming complexity, brains have two properties in particular that make it difficult for many persons to accept behavioral science as a true science: consciousness and free will. What kind of a science can be built around a subjective, will-o-the-wisp-like consciousness that seems to resist even a satisfactory definition? And, equally disturbing, how can one construct scientific laws for the kind of system that, under the most controlled laboratory conditions, reacts to a given invariant stimulus according to its whim of the moment?

The answer of psychology and the behavorial sciences in general has simply been to deny the existence of both consciousness and free will. A purely objective approach is adhered to, with subjective introspective description being strictly excluded. The working premise holds that a complete conceptual model of brain function is possible in purely mechanistic physical and chemical terms without any reference to consciousness. Every choice we make is assumed to have its causes, like



Split-brain monkeys are used to study coordination between right and left hands. This monkey attempts to catch with the lower hand a falling peanut that has been found by touch and poked loose with the upper hand. The two hands are prevented from making any contact. With vision excluded, the location of the target is known only from position-sense in the other hand. The cerebral pathways involved are determined by selective surgery.



In automated experiments to test cerebral processing of color, a monkey presses a red or green push button to match the color flashed on a signal panel. The colors on the panel change at random. The color information received by the monkey, split and projected in the visual pathways partly to one hemisphere and partly to the other by means of polarizing light filters, must be put together centrally for a correct response.

all behavior, in the preceding brain states and surrounding circumstances.

I subscribe to the view that each mental choice is causally determined, but on condition that the phenomena of consciousness are not excluded from the causal sequence. It is my contention that the kind of causality that prevails in brain function must be extended to include mental forces, these being defined as either "emergent" or "entitive" properties of the living, alert brain in action. Like the roundness of a sphere or like the weight, speed, or design of an airplane, these overall "boundary" properties of the brain circuits in action largely control the course and the fate of their inner molecular and other constituents. Similarly these pattern properties of the brain circuitry are subject in principle to objective scientific investigation; it only remains to develop a technology to record them.

Philosophic but not idle, such matters carry important implications for the future place of psychology in scientific institutions. If it proves true that mental phenomena (ideas, sensations, feelings, etc.) are real entities and are potent as controlling factors in the objective chain of command in the brain, it would restore mind and mental phenomena to the domain of experimental science from which they have been largely excluded by the behaviorist movement for over half a century. This change of position would also resolve some of the longstanding disparities between the objective analytic approaches in psychology and the more subjective humanistic and clinical approaches that deal with inner experience and the whole person. Extensive fallout can be seen also with reference to questions concerning human values.

It is apparent that our current thinking on the mind/brain (psycho-biologic) relationship has not advanced beyond a hypothetical answer posed in abstract principle only. The explicit engineering trick by which brain circuits generate conscious properties remains a major guideline problem for the future. The trick may be of such nature that it is impossible to replicate except with living hardware in living brains. On the other hand, it remains possible that conscious awareness is essentially a property of communication networks, in which case consciousness might some day be simulated in the growing technology of artificial intellect. □

AND MUCH MORE TO TELL

The 1968 Annual Report of the Division of Biology contains some 225 research reports by nearly 200 investigators. Clearly the preceding articles represent but a selected sample of the many and diverse researches currently probing the frontiers of biology in the Caltech laboratories.

Professor Albert Tyler has continued his important studies of the event that defines the creation of every individual—the fertilization of the egg. Professor Giuseppe Attardi is actively exploring the functions of the mitochondrial DNA described in Professor Jerome Vinograd's article in this issue, and Professor Daniel McMahon studies the origin and development of these mitochondria and of chloroplasts. Professor Charles Brokaw inquires into the bases of cellular motion: How do cilia beat and flagella propel?

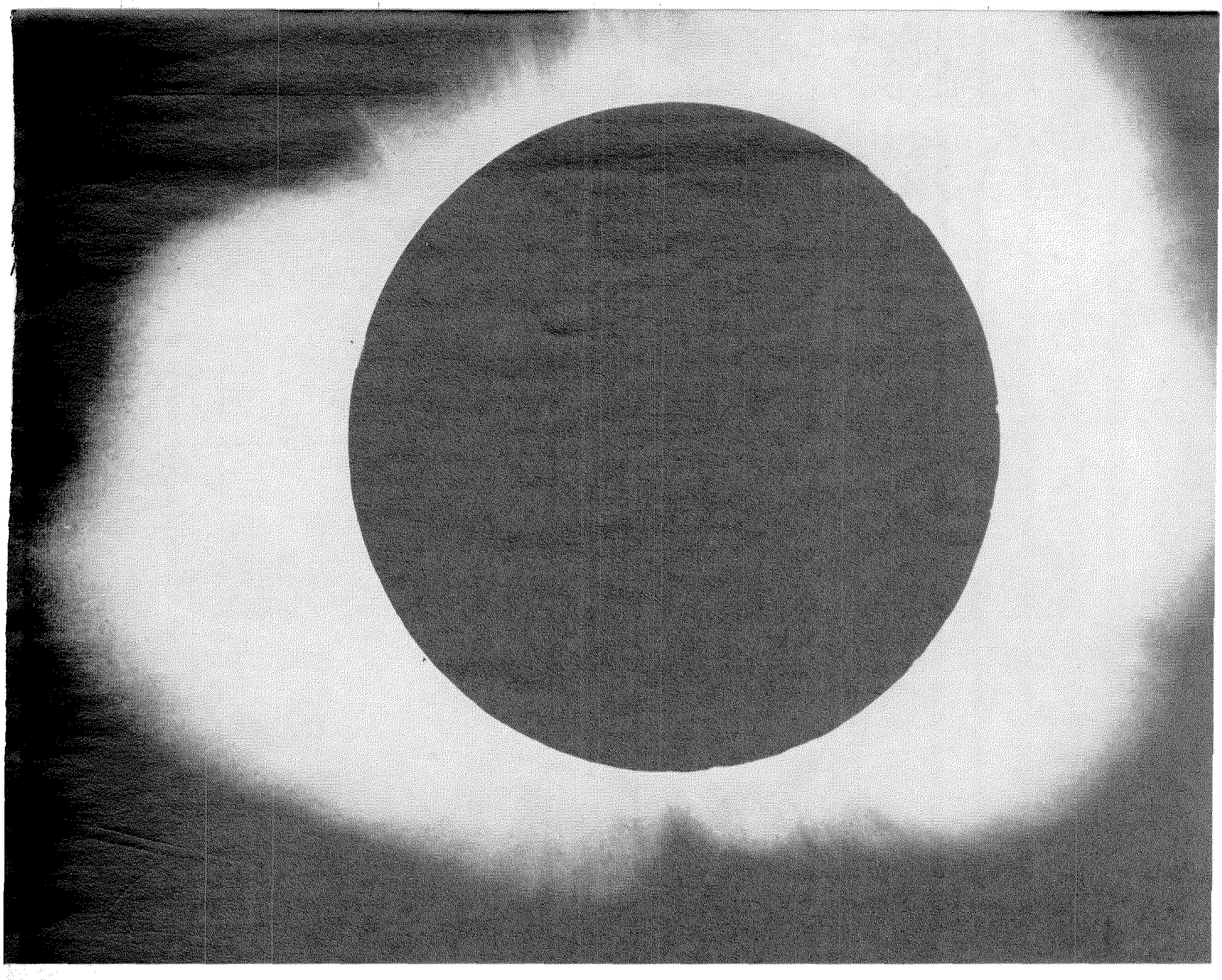
Professor William Dreyer probes the detailed structures of the antibodies that confer immunity and of the proteins that form the protective membranes around all cells, while Professor Robert Sinsheimer analyzes and inquires into the detailed structures of DNA and RNA and the means whereby such molecules can replicate. In addition to gene replication there is gene conversion, and Professor Sterling Emerson has developed special techniques to analyze this process, free of selective bias.

Cell differentiation, based upon differential DNA expression as described in Professor James Bonner's article in this issue, can be studied at many levels. Professor Norman Horowitz seeks to understand the adaptive differentiation represented by the formation of a fruiting body in *Neurospora* in response to certain environmental conditions. Biochemical changes during the development of *Drosophila* are studied by Professor Herschel Mitchell, while Professor Edward Lewis studies the genetic bases of developmental stages in the same organism.

Aplysia, the sea hare, has a central ganglion with large, identifiable neurons, and Professor Felix Strumwasser wonders about the distribution of function among these cells, about the biochemical basis of their rhythmic activities and the origin of their electric pulses. The structural and physiological interactions of neurons in the central nervous system challenge Professor Anthonie van Harreveld. In the visual response to images stabilized on the retina under varied conditions, Professor Derek Fender has developed a powerful tool for the analysis of visual perception.

A diverse group of scientists—using a kaleidoscopic array of techniques to ask a galaxy of questions—they are nonetheless united by their open curiosity about, their fascination with, and their profound respect for the extraordinary phenomena of life.

Professor Albert Tyler's sudden and unexpected death on November 9, 1968, has deprived the biology division of one of its ablest faculty members whose career spanned the entire 40 years of biology at Caltech.



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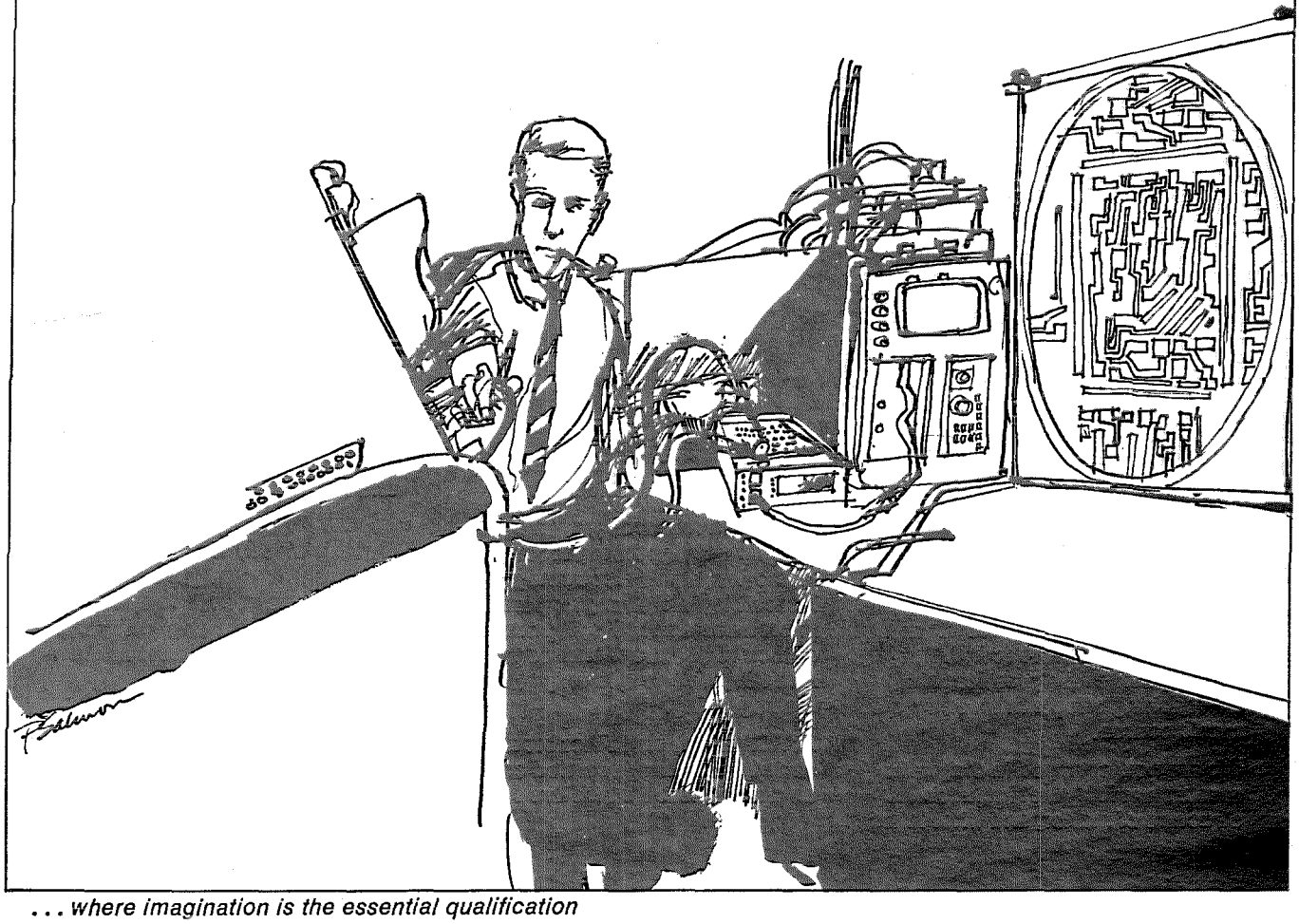

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18 Caltech Lecture Series: Richard H. Jahns, dean, School of Earth Sciences, Stanford—"How Firm Is Terra Firma?" 8:30 p.m.

19 Silent Film Series: "The Iron Horse," directed by John Ford. 8:00 p.m.

25 Caltech Lecture Series: Norman H. Horowitz, professor of biology and chief, bioscience section, JPL—"The Biological Exploration of Mars."

26 Silent Film Series: "Wild and Woolly," starring Douglas Fairbanks, also excerpts from film classics. 8:00 p.m.

December

3 Silent Film Series: "The Big Parade," directed by King Vidor. 8:00 p.m.

7 Leonard Pennario, pianist. 8:30 p.m.

10 Silent Film Series: An evening of comedies. 8:00 p.m.

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1. DATE OF FILING: Sept. 27, 1968
2. TITLE OF PUBLICATION: Engineering and Science Magazine
3. FREQUENCY OF ISSUE: Monthly - October through June
4. LOCATION OF KNOWN OFFICE OF PUBLICATION *(Street, city, county, state, ZIP code)*: 1201 E. California Blvd., L. A. County, Calif. 91109
5. LOCATION OF THE HEADQUARTERS OR GENERAL BUSINESS OFFICES OF THE PUBLISHERS *(Not printers)*: Same
6. NAMES AND ADDRESSES OF PUBLISHER, EDITOR, AND MANAGING EDITOR

PUBLISHER *(Name and address)*: Alumni Assn., California Institute of Technology, Pasadena, Calif.

EDITOR *(Name and address)*: Edward Hutchings Jr., 1201 E. California Blvd., Pasadena, Calif.

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POD Form 3526 *Mar 1968*

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