

Biology Today



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Biology Today



CRM BOOKS

Del Mar, California

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Communications Research Machines,
Inc., Del Mar, California 92014. Library
of Congress Catalog Card Number:
72-176334 Manufactured in the United
States of America
Second Printing

Preface

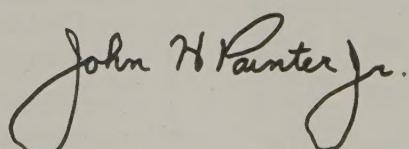
Like religion or politics or group therapy, science is a peculiarly human endeavor that seeks newer and better solutions to the problems plaguing us all. The horizons of science continue to expand as it becomes ever more responsive to social priorities of the day.

The preeminence of the physical sciences in the 1940s was triggered in part by the sense of urgency to end a horrifying world war. The technological means to do so had emerged out of the work of the Rutherfords, the Bohrs, the Einsteins of physics. After the war officially came to a close, many physical scientists found that they could apply their knowledge to biological problems. In exploring the mysteries of atoms, physicists reinforced the interrelatedness of the whole of scientific, and human, endeavor. Their work also provided incredible perceptions for the biological sciences. Before, studies of all living things had floated disconnectedly above the physical and chemical substrate of life, and the biologists were left to map the manifest diversity of it. Armed with perceptions at the molecular level, biology—traditionally a science of

careful and elaborate observation—is now finding solutions to problems of immediate concern.

Many of these advances in biology directly or indirectly involve information processing. Man has access to most of the information in the biosphere. If earth is to be preserved as a habitat for humans and other living things, then humans must better examine, codify, interpret, and apply this information. Watson and Crick's insight into the structure of DNA was a dramatic step forward in our understanding of the basic units of biological information. When we discover how these and other units are rearranged against randomness—how they form an amoeba, a potato, or a man, or by extrapolation, quantum mechanics or a Bach concerto—then we will know better how to utilize this information in problem-solving.

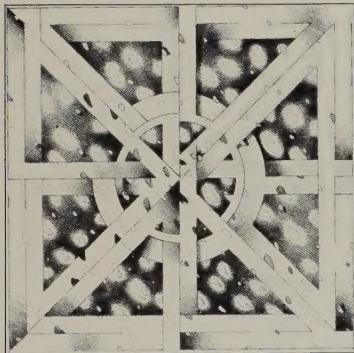
Virology, cancer research, immunology, applied genetics—these are a few of the common denominators in the existing equation of social priorities for biological inquiry. Yet, unhappily, we have come to realize that economic and technological man, reckless of biological balance, threatens the existence of all living things, himself included. Constructive change can only come through intelligent commitment—a commitment that requires an understanding of and reverence for the basic mechanisms of life. The goal of this book is to help convey that essential understanding.



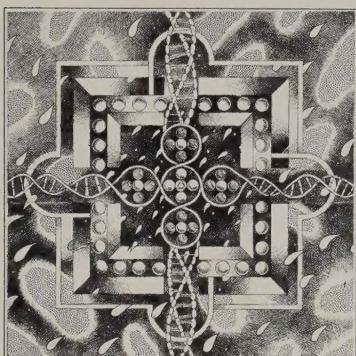
Publisher
Del Mar, California

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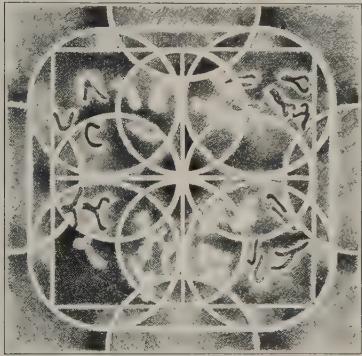
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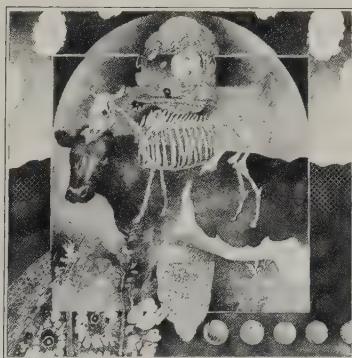
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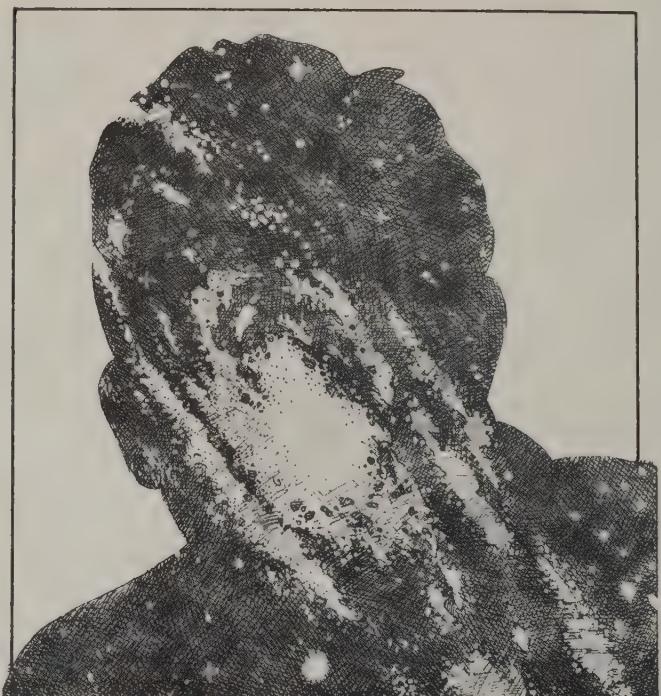
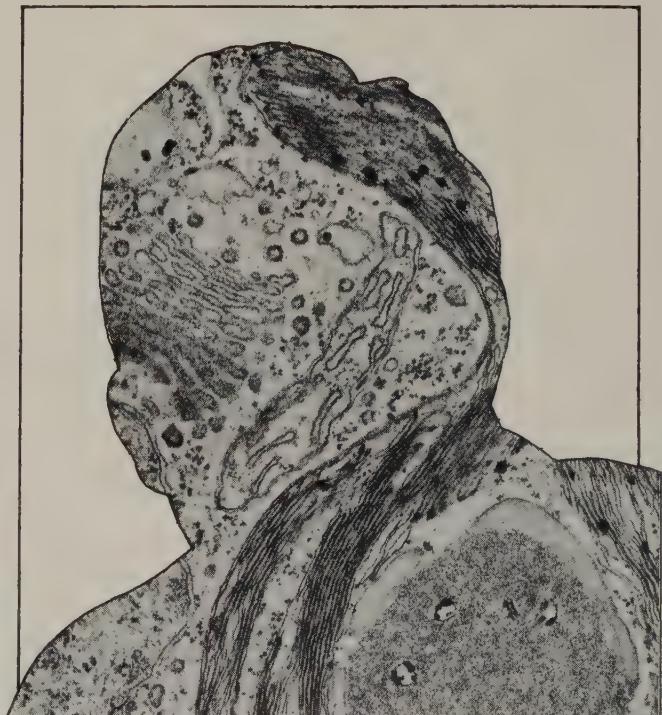
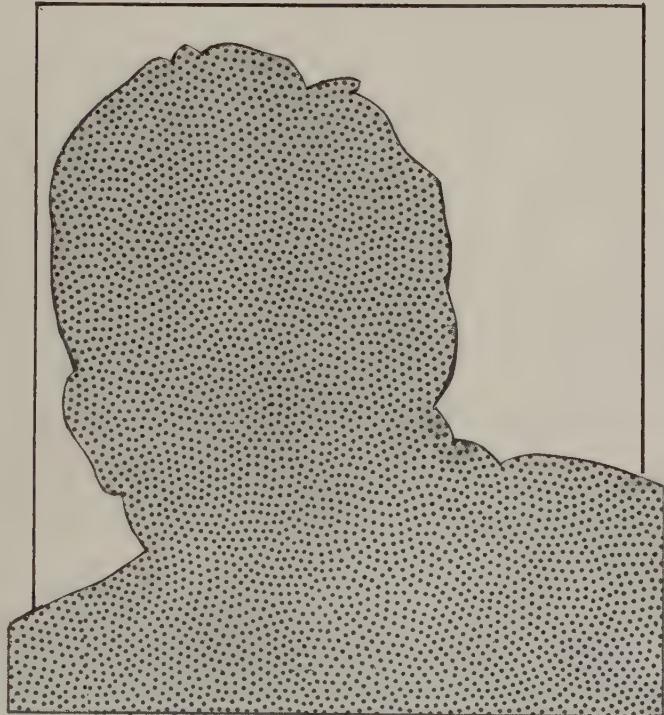
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What Is Life?



*Albert Szent-Györgyi, who considers himself to be an American, was born into a Hungarian family of prominent scientists 77 years ago. He was unwillingly involved in his early life in the two World Wars and in the political intrigues of the big powers engaged in these wars. Throughout his life, he has been quietly searching for the principles on which nature and all of life are organized. In 1937 Szent-Györgyi's search resulted in the isolation of vitamin C, for which he was awarded the Nobel Prize. His protest against man's "idiocy," which he believes is evident in the irrational pursuit of war and politics that characterizes our Western culture, has been capsulized in his two short books, *The Crazy Ape* and *What's Next*. The Crazy Ape warns youth against the gerontocracy that rules the world and urges mankind to take advantage of technological skills in order to create a psychologically and socially progressive world where humanistic values are paramount. To him, research is "not a systematic occupation but an intuitive artistic vocation."*



"What is life?" The question has been asked innumerable times but has been answered to the satisfaction of few. Science is based on the experience that nature gives intelligent answers to intelligent questions. To senseless questions, nature gives senseless answers—or no answers at all. If nature has never provided an answer to this question, perhaps something is wrong with the question.

The question is wrong indeed. It has no sense, for life in itself does



not exist. No one has seen or measured life. Life is always linked to material systems; what man sees and measures are living systems of matter. Life is not a thing to be studied; rather, "being alive" is a quality of some physical systems.

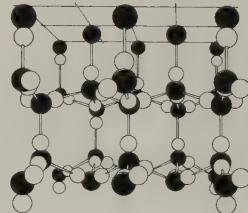
A look at the living world reveals an incredible variety of shapes, sizes, forms, and colors. There seems to be an infinite variability among living systems. How can man approach such complexity? How can he ask intelligent questions?

One key to an intelligent approach may be the simple fact that things can be put together in two different ways: randomly or meaningfully. Things put together in random fashion form a senseless heap. Nine persons selected at random and placed together probably will form nothing more



than a slightly puzzled collection of nine individuals. Nine persons selected and combined in a meaningful fashion may form a championship baseball team. The whole in this case is more than the sum of its parts—it is what is called organization.

If an atomic nucleus is combined with electrons, an atom is formed. This atom is something entirely new, quite different from electrons or nuclei alone. When atoms are combined, molecules are formed. Again, a new thing is generated with strikingly different qualities. Smaller molecules—say, amino acids—may be combined to form a “macromolecule”—perhaps a protein. This macromolecule has a number of amazing qualities. It demonstrates self-organization—the ability to create more complex, higher structures. It may act as an enzyme to speed up a particular



chemical reaction, or it may act as an antibody to neutralize the effects of some other specific protein molecule. Proteins can be created in a literally inexhaustible variety of forms, each with its own qualities.

Macromolecules may be combined to form small “organelles,” such as mitochondria or muscle fibrils. When they are combined, the result is a cell—the unit of life, the miracle of creation—capable of reproduction and of independent existence.

The more complex the system, the more complex its qualities. Organs may be built from cells; from organs may come an individual organism, such as a human being. Individuals in turn may be combined



to form societies or populations, which again have their own rules. At each level of complexity are new qualities not present in the simpler levels. The study of each level yields new information for the biologist.

The history of biology has been marked by a penetration into ever smaller dimensions. In the sixteenth century, Vesalius was dependent on his unaided eyesight for his study of the human body. In the following century, the optical microscope led to the discovery of many new details of structure. Marcello Malpighi observed the capillary vessels that complete the cycle of blood circulation and showed that even such tiny insects as the silkworm have an intricate internal structure.



Anton van Leeuwenhoek described blood cells and the compound eyes of insects. Robert Hooke described the cellular structure of plants.

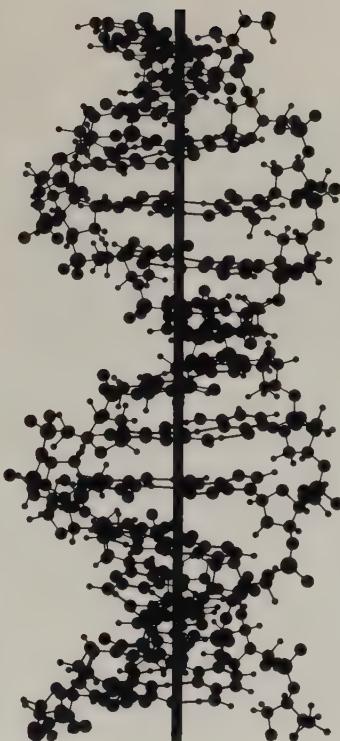
As microscopes were improved, more and more details of structure were described. By the nineteenth century, it was becoming clear that all complex organisms are composed of semi-independent units called cells. The major structural features of cells were established. Bacteria were discovered and studied.

In this century, the electron microscope has taken the scientist down to molecular dimensions, and he has learned to observe with x-rays as well as with visible light. Organic chemistry was established in the nineteenth century, and by the beginning of this century, it was clear that this approach could be applied to the study of living systems. Biologists have had to learn



a new anatomy—the anatomy of molecules. Chemists and physicists have penetrated the atom, first finding the elementary particles and then moving still deeper into the realm of wave mechanics. The discovery of the wave properties of the electron has given a deep insight into the nature of biological reactions.

As scientists attempt to understand a living system, they move down from dimension to



dimension, from one level of complexity to the next lower level. I followed this course in my own studies. I moved from anatomy to the study of tissues, then to electron microscopy and chemistry, and finally to quantum mechanics. This downward journey through the scale of dimensions has its irony, for in my search for the secret of life, I ended up with atoms and electrons, which have no life at all. Somewhere along the line, life has run out through my fingers. So, in my old age, I am now retracing my steps, trying to fight my way back toward the cell.

I have concluded that life is not linked to any particular unit; it is the expression of the harmonious collaboration of all. As I descended through the levels of complexity, I studied simpler units and found myself speaking more and more in the language of chemistry and physics.

J. F. Danielli has shown that the subcellular organs of various cells are interchangeable. They can be transferred from one cell to another, much as organs can be transplanted from one human individual to another. The parts of the cell have no individuality. The



quality of individuality resides in the higher organization—in the cell or the individual.

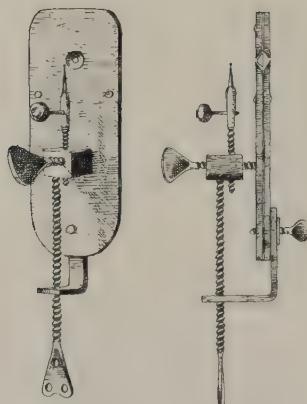
No one yet knows the higher principle that holds a cell together. Perhaps the answer will be found in irreversible thermodynamics. The good working order of a living cell may correspond to a stable state with a high probability of occurrence. Perhaps some new principle—as yet undiscovered—keeps the cell together. Living systems do not only maintain their good working order but they all tend to improve it, to make the working structure more complex. When the fundamental principle that holds the cell together is found, perhaps we



will then also understand what brought together the first living system and understand what drives living systems toward self-perfection.

Scientists know today that rather complex molecules — amino acids, nucleic bases, even macromolecules — can under certain conditions be built without intervention of living systems. They are still seeking the principle that brought them together for the first time and that makes these systems improve themselves by building more complex structures, capable of more complex functions.

Biology is a very young science. It has called itself a separate science for only some eight decades. No one



can expect it to find the answers to all questions. The most important questions are yet unanswered — perhaps unasked. What biologists can do — what they are doing at present — is to ask questions that seem answerable with present techniques. They ask questions about structure and function, from the nature of consciousness down to the behavior of electrons, hoping that some day all of this detailed knowledge will come together in a deeper understanding.

Perhaps some day they will find a new way of looking at things. The best scientists, with the aid of giant computers, cannot yet fully explain the behavior of three electrons moving within an atom. Yet those three electrons — even dozens of electrons — know exactly what to do and never miss. In the essence, nature may be far simpler than is believed.

To see the solutions, scientists must preserve a certain naïveté,



childish simplicity of the mind, an ability to recognize a miracle when they see it every day. The solution may be far closer than it seems. It was a hundred years ago that H. P. Bowditch, one of the first American physiologists, showed that after a frog's heart has been stopped for a while, its first beats are rather weak. The heart gradually regains its original strength, with the record of the heartbeat rising like a series of

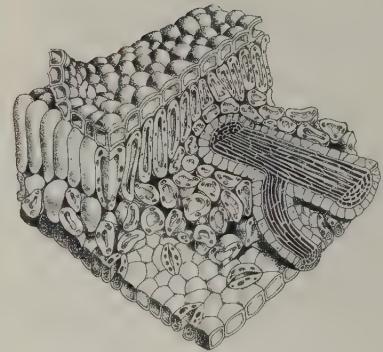


stair steps. Bowditch called this phenomenon "the staircase."

One might expect the heart to be stronger after a rest. Yet what is observed here can be considered a general quality of living systems. Life generates life: rest or inactivity causes life to fade away. Muscles weaken if they are not used; they become stronger if exercised regularly.

This principle is one of self-organization, one of the most striking differences between a living system and a nonliving one. A machine is worn out by usage. A living system is worn out by inactivity. Living systems are able to organize and improve themselves.

S. J. Hajdu and I tried to discover the mechanism that produced the Bowditch staircase. We found that potassium leaks out from the heart fibers into the surrounding fluid



when the heart is not beating. When the heart resumes its activity, the potassium is pumped back into the heart fibers. The change in strength of the heartbeats is caused by this change in the distribution of potassium. The movement of the potassium back into the fibers, against the potassium concentration gradient, increases the amount of organization or order in the system. The entropy of the system is decreased. Ernst Schrödinger in 1944 suggested that the ability to decrease entropy is the most characteristic feature of living systems. In a living system, the decrease of entropy leads to further decrease of entropy, to greater order. The increase of



entropy leads to further increase of entropy, the maximum state of entropy being death.

Further pursuit of these thoughts would lead into abstract speculation. I would like to point out, however, that these abstruse questions are not at all far from the sickbeds of suffering patients. One of our most important drugs is digitalis, which is used to stimulate failing hearts. Hajdu and I showed that digitalis helps the heart to pump potassium back into its fibers and to retain it. The inability of a heart to

maintain its potassium concentration may be one cause of its failure.

Living systems are clearly different from nonliving systems. There must therefore be a difference in the way these two kinds of systems are thought about. Physics is undoubtedly the most basic science. In a way, biology is only an applied science, applying physics as a tool for the analysis of living systems. However, there are distinctions between physical and biological events.

Suppose a process, left to itself, is likely to occur 999 times in one way for each time that it occurs in another. The physicist concerns himself primarily with the first way. Physics is the science of the probable.

Biology is the science of the improbable. On principle, biological reactions must be improbable. If man's cells worked only through the probable reactions, they would soon run down. In order to regulate itself, a cell must use improbable reactions and must make them take place through very specific tricks. The cell may make use of just that one way in a thousand that the physicist ignores.



The cell finds a way to make the reaction go at just the right moment and at the desired rate. The reaction may be improbable, but if it is thermodynamically possible, the cell will find a way to use it.

Physically, all of us—you and I—are improbable. The probability of atoms happening to come together in the complex structure that makes up my body is so tiny that it is practically equal to zero.

Another difference between the physical and biological approaches to the study of a reaction lies in the matter of isolation. The physicist is apt to attempt to isolate the reaction he wishes to study. In biology, single reactions are rarely encountered. Most biological reactions are parts of



complex chains. They cannot be fully understood except as members of the chain, or even as parts of an entire living system—the living biological entity.

For example, one of the most important biological reactions is the “electron flow” that underlies photosynthesis and biological oxidation. These processes generate the energy that keeps living systems going. In these reactions, electrons “flow” from molecule to molecule. If an electron moves from molecule A to molecule B, it leaves a positive electric charge on molecule A. This charge tends to pull the electron back toward A. Electrons could not move against such a strong electrostatic attraction. However, if molecule A is a member of a chain and simultaneously receives an electron from a third molecule, its positive charge will be neutralized. There will then be nothing to pull



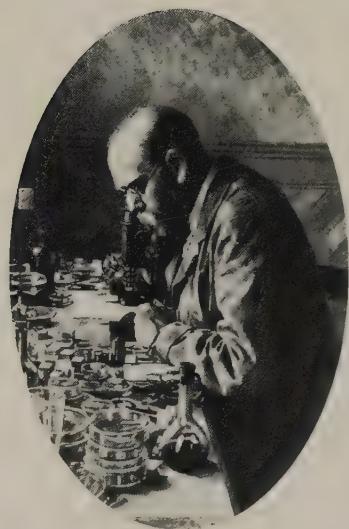
the electron back toward A. Thus, where electrons cannot move from A to B in an isolated system, they can “flow” without difficulty if A and B are members of a chain.

Like many biological researchers, I have often worked for long periods, using all the tricks of chemistry, wave mechanics, and mathematics to understand a certain reaction. In the end, I have found that the cell carries out this reaction in the only way that it could be accomplished. In my long research career, one of the greatest mysteries to me was the

way in which a living cell—without the aid of computers or even a brain—could find this single path to the necessary result.

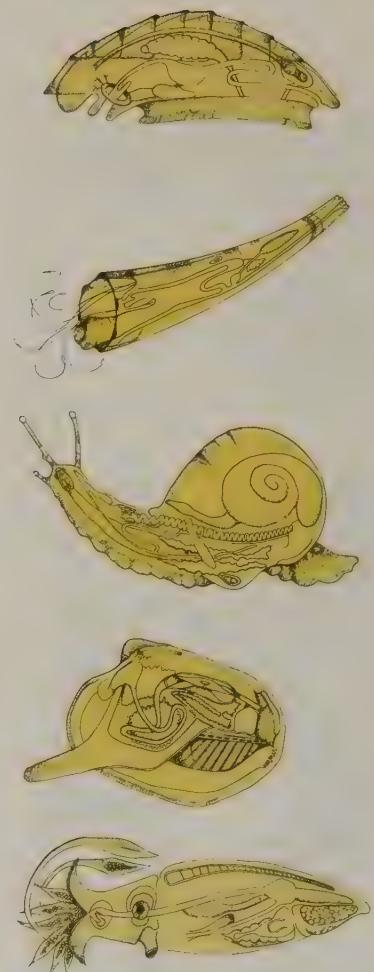
In one way, the discoveries of genetics have made our understanding of evolution even more difficult. A cell does not directly alter the molecules in evolution. Instead, it alters the code of the nucleic acid in its chromosomes. Then all of the descendants of that cell make the appropriate changes in the molecules involved in the reaction. In most cases, a number of genes must be altered to accomplish a meaningful change in a chemical reaction. If all of these genes were not changed simultaneously, only confusion would result.

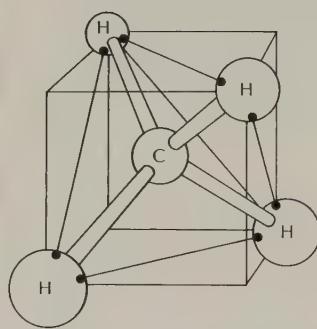
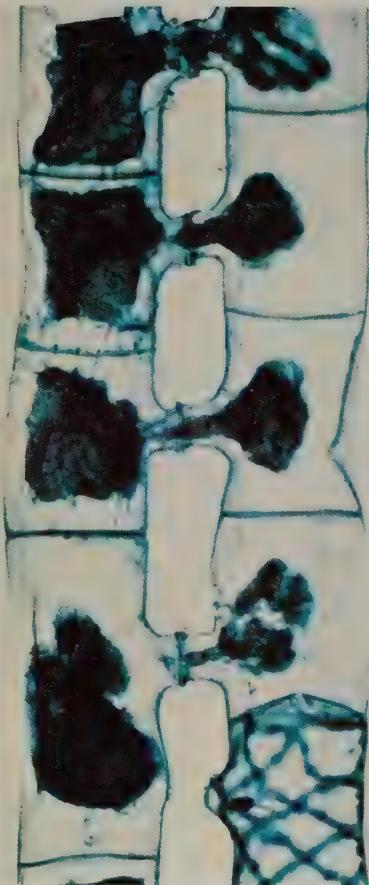
According to present ideas, this change in the nucleic acid is accomplished through random variation. The nature of protein molecules formed in the cell is determined by the code of the nucleic acid. The protein cannot alter the nucleic acid. If I were trying to pass a biology examination, I would vigorously support this theory. Yet in my mind I have never been able to accept fully the idea



that adaptation and the harmonious building of those complex biological systems, involving simultaneous changes in thousands of genes, are the results of molecular accidents.

The feeding of babies, for example, involves very complex reflexes. These reflexes require extremely complex mechanisms, both in the baby and in the mother, which must be tuned to one another. Similar mechanisms are involved in the sexual functions of male and female animals. These mechanisms must be tuned precisely to one another in order to achieve successful copulation. Thousands of genes must be involved in the coding of these mechanisms. The probability that all of these genes should have changed together through random variation is practically zero, even considering that millions or billions of years may have been available for the changes.





I have always been seeking some higher organizing principle that is leading the living system toward improvement and adaptation. I know this is biological heresy. It may be ignorance as well. Yet I think often of my student days, when we biologists knew practically nothing. There was then no quantum theory, no atomic nucleus, and no double helix. We knew only a little about a few amino acids and sugars. All the same, we felt obliged to explain life. If someone ventured to call our knowledge inadequate, we scornfully dismissed him as a "vitalist."

Today also we feel compelled to explain everything in terms of our



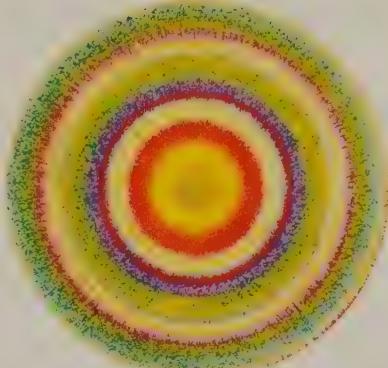
present knowledge. Identical twins are often exactly alike in the smallest details of physical appearance, indicating that the instructions for building this entire structure must have been encoded in the genetic materials that they share. All the same, I have the greatest difficulty in imagining that the extremely complex structure of the central nervous system could be totally described in the genetic codes. Thousands of nerve fibers grow for long distances in order to find the nerve cell with which they can make a meaningful junction. Surely the nucleic acid did not contain a blueprint of this entire network. Rather, it must have contained instructions that gave the nerve fiber the "wisdom" to search for and locate the only nerve cell with which it could make a meaningful connection. Perhaps this guiding principle also is related to the way in which the first living system came together.

I do not think that the extremely complex speech center of the human brain, involving a network



formed by thousands of nerve cells and fibers, was created by random mutations that happened to improve the chances of survival of individuals. I must believe that man built a speech center when he had something to say, and he developed the structure of this center to higher complexity as he had more and more to say. I cannot accept the notion that this capacity arose through random alterations, relying on the survival of the fittest. I believe that some principle must have guided the development toward the kind of speech center that was needed.

Walter B. Cannon, the greatest of American physiologists, often spoke of the "wisdom of the body." I doubt whether he could have given a more scientific definition of this "wisdom." He probably had in mind some guiding principle, driving life toward harmonious function, toward self-improvement.



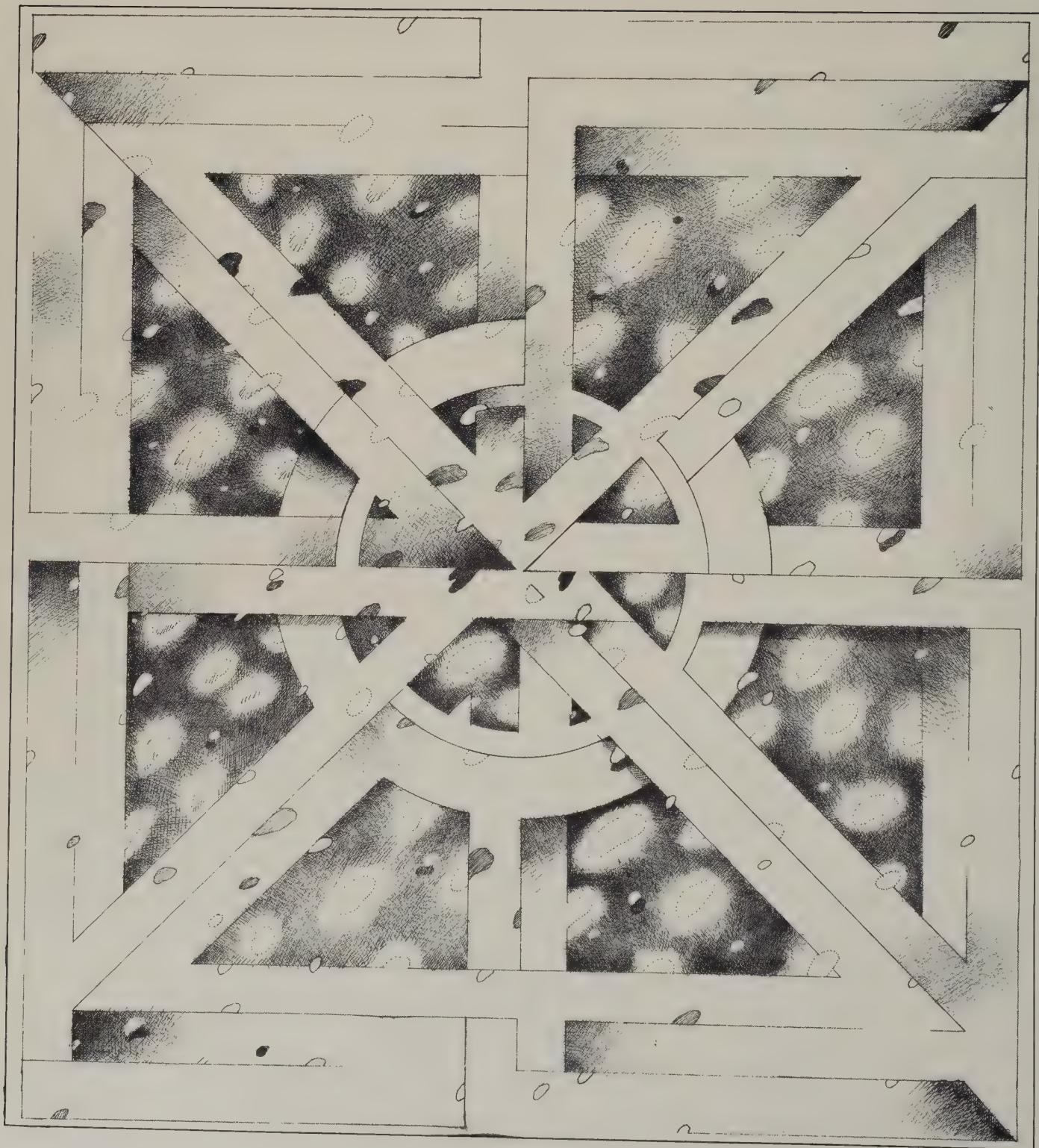
Life is a wondrous phenomenon. I can only hope that some day man will achieve a deeper insight into its nature and its guiding principles and will be able to express them in more exact terms. It is this mysterious quality of life that makes biology the most fascinating of sciences. To express the marvels of nature in the language of science is one of man's noblest endeavors. I see no reason to expect the completion of that task within the near future.



and here we are

— Albert Szent-Györgyi
Woods Hole, Massachusetts

Biology Today



The Study of Life

Life is unified in two ways. In the first place, organisms are dependent upon one another for the chemical substances which are essential to their existence, and each type of organism plays a part in maintaining the entire world of life as a going concern.

In the second place, all organisms are related to one another through a long line of evolutionary descent. A billion years ago or more, the first organisms appeared upon the earth. All the evidence indicates that they were simpler than even the humblest of the organisms with which we may become acquainted by viewing them through the most powerful microscopes. Through the long aeons that have passed since that time, the descendants of those organisms have developed through evolution to become the myriad of living forms both great and small which populate the earth today.

— Clarence W. Young (1938)

1

Vitalists and Mechanists



Biology is often defined as "the study of life"—but biology has never succeeded in proposing a simple definition of life. In fact, biology today is tending toward the view that there is no sharp dividing line between living and nonliving systems.

Francis H. C. Crick, who shared the 1962 Nobel Prize for his work in discovering the molecular structure of deoxyribonucleic acid (DNA), has said: "It is notoriously difficult to define the word 'living.' In many cases we all know whether something is alive or dead. You are alive; cats and dogs are alive; whereas a rock or a pane of glass is dead. But the word 'dead' is a bad one, because it half implies that the object was once alive and is now dead. It is interesting that there is no *simple* word for something that is not alive and never has been" (Crick, 1966).

In the past, biologists argued that living things are alive because they contain a "vital force." Few modern biologists, if any, would count themselves as vitalists. Crick continues: "Vitalism implies that there is some special force directing the growth or behavior of living systems which cannot be understood by our ordinary notions of physics and chemistry. Exact knowledge is the enemy of vitalism."

Crick is an outstanding example of the school of biological thought that has largely replaced vitalism. This new group of biologists believe that living as well as nonliving systems can be explained by physical and chemical laws. Because this view implies that biology eventually can be reduced to statements about physics and chemistry, its adherents often are called reductionists or mechanists.

Although most modern biologists hold that living systems operate according to basic laws of chemistry and physics, they also recognize that living systems are distinguished by their incredible complexity. In fact, Crick points out, "It is essentially true that *all* very highly ordered complex objects are biological." Complexity and organization are the features most characteristic of living systems. They are so complex and highly organized that in many ways they seem to have properties quite different from those of simpler, nonliving systems.

Not many years ago, cynics were fond of pointing out that a man is made up of nothing more than 97 cents' worth of chemicals. Since then the price of the chemicals has risen, but the statement can be criticized for other reasons as well. Obviously, a human being is not a random heap of simple chemicals, any more than a painting by Picasso or Van Gogh is a random splash of a few pennies' worth of paint. The unique properties of a living system are due primarily to the organization of its chemical components.

When chemists began to study the properties of matter, they quickly realized that the substances in living systems are quite different from those in the nonliving world. The substances that make up the earth, sea, and air are relatively stable. Water can be frozen to ice or boiled to vapor, but it can always be returned to its original liquid form by reversing the procedure that changed it. Metals, salts, and other minerals also can be melted, even vaporized, but will recrystallize in their original forms when cooled. On the other hand, most of the substances making up living systems are very unstable. When materials such as wood and sugar are heated, they char and burn. These processes are not readily reversible; the ashes and smoke that result cannot be restored to the original material by cooling or by any simple chemical or physical process.

In 1807 a Swedish chemist, Jöns Jakob Berzelius, suggested that the

Figure 1.1 (left). Illustration depicting the costume worn by physicians during the Marseilles plague in 1720.

Figure 1.2 (right). A modern model of the cell.



substances derived from living systems be called *organic* substances; all other chemicals he called *inorganic* substances. Although organic substances can be easily converted into inorganic ones (through burning, for example), Berzelius found it impossible to create organic substances from inorganic ones. He suggested that only living systems have the power to produce organic substances.

Alchemists and early chemists had long recognized that all matter is made up of a limited number of simple substances called *elements*. The organic substances proved to be composed almost entirely of only four elements: carbon, oxygen, hydrogen, and nitrogen—elements that also are found in inorganic substances. In fact, analysis of organic substances revealed that they contain no unique material substances; any element that is found in organic substances also is found in inorganic matter. Most early

biologists therefore concluded that the unique properties of living systems are caused by some “vital force” that can be imparted to matter only by another living system.

VITALISM AND SPONTANEOUS GENERATION

From the early Greek philosophers through the natural philosophers of the seventeenth century, most educated or observant men believed that living organisms are generated spontaneously from nonliving matter. Frogs and insects emerge from the mud after a spring rain. Maggots or worms arise spontaneously in decaying meat. In 1668 this belief was challenged through a series of brilliant experiments performed by Francesco Redi, an Italian poet, physician, and naturalist. Redi’s experiments still are cited as examples of careful and valid scientific investigation (Interleaf 1.1). Redi showed that worms arise in decaying meat only if adult insects are able to lay their eggs in the meat. Other researchers soon showed that aphids, fleas, lice, and the larvae in plant galls are produced only from eggs laid by adult animals.

For a short time, the theory of spontaneous generation of living creatures was discredited. Then in 1677 Anton van Leeuwenhoek, using hand-ground lenses, discovered organisms of microscopic size. Microscopic examination of any sample of broth or water revealed a teeming population of microorganisms. These microorganisms—bacteria, yeasts, and protozoans—are so much smaller and simpler in appearance than other organisms that their spontaneous generation seemed quite plausible.

For two centuries, controversy raged about the spontaneous origin of microorganisms. Some experimenters showed that filtering, boiling, or chemically treating samples of broth or water killed the microorganisms. Other experimenters, however, showed that the microorganisms reappeared in many cases despite the most careful attempts to avoid contamination by living microorganisms.

Finally, in the early nineteenth century, Lazzaro Spallanzani and Theodor Schwann showed that organisms do not appear in a broth that has been

Figure 1.3 (right). Anton van Leeuwenhoek’s hand-ground lenses.

Figure 1.4 (lower left). Lazzaro Spallanzani.

Figure 1.5 (lower right). Theodor Schwann.

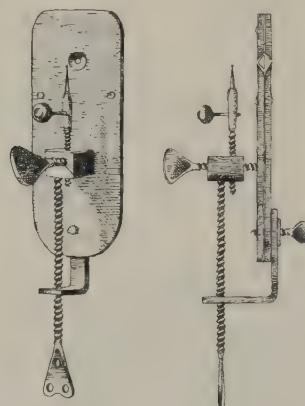


Figure 1.6. Francesco Redi.



Interleaf 11
**FRANCESCO REDI'S
EXPERIMENTS ON SPONTANEOUS
GENERATION OF INSECTS**

Francesco Redi (1621–1697) of Italy was a distinguished scholar, philologist, physician, and poet, as well as a naturalist of wide interests. As a scientist, he is best known for a series of experiments in which he showed quite conclusively that maggots are not spontaneously generated in decaying meat. Redi set out in 1668 to test this hypothesis experimentally. At the time, there was little clear understanding among scientists of the importance of experimentation or of the best way in which to carry out experiments. Redi's work is of importance not only for his conclusions but because he designed his experiments so carefully that there could be little doubt of the correctness of his conclusions. The quotations from Redi's report that follow are taken from the English translation by Bigelow (1909).

Redi began with a simple observation to test the truth of the ancient belief that "the putrescence of a dead body, or the filth of any sort of decayed matter engenders worms." He obtained three dead snakes ("the kind called eels of Aesulapius") and put them in an open box to decay. "Not long afterwards I saw that they were covered with worms of a conical shape and apparently without legs. These worms were intent on devouring the meat, increasing meanwhile in size, and from day to day I observed that they likewise increased in number; but although of the same shape, they differed in size, having been born on different days."

After the meat had been consumed by the worms, leaving only the bones, the worms disappeared, apparently escaping from the box. To discover what happened to the worms, Redi repeated the experiment, this time carefully sealing all holes through which the worms might escape. After three days, when the decaying meat became covered with worms, Redi noted this time that there were two different kinds of worms alike in form, but one kind large and white and the other kind smaller and pink. When the meat was gone, the worms tried to escape from the box but were unable to do so. "On the nineteenth day of the same month some of the worms ceased all movements, as if they were asleep, and appeared to shrink and gradually to assume a shape like an egg. On the twentieth day all the worms had assumed the egg shape, and had taken on a golden white color, turning to red, which some darkened, becoming almost black. At this point the red, as well as the black ones, changed from soft to hard, resembling somewhat those chrysalids formed by caterpillars, silkworms, and similar insects."

Redi separated the red and black egg-shape objects and put them in glass vessels sealed with paper. After eight days, each of the red objects broke open "and from each came forth a fly of grey color, torpid and dull, misshapen as if half finished, with closed wings; but after a few minutes they commenced to unfold and to expand in exact proportion to the tiny body which also in the meantime had acquired symmetry in all its parts. Then the whole creature, as if made anew, having lost its grey color, took on a most brilliant and vivid green; and the whole body had expanded and grown so that it seemed incredible that it could ever have been contained in the small shell." The black objects broke open after 14 days "to produce certain large black flies striped with white, having a hairy abdomen, of the kind that we see daily buzzing about the butcher's stalls."

Redi concluded from these observations that the worms are the immature forms of flies and "began to believe that all worms found in meat were derived directly from the droppings of flies, and not from the putrefaction of the meat." He remembered seeing both the green and the large black flies hovering over the meat before the worms appeared. To test this new hypothesis, Redi set up another experiment, for "belief would be in vain without the confirmation of experiment."

He prepared four large, wide-mouth flasks, each containing a different kind of meat: snake, fish, eels, and milk-fed veal. Each of the flasks was carefully closed and sealed. Then Redi prepared an identical set of flasks, but he left this set open. Within a few days, the meat in the open flasks became covered with worms, and flies were seen freely entering and leaving the open flasks. However, "in the closed flasks I did not see a worm, though many days had passed since the dead flesh had been put in them. Outside on the paper cover there was now and then a deposit, or a maggot that eagerly sought some crevice by which to enter and obtain nourishment. Meanwhile the different things placed in the flasks had become putrid."

This experiment is noteworthy because it represents one of the earliest examples of the deliberate use of a control group in a biological experiment. Redi prepared two identical sets of flasks; one set was open and the other set was sealed. By using various kinds of meat, Redi was able to test the effect of another variable. His results showed that the appearance of worms occurred only in open flasks but was not dependent upon the kind of meat used. Redi repeated his experiment many times, using different kinds of vessels and different kinds of meat and keeping the vessels under different weather conditions at different seasons of the year. He even tried burying pieces of meat underground. The results were always consistent with his hypothesis: worms appeared in decaying meat only if adult flies were able to place their droppings on the meat. He even tried using dead maggots and flies as the meat in the flasks, but he observed the same results.

Although these experiments might seem to offer indisputable evidence that worms cannot arise in dead flesh of any kind unless flies are allowed to make deposits in the flesh, Redi recognized another possible interpretation of his observations. In every case, the sealing of the meat to prevent the entry of flies had also prevented the free entry and circulation of air. It could be argued that only the lack of fresh air kept maggots from arising spontaneously in the sealed meat samples.

Therefore, Redi set up another experiment. He put samples of meat and fish in a large vase closed with a fine veil through which air could circulate freely, but with holes too small for flies to penetrate. He put the vase inside a framework covered with the same kind of netting. "I never saw any worms in the meat, though many were to be seen moving about on the net-covered frame. These, attracted by the odor of the meat, succeeded at last in penetrating the fine meshes and would have entered the vase had I not speedily removed them." Redi noticed that some flies left deposits ("fly specks") on the netting, whereas others left live worms. "I noted that some left six or seven [live worms] at a time there and others dropped them in the air before reaching the net. Perhaps these were of the same breed mentioned by Scaliger, in whose hand, by a lucky accident, a large fly deposited some small worms, whence he drew the conclusion that all flies bring forth live worms directly and not eggs. But what I have already said on the subject proves how much this learned man was in error. It is true that some kinds of flies bring forth live worms and some others eggs, as I have proved by experiment."

Redi's report is a model of good scientific procedure in several respects. Not only are his experiments cleverly designed to provide unambiguous tests of his hypotheses, but he describes exactly what he did and what results he observed. He separates his interpretations and beliefs from these factual accounts of the experiments. Science has been built upon this sort of reporting; each investigator is expected to describe his work and observations meticulously and factually, without letting his own ideas or beliefs color the facts. Later researchers may disagree with his interpretations, but their own theories must be consistent with his experimental observations.

With the hindsight of a few centuries of experience, it is easy to see how unrecognized assumptions and beliefs crept into Redi's supposedly factual accounts of his observations. It is not nearly as easy to recognize the assumptions and beliefs that underlie contemporary "factual" accounts. However, the cumulative progress of science would be impossible if it were necessary for each scientist to distrust the reports of those who disagree with him and to repeat all of their experiments to be sure that they did not "fudge" the results. To permit other scientists to look for other possible interpretations of his results, the modern biologist is careful to include in his report a great many details that Redi did not think worth reporting. For example, he would probably include information on the weather conditions, the species names of the organisms used or observed, the exact sizes of the flasks and of the openings in the netting, and the exact numbers and sizes of the worms and flies that were observed. He would be sure to note whether the flasks were observed continuously and to explain just how he determined, for example, that *all* of the red objects broke open to release green flies. Because of this exact reporting, other scientists are able to think of possible alternative interpretations of the experiments without as much need to perform a new experiment in order to test each one.

Figure 1.7 (left). Pierre Berthelot at work in his laboratory.

Figure 1.8 (right). Friedrich Wöhler.

heated to the temperature of boiling water for nearly an hour—if all air reaching the broth has been similarly heated. The proponents of spontaneous generation could argue only that the heated air was somehow damaged and inadequate for the support of life. Schwann countered this argument, however, by showing that heated air is suitable for breathing.

The evidence against spontaneous generation of life was consistent with the prevailing theory of vitalism. This theory holds that living systems can arise from nonliving matter only through the intervention of an already living organism to impart a vital force to the matter. In short, each organism can arise only as the offspring of a parent organism similar to itself.

But how did all the various kinds, or species, of organisms originate? If the ancestors of modern organisms were not generated spontaneously—if each organism has always arisen from parents like itself—it seems necessary to suppose either that life has always existed in its present forms or that there was a supernatural creation of the many different species at some time in the past. Those who disproved spontaneous generation were unable to present a convincing scientific explanation of the origin of life.

THE RISE OF MECHANISM

Even while the theories of vitalism were being developed, they received a damaging blow from an unexpected quarter. In 1828 a German chemist, Friedrich Wöhler, was studying an inorganic substance now called ammonium cyanate. Wöhler analyzed the crystals that are produced when this substance is heated. He was startled to find that the crystals were urea, the major solid component of mammalian urine and a substance definitely considered to be organic.

Other chemists attempted to create organic substances from inorganic materials, and reports of their successes soon began to accumulate. All doubt was removed by the late 1850s, when Pierre Berthelot succeeded in producing such organic substances as alcohols, methane, acetylene, and

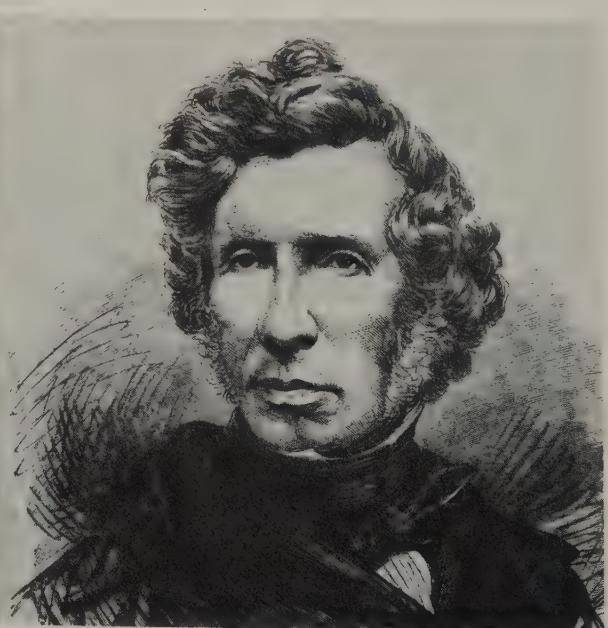


Figure 1.9. Svante August Arrhenius.

benzene from inorganic chemicals. It was clear that at least the simpler organic substances can be produced without the addition of a vital force.

Vitalism, however, was not completely discredited. Many biologists still felt that the more complex organic substances—particularly the proteins, which play important roles in living systems—could not be synthesized outside a living organism. Nevertheless, the weight of scientific opinion began to shift toward a mechanistic view of life. This approach holds that organic substances possess no special vital force but can be described in terms of the same chemical and physical laws that apply to inorganic substances. Organic substances, then, are distinguished only by the complexity of their organization.

In 1858 Charles Darwin and Alfred Russel Wallace simultaneously published the theory of evolution through natural selection, which each man had developed independently. This theory proposed a mechanism to explain how the vast array of organisms currently inhabiting the earth could have developed from simpler organisms that lived in the past. In each generation, those individuals possessing variations that helped them to survive or to reproduce would be likely to produce more offspring than those with unfavorable variations. If it is assumed that the offspring inherit characteristics similar to those of their parents, this natural selection of favorable variations, carried on over many thousands of generations, could lead to the eventual development of all modern organisms from a single, simple ancestral living system.

As this theory gained acceptance, the problem of the origin of life became both simpler in form and more difficult to solve. It was no longer necessary to account for the independent origin of many species but only to explain how the first, simple organism arose. Yet at almost the same time, the experiments of Louis Pasteur proved beyond reasonable doubt that even microorganisms cannot arise spontaneously (Interleaf 1.2).

Most scientists felt that it was futile to inquire into the origin of life. It was even suggested that life, like matter, has always existed. A Swedish chemist, Svante August Arrhenius, proposed the idea that life-bearing particles are scattered through space. They fall on all planets and germinate to produce organisms wherever conditions are favorable. Arrhenius' theory, however, does not account for the origin of life; it merely pushes the origin of the first living system further away in time and space.

By the late nineteenth century, most biologists had accepted a mechanistic view of living systems and had rejected the theories of vitalism and spontaneous generation. Yet as biologist George Wald (1954) comments: "Most modern biologists, having reviewed with satisfaction the downfall of the spontaneous generation hypothesis, yet unwilling to accept the alternative belief in special creation, are left with nothing." The question of the origin of life faded into the background as biologists applied themselves to the more rewarding task of investigating the mechanisms by which living systems operate. Application of the techniques and theories of the physical sciences to the study of organisms proved remarkably fruitful in terms of new understanding.

The mechanistic view of life was widely accepted by biologists long before experimenters actually succeeded in creating complex organic molecules from inorganic substances. In fact, it was not until 1969 that biochemists succeeded in synthesizing one of the complex protein molecules that plays an active chemical role in living organisms (Hirschmann, et al.,



Figure 1.10. A caricature of Louis Pasteur.



Interleaf 1.2

LOUIS PASTEUR'S EXPERIMENTS ON SPONTANEOUS GENERATION OF MICROORGANISMS

The controversy about spontaneous origin of life raged for a long time after the brilliant experiments of Redi (Interleaf 1.1). Although Redi demonstrated that maggots arise in decaying meat only if flies lay their eggs or deposit live young there, he continued to believe in the spontaneous generation of other kinds of insects. By the early eighteenth century, other researchers had demonstrated that insects of many different kinds arise only as offspring of adult insects. However, the microorganisms discovered by Anton van Leeuwenhoek in 1677 seemed to be a different case. Attempts to carry out experiments modeled after those of Redi on the origin of microorganisms led to conflicting results.

The major figures in a great debate that went on near the middle of the eighteenth century were John Needham, an English Catholic priest and naturalist, and Lazzaro Spallanzani, an Italian abbot and biologist. Needham, with the help of the Comte de Buffon, attempted to apply Redi's experimental methods to the study of the origin of microorganisms. He boiled mutton broth, thus killing all the microorganisms present in it. He placed the sterile broth in a well-sealed flask and left it for a few days. When he opened the flask, he found the broth swarming with microorganisms. He obtained similar results when he repeated the experiment with a variety of organic solutions—water in which various animal and vegetable substances had been soaked. Thus, Needham concluded that microorganisms do arise spontaneously.

Spallanzani realized that it would be difficult to be sure that no microorganisms enter the broth after it has been boiled. The adult microorganisms were so tiny that they could barely be seen in the microscopes of the time, and it seemed likely to Spallanzani that the eggs or other reproductive forms of these creatures might even be undetectable under the microscope. He repeated Needham's experiments, boiling the liquid for half an hour and then placing it in loosely corked flasks. After eight days, he examined the liquids and found them filled with microorganisms. Spallanzani next prepared five flasks containing a liquid prepared by soaking seeds in water. He left one flask open and sealed the other four completely by melting the glass and closing the openings. He then boiled the liquid in the flasks, varying the length of boiling for the four closed flasks. After two days, he found the open flask swarming with microorganisms. A closed flask boiled for half a minute contained only the smaller kinds of microorganisms, and the other closed flasks boiled for one to two minutes contained only extremely minute organisms. In further experiments, he found that boiling a sealed flask for 30 to 45 minutes is sufficient to ensure that no new microorganisms will appear in the flask so long as it remains sealed.

Other theorists objected to Spallanzani's experiments on the grounds that heating the air in the sealed flasks makes it unfit to support life. Theodor Schwann demonstrated that no microorganisms arise in broth to which only heated air is allowed access. Furthermore, he demonstrated that animals can live in such previously heated air. The work of Spallanzani and Schwann might seem to have disproved the possibility of spontaneous generation, but many scientists were unconvinced. When others tried to repeat these experiments, they often observed the generation of microorganisms. Although it could be argued that some leak in the flasks or some flaw in the procedure had permitted microorganisms to get in from the outside, such arguments were unlikely to convince the researcher who believed that he had done his work carefully. A prominent advocate of spontaneous generation was the naturalist Félix Archimède Pouchet, who published a large book in 1859 describing the process of spontaneous generation and the conditions under which it occurs.

Related to the debate over spontaneous generation was another conflict about the nature of fermentation and decay. The most common view was that set forth by the noted German chemist Justus von Liebig, who argued that decay and fermentation are peculiar processes that occur only in organic matter as the last stages of the process of death. On the other hand, Spallanzani and Schwann found that no decay occurs in the microorganism-free broths that have been boiled in sealed flasks. Therefore, they concluded that decay is a process carried out by living microorganisms. Schwann went on to show that yeast is composed of microorganisms and that the presence of live yeast organisms is necessary for the alcoholic fermentation of sugar.

Figure 1.11. Louis Pasteur in his laboratory.

Both of these debates were to be resolved essentially as a result of the work of one man, Louis Pasteur. A physical chemist, Pasteur began his career with research on the optical properties of certain crystals that are produced in the course of fermentation. He then became interested in the process of fermentation itself and in 1857 published a paper on the souring of milk. He was able to isolate the substance that produces lactic acid, which in turn makes the milk sour. The substance proved to be a ferment, and it was later shown to be a mass of bacteria. Pasteur then turned to the study of the alcoholic fermentation of sugar as it occurs in the process of wine making. He found that the fermentation is dependent upon the presence of certain yeasts or molds that live on the skins of ripened grapes. Pasteur's study of the defects of some wines convinced him that a number of different organisms, including some kinds of bacteria, participate in the fermentation process and that these organisms must be present in the proper proportions if a good wine is to be formed. Pasteur (1866) concluded that both fermentation and decay are the results of activity carried out by microorganisms that reach foods and dead flesh largely in the form of tiny airborne germs.

To confirm his hypothesis, Pasteur filtered air by drawing it through guncotton. He dissolved the guncotton and showed that a residue of microscopic spherical and rod-shape objects remained. He also drew air that had already been filtered through guncotton and showed that in this case no microscopic residue remains when the guncotton is dissolved. Thus, he confirmed that the air is full of microscopic objects that could be the germs of his hypothesis.

Still the supporters of spontaneous generation were no more convinced by Pasteur's experiments than they had been by those of Spallanzani and Schwann. They argued that Pasteur had merely shown the air to contain microscopic objects but that he had provided no evidence that these objects are alive or can become alive. They still argued that the creation of microorganisms in decaying organic material occurs spontaneously and that decay is caused by a vital force leaving the dying matter to enter the newly created microorganisms.

In answer to these objections, Pasteur conducted another series of experiments that were similar in design to the original experiments of Redi. He placed fermentable substances in flasks, then drew the neck of the flask out into a long, narrow S shape. Air can enter and leave the flask through the narrow opening, but any particles in the air are likely to be trapped on the walls of the long, curving neck. The flasks and their contents were heated to the temperature of boiling water for some time, then left sitting in still air. No fermentation was observed in the flasks. However, if the necks were broken off so that atmospheric dust and other small particles could enter with the air, the contents began to ferment within a few hours and microorganisms abounded in the liquid (Pasteur, 1861). Although this experiment did not immediately convince all of the proponents of spontaneous generation that they were wrong, it did carry great weight with the majority of the scientific community. Within 20 years after the publication of Pasteur's work, the idea of spontaneous generation had essentially been abandoned.

Pasteur's work carried great impact not only because of the elegance of his experimental technique but also because of his success in confirming other implications of his germ theory. Pasteur's work on fermentation led to important practical improvements in many industrial processes, and his application of the germ theory to medical practices had dramatic results. Furthermore, as biologists learned more about microorganisms, these little creatures began to seem more and more similar to larger organisms, and the idea that they might form spontaneously began to seem quite peculiar.



Figure 1.12. Linus Pauling (left) in Oslo to receive the 1962 Nobel Prize.



1969). In 1970 other researchers succeeded in synthesizing a gene, one tiny part of the complex DNA molecule that carries hereditary information in a living organism (Agarwal, et al., 1970). It may be many years before confirmation of the mechanistic view is obtained through the complete synthesis of a simple living creature from inorganic materials. However, few biologists today doubt that such a synthesis is theoretically possible.

THE ORIGIN OF LIFE

By the middle of the twentieth century, sufficient progress had been made in the understanding of the chemical and physical mechanisms of life to permit biologists to make some progress toward a theory about the origin of life. Extrapolation of the evolutionary process backward in time leads to the hypothesis of a single species of living systems—probably quite simple in nature—that were the ancestors of all later life on earth. It is possible to suppose that these first living systems were one of a great variety of complex physiochemical systems—most of which were not alive—that had been generated by the physical and chemical processes on the primitive earth.

Darwin anticipated this view in a letter written in 1871 to J. D. Hooker, the botanist who studied and classified the plant samples Darwin collected during the voyage of the *Beagle*. “It is often said,” wrote Darwin, “that all the conditions for the first production of a living organism are now present, which could ever have been present. But if (and oh what a big if) we could conceive in some warm little pond with all sorts of ammonic and phosphoric salts,—light, heat, electricity &c. present, that a protein compound was chemically formed, ready to undergo still more complex changes, at the present day such matter would be instantly devoured, or absorbed, which would not have been the case before living creatures were formed.”

In essence, the modern view is that spontaneous generation of simple organisms may have been possible under the very different chemical and physical conditions that are thought to have existed on the primitive earth (Chapter 32). From a variety of evidence, scientists now believe that the earth, the other planets, and the sun were all formed about 5 billion years ago from a cloud of cosmic dust at low temperatures. The oldest rocks yet found to contain apparent remains of living organisms were formed about 3.1 billion years ago. Because the time between the formation of the earth and the appearance of living systems was probably only 1 or 2 billion years or less, it has been suggested that the organic substances from which life formed were a part of the dust cloud that condensed into the earth (Robinson, 1966). Arrhenius’ hypothesis that life may have come in the form of spores or seeds from elsewhere in space is not totally disproven either. One major reason for the careful sterilization of unmanned rockets to the moon and to other planets has been the hope of eventually testing Arrhenius’ theory by looking for such spores on planets where life has not existed.

MODERN MECHANISM AND REDUCTIONISM

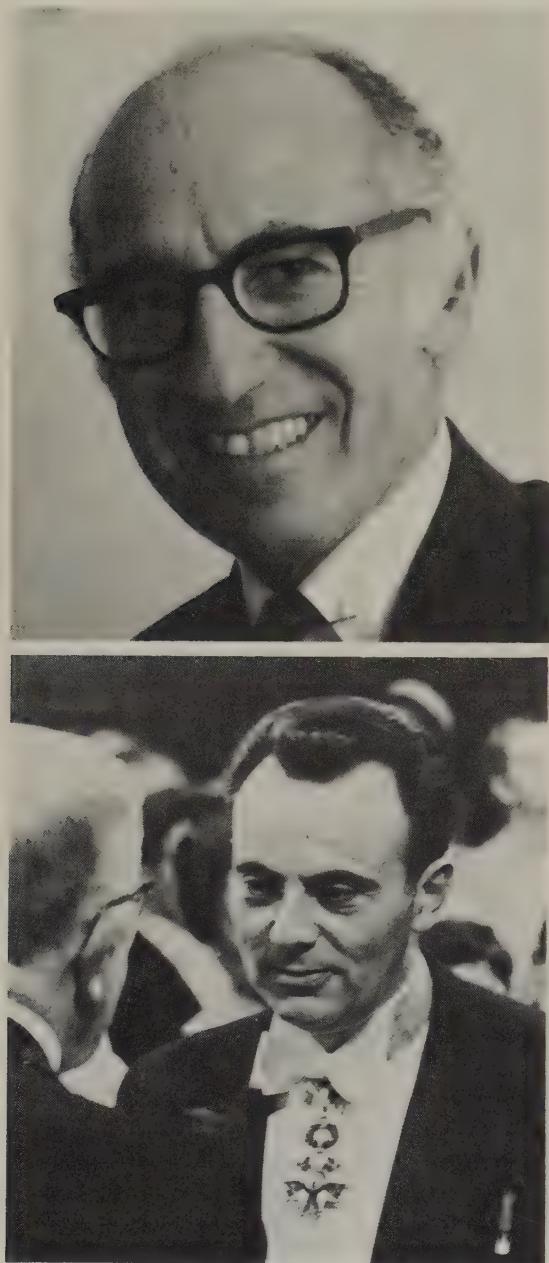
Over the past two centuries, the mechanistic approach to biology has steadily gained adherents as it has accumulated an impressive record of successes in explaining living systems. It is now generally accepted that both living and nonliving systems are governed by the same physical and chemical principles.

Indeed, the most spectacular advances in biology during this century have come through mechanistic and reductionistic approaches. Important

Figure 1.13. Apollo 12 lunar sample seen under polarized light.



Figure 1.14. Two prominent modern biologists. Jonas Salk (above) developed the Salk polio vaccine. François Jacob (below) is seen receiving the 1965 Nobel Prize.



advances in molecular biology and molecular genetics have come as the most modern understandings of chemistry and physics were applied to the study of living cells. In more and more cases, it is becoming possible to explain some characteristic of an organism in terms of the physiological systems within the organism, to explain these systems in terms of cellular interactions, and to explain the cellular interactions in terms of chemical and physical processes. On the other hand, there has not been notable success in moving toward new generalizations at higher levels of organization. That is, biologists are still struggling to delineate the general principles that govern the functioning of populations and of the ecosystem.

Because of the successes of the reductionistic approach, many scientists have concluded that the goal of biological study should be the eventual explanation of living systems entirely in terms of chemistry and physics, with everything fully measured and quantified at the molecular level. On the other hand, another large group of biologists argues that living systems are unique, and they may be expected to obey certain principles at higher levels of organization that cannot readily be reduced to simpler physical laws. Although it might be theoretically possible to explain the outcome of a presidential election in terms of a complete biochemical understanding of the American population, there is great practical benefit in having principles of behavioral, political, and social sciences to apply to such studies. Furthermore, the statistical processes involved in interactions of large numbers of cells or individual organisms may make complete reductionism impossible. Thus, it may be possible to explain in terms of physiological mechanisms why an individual is likely to behave in certain ways, but it may still be necessary to have general principles of social behavior to explain the particular distributions of behavior observed in a population of interacting individuals.

Advance in any science requires an interplay between theory and experiment, between imaginative guesswork and careful testing, between broad generalizations and exact measurements. Any good experiment is designed because the experimenter wants to test some hypothesis that he has created. Even simple observation of nature must be guided by some preconceptions about what is sufficiently significant to be observed. On the other hand, even the most brilliant theoretical scheme is of little scientific interest unless it can be tested by experimentation or observation in the real world.

Biology today is a complex, diverse, and incomplete field of knowledge. Many different groups of researchers and theorists have explored different aspects of living systems. They have used different assumptions about how to ask their questions, different vocabularies, and very different experimental techniques. As a result, biological knowledge seems somewhat like a huge jigsaw puzzle with a great many missing pieces. Here and there, segments of the picture can be fitted together very nicely. The broad relationships between these segments are fairly clear, but there still are many gaps and scattered loose pieces whose proper position seems very uncertain.

In the chapters that follow, the broad picture has been outlined and the details filled in here and there. It is important to keep in mind that behind almost every sentence stating some apparently simple fact may lie a history of experimentation and debate as complex as the controversy about spontaneous generation. What seems today to be a safe generalization may tomorrow be proven by some critical experiments to have been a faulty interpretation of the available evidence. What seems today to be a minor

Figure 1.15. The mapping of molecules.



exception to some general rule may prove tomorrow to be the key to an important new understanding of living systems.

From the level of molecular interactions on a time scale of seconds or minutes to the level of population interactions on a time scale of millions of years, the biologist is involved in the study of the most complex systems known to exist in the universe. The understanding accumulated in the past few centuries is staggering in its scope and variety, but the amount yet to be learned about life is far greater. Very few of the chapters in this book represent summaries of aspects of living systems that are thoroughly understood. Although most modern biologists are mechanists and reductionists, it will be a very long time before an introductory biology book can be written in which all important facts about even a single living system are set forth clearly and simply as the logical result of chemical and physical laws.

FURTHER READING

Asimov (1964) provides a brief and very readable summary of the history of biology. For more detailed information on the development of the scientific study of living systems, see Commoner (1966), Crick (1966), Crombie (1959), Dampier (1958), Eiseley (1969), Hall (1954), Rook (1963), Simpson (1969), and Singer (1959). More general discussions of the nature of science will be found in books by Butterfield (1957), Conant (1951), Nagel (1961), and Nash (1963).

2

The Variety of Life



The instructor stepped up to the podium, shuffled his notes, and began his lecture: "Without grass, life as we know it would be impossible."

The resulting explosion of laughter from the students was somewhat disconcerting, for he had been about to launch into a very serious discussion of ecological food pyramids. Communication gaps such as this one occur with unfortunate regularity in daily life, but they usually are cleared up by further conversation. In scientific circles—where thousands of individuals must communicate via the written word in monthly periodicals and must rely upon the reports of others for reliable information—the problem becomes more severe. It may be vitally important to know exactly what kind of organism is meant by a name such as "grass" in a scientific report.

In attempting to categorize the incredible variety of living things that exist in the world, biologists have developed a complex system of classification and terminology. It has been said that a first-year biology student must learn more new words than does a student in an introductory language course. This statement may be a slight exaggeration, but it does not seem so to many students, who wonder why things cannot be said in "plain English." Present systems of nomenclature and classification were worked out slowly as it became necessary to deviate from the "everyday" language of other kinds of writings.

The present system of classification was developed in the eighteenth century. Although the system has been revised extensively since then, the basic principles and rules of *biosystematics* (biological classification and nomenclature) have remained essentially unchanged since 1758.

PRINCIPLES OF CLASSIFICATION

Biological classification systems are hierarchical. That is, an individual is assigned to one small set of very similar individuals, and that set is grouped with other sets to form a larger set of individuals with some similarities, and so on. Hierarchical classification schemes are common in many areas of life and are built into the everyday language. The hierarchy used in biological classification has several levels of sets, with a general name given to each level, or taxon. A set at one level in the hierarchy is made up of one or more sets of the level below it. For example, an order contains one or more families, and each family contains one or more genera. Thus, the kingdom is the most inclusive taxon, and the species is the least inclusive (most specific) taxon. Two individuals classified as members of the same species are very similar to one another in many ways, whereas two individuals classified as members of the same kingdom may share only a few common characteristics. Additional levels of classification—such as suborders, superfamilies, and so on—are added to the hierarchy in cases where it seems important to emphasize certain similarities or differences.

The modern system of classification, or *taxonomy*, is based upon a system first worked out in the eighteenth century by Carolus Linnaeus. Each species is assigned a two-part, or *binomial*, name. The first part of the full name is that of the genus, and the second part of the name is that of the species. Linnaeus wrote in Latin—the common scientific language of his age; much as English is today—and formal systematic names are still written in Latin.

The basic unit of classification is the species. All higher taxa represent abstractions—groupings created in the human mind and not corresponding

to any actual aggregations in nature. Species also are abstractions, but they are more closely related to natural groupings than are higher taxa. For most kinds of organisms, the individual is a unit that has clear physical significance. Individuals live together in populations, which can be defined relatively clearly for sexually reproducing organisms. A *population* consists of those individuals living in the same area who actually or potentially are related to one another. In other words, any two individuals who can mate with one another are members of the same population, as are their parents, offspring, and other ancestors and descendants. A species can be defined simply as the group including all populations that could interbreed if they lived in the same area.

Each species is named by genus and species, and an elaborate set of rules exists to ensure that no two species will be assigned the same name. The name of the human species, for example, is *Homo sapiens* — a name originally assigned by Linnaeus. There are no other species of the genus *Homo* living today, but some fossil remains are thought to represent other species of *Homo*. This genus is grouped in the family Hominidae (hominids) with some other genera of fossil creatures similar to man but not similar enough to be considered part of the genus *Homo*. The family Hominidae is part of the superfamily Hominoidea (hominoids), which includes the great apes as well as the various manlike genera. The superfamily Hominoidea is part of the suborder Anthropoidea (anthropoids), which includes all of the tailless apes that walk erect or semierect. This suborder is part of the order Primates. Among other characteristics that distinguish the primates are flat nails instead of claws, forward facing eyes, and large brains. This order is part of the class Mammalia (mammals), which includes all animals that suckle their young. The Mammalia are grouped in the phylum Chordata (chordates), which includes all organisms having a vertebral column or a dorsal, tubular nerve cord. Finally, the Chordata are part of the kingdom Animalia (animals).

At each taxon, the systematic classification indicates a range of other organisms that share certain characteristics with the species being classified. Certain characteristics have been chosen as the basis for classification, whereas others have been largely ignored. Thus, organisms of similar size or color are not likely to be grouped together at any level of this system. Originally, groupings were based upon characteristics that seemed most basic or “natural,” but the modern system is based upon an attempt to indicate evolutionary relationships.

THE CONSTANCY OF SPECIES

Today, the system of classification is based chiefly upon theories of evolution. Species grouped in the same genera are considered to be “more closely related” than are two species in different genera. Two species grouped in the same genus are thought to have shared a common ancestral species in the relatively recent past. All species grouped in a single family presumably shared common ancestors in the more distant past. Because theories of the evolutionary history of organisms are continually being revised and debated, the higher levels of taxonomy are also in a state of continual revision. At any given time, several different schemes of classification are likely to be proposed and defended by various biologists. To some extent, this continual revision and debate counteracts the advantages of a uniform system of nomenclature. However, the rules by which names are

Figure 2.1. The two-kingdom system of classification.

proposed and adopted ensure that the meaning of any particular name will be clear to those who read a paper in which it is used, although a considerable amount of research in the voluminous literature of taxonomy may be necessary to determine the exact meaning of a particular item.

It is more difficult to define species in organisms that reproduce asexually. In fact, there are a number of difficulties related to a precise definition of species. However, it is now generally agreed that populations are significant, natural groupings of individuals, and that in most cases species can be defined as meaningful, unambiguous groupings, where members of one species are reproductively isolated from those of another species. Higher taxa are much more arbitrary and reflect current views of significant functional and structural similarities. The system of classification used in this book represents a compromise among some of the newer proposals that seem to be gaining favor among biologists. This system reflects current theories about the evolutionary relationships among organisms, but it is only one of many similar schemes currently in use. The evolutionary nature of taxonomic systems can be illustrated through a brief consideration of the changes that have occurred in the groupings of the most general taxon, the kingdoms.

HOW MANY KINGDOMS?

Early students of life were aware of little more than the higher plants and animals. It was natural for them to divide all organisms into two great realms, or kingdoms, of life—the immobile plants that gather nutrients through roots and the active, food-ingesting animals. Such a major division was recognized by Aristotle and became part of the formal system of classification in the work of Linnaeus.

As early as 1860, various authors began to suggest that the single-cell organisms should be regarded as a separate kingdom—neither plant nor animal—and the names Protista and Protoctista were suggested for this kingdom. In some schemes, the simpler multicellular organisms—such as fungi and algae—were included in this third kingdom. However, through the early twentieth century, many biologists continued to think only in terms of two kingdoms—plants and animals—with some uncertainties of

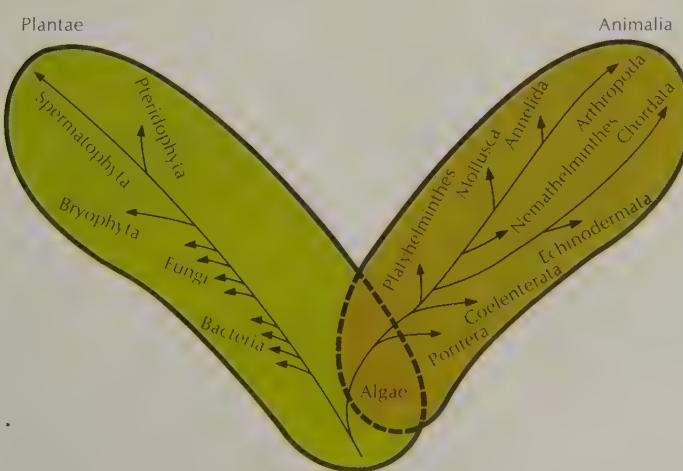


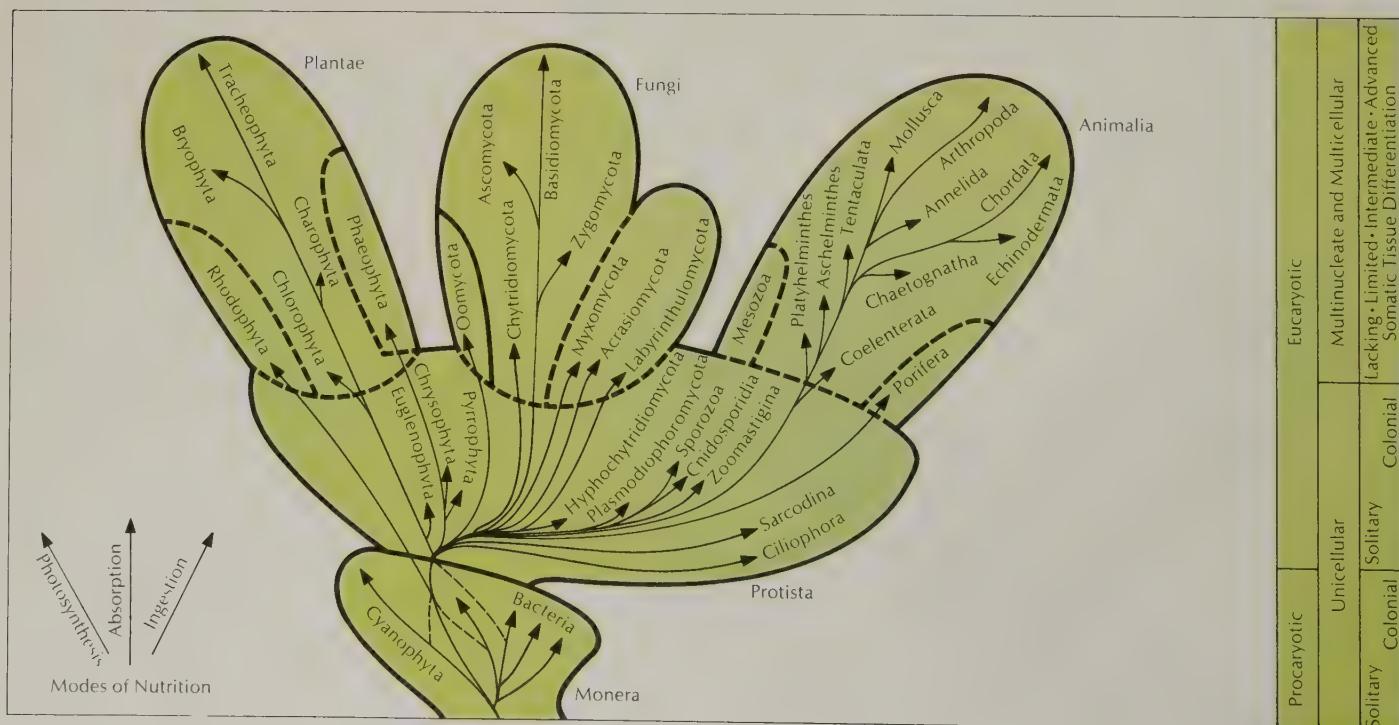
Figure 2.2. Whittaker's five-kingdom system of classification.

classification among simpler organisms. In evolutionary terms, this method of classification implies that plant and animal lines diverged very early in the history of life (Figure 2.1).

As more was learned about microorganisms, simple multicellular organisms, and evolutionary history, the ranking of the Protista as a separate kingdom became more and more common. But even as the three-kingdom system came into common use, evidence was accumulating that other major divisions should be made in the grouping of organisms. Studies of single-cell organisms revealed two very different kinds of cell structure. Prokaryotic organisms—bacteria and blue-green algae—lack a cell nucleus and are fundamentally different from other cells in many important ways (Chapter 8). The distinction between prokaryotic cells and the eukaryotic (nucleated) cells of all other organisms was shown to represent very fundamental and extensive differences in basic organization.

Further study of fungi has revealed that these organisms depend upon a supply of organic molecules in their environment as animals do, yet they feed by absorbing substances through cell walls much as plants do. The complexity of fungi makes it unreasonable to group them with the Protista, yet it seems clear that they represent an independent line of evolution that diverged from plants and animals early in the history of life.

On the basis of the most recent information about evolutionary relationships, R. H. Whittaker (1969) has proposed a five-kingdom system that recognizes the fungi as a separate group of equal importance, with plants and animals as evolutionary lines of multicellular organisms that have developed from single-cell organisms (Figure 2.2). Whittaker's system appears to be gaining favor among biologists, and a modified version of his system follows (Whittaker, 1969).



KINGDOM MONERA [procaryotes]

- Phylum Schizophyta, or Schizomycetes [bacteria]
- Phylum Cyanophyta, or Myxophyta [blue-green algae]

KINGDOM PROTISTA [protists]

- Phylum Euglenophyta [euglenophytes]
- Phylum Chrysophyta [golden algae and diatoms]
- Phylum Xanthophyta [yellow-green algae]
- Phylum Pyrrophyta [dinoflagellates and cryptomonads]
- Phylum Hyphochytridiomycota [hyphochytrids]
- Phylum Plasmodiophoromycota [plasmidiophores]
- Phylum Sporozoa [sporozoans]
- Phylum Cnidosporidia [cnidosporidians]
- Phylum Zoomastigina [animal flagellates]
- Phylum Sarcodina [rhizopods]
- Phylum Ciliophora [ciliates and suctorians]

KINGDOM PLANTAE [plants]

- Phylum Rhodophyta [red algae]
- Phylum Phaeophyta [brown algae]
- Phylum Chlorophyta [green algae]
- Phylum Charophyta [stoneworts]
- Phylum Bryophyta [liverworts, hornworts, and mosses]
- Phylum Psilophyta [psilophytes]
- Phylum Lycopodophyta [club mosses]
- Phylum Arthrophyta [horsetails]
- Phylum Pterophyta [ferns]
- Phylum Cycadophyta [cycads]
- Phylum Coniferophyta [conifers]
- Phylum Anthophyta [flowering plants]

KINGDOM FUNGI [fungi]

- Phylum Myxomycophyta [slime molds]
- Phylum Eumycophyta [true fungi]

KINGDOM ANIMALIA [animals]

- Phylum Mesozoa [mesozoans]
- Phylum Porifera [sponges]
- Phylum Archaeocyatha [extinct organisms]
- Phylum Cnidaria [coelenterates]
- Phylum Ctenophora [comb jellies]
- Phylum Platyhelminthes [flatworms]
- Phylum Nemertea [ribbon worms]
- Phylum Acanthocephala [spiny-headed worms]
- Phylum Aschelminthes [pseudocoelomate worms]
- Phylum Entoprocta [pseudocoelomate polyzoans]
- Phylum Bryozoa [sea mosses, or moss animals]
- Phylum Brachiopoda [brachiopods, or lampshells]
- Phylum Phoronida [phoronid worms]
- Phylum Mollusca [molluscs]
- Phylum Sipunculoidea [peanut worms]
- Phylum Echiuroidea [spoon worms]
- Phylum Annelida [segmented worms]
- Phylum Arthropoda [arthropods]
- Phylum Brachiata [beard worms]
- Phylum Chaetognatha [arrow worms]
- Phylum Echinodermata [echinoderms]
- Phylum Hemichordata [acorn worms]
- Phylum Chordata [chordates]

Monera

Figure 2.3 (above). Blue-green algae *Anabaena*, representing the phylum Cyanophyta, and green algae on a pond.

Figure 2.4 (below). Colonial blue-green algae. Some of the blue-green algae may function as nitrogen-fixers in aquatic ecosystems and thus play a role in the global nitrogen cycle.

Protista

Figure 2.5 (opposite left). Diatoms. Note the variety in form among the types represented. These organisms are of major economic importance because they form the base of the oceanic food chain and thus support much of the marine flora and fauna.

Figure 2.6 (opposite right). Close-up photograph of a radial diatom.

Whittaker regards plants, fungi, and animals as three groups of organisms that, through evolution, have come to specialize in three different modes of nutrition: photosynthesis, absorption, and ingestion. Protista include a variety of diverging lines of evolution, all composed of organisms that share a eucaryotic, single-cell level of organization. Monera are the simplest known organisms and are presumed to be similar to the early organisms from which the other kingdoms evolved.

Further revisions of kingdoms may result as more is learned about the early evolution of life. Whatever system is accepted, however, it should be emphasized that systems of classification represent categories superimposed on nature by the human mind in its search for order, and any system may be expected to fit nature somewhat imperfectly. The classification and naming of organisms in itself does not represent significant knowledge about the living world. A classification system simply provides a means of summarizing existing knowledge and provides a stimulus for new researches and theories. It is a good idea to keep in mind that learning the name of an organism tells one little about the thing itself, although it may explain something about the theories of the person who invented the name.

Various groups of organisms are considered in detail elsewhere in this book, but a brief pictorial survey of the five kingdoms will help to clarify the nature of the classification system and to illustrate the diversity of life.



Figure 2.7 (middle). A radiolarian test or shell. Radiolarians are marine amoebas with silica shells. (Eric Gravé)

Figure 2.8 (middle left). Close-up photograph of the extended pseudopod of the shelled amoeba *Arcella* (Phylum Protozoa). (Eric Gravé)

Figure 2.9 (middle right). *Paramecium aurelia*, representing the ciliate class of protozoans. (Eric Gravé)

Figure 2.10 (lower left). A representative marine ciliate. Note the winglike extensions of the pellicle (surface

membrane), which function as flotation devices. (Eric Gravé)

Figure 2.11 (lower right). *Euplotes*, an advanced ciliate. The ciliophora are structurally the most complex of the protozoa. (Eric Gravé)



Fungi

Figure 2.12 (above). Photograph of the plasmodium, or vegetative body, of a slime mold (Kingdom Fungi). Most slime molds are free-living saprophytes, feeding on dead organic matter such as wood or leaf litter on the forest floor.

Figure 2.13 (middle). Close-up photograph of the sporangia, or fruiting bodies, that develop from the slime mold plasmodium during the reproductive period.

Figure 2.14 (lower left). Bracket fungi growing on the

trunk of a tree. This organism feeds on the dead bark tissue of the tree.

Figure 2.15 (lower right). The basidium, or club-shape fruiting body, commonly called a mushroom. These fungi act as saprophytes in breaking down dead organic matter to obtain their energy.

Plantae

Figure 2.16 (opposite left). A desmid, one of the green algae (Phylum Chlorophyta) in the Kingdom Plantae. (Eric Gravé)



Figure 2.17 (upper middle). Close-up photograph of strands of *Spirogyra*, a common fresh-water green alga. (Eric Gravé)

Figure 2.18 (upper right). Close-up photograph of a thallus (sheetlike) liverwort (Phylum Bryophyta). Note the globular, fingerlike reproductive units.

Figure 2.19 (middle left). *Lycopodium*, a club moss. Note the terminal spore sacs.

Figure 2.20 (center). Venus flytrap. This insectivorous

plant is a native of marsh regions, and it supplements its nitrogen intake with insect protein.

Figure 2.21 (middle right). *Welwitschia mirabilis*, a unique desert plant indigenous to the Kalahari Desert in southwest Africa.

Figure 2.22 (lower left). Snow plant, of the alpine zone.

Figure 2.23 (lower middle). Passion flower.

Figure 2.24 (lower right). Elephant-foot tree, a member of the palm family and native to Mexico.



Animalia

Figure 2.25 (upper left). A simple vase-shape sponge. These organisms are filter-feeders, straining out planktonic organisms from the water.

Figure 2.26 (upper right). The medusa, or jellyfish, form of a coelenterate.

Figure 2.27 (middle left). *Obelia* polyp. Note the ring of tentacles surrounding the mouth.

Figure 2.28 (center). Coral polyps, the well-known reef-building coelenterates.

Figure 2.29 (middle right). Sea pens, colonial coelenterate polyps that feed extended as shown, but retreat into a central stalk when disturbed.

Figure 2.30 (lower left). The honeycomb worm, a marine tube-building annelid worm.

Figure 2.31 (lower middle). The white-lined nudibranch, a gastropod member of the phylum Mollusca. Note the fingerlike gills on its back.

Figure 2.32 (lower right). Close-up photograph of the Great Scallop, a representative molluscan bivalve.





Figure 2.33 (above). Octopus, a cephalopod mollusc without an external shell covering.



Figure 2.34 (below). The mystery snail, another gastropod mollusc.

Arthropod members include:

Figure 2.35 (upper left). Spiny lobster, a crustacean.

Figure 2.36 (upper middle). The crustacean shrimp.

Figure 2.37 (upper right). A wolf spider, an arachnid.

Figure 2.38 (middle left). The ox beetle, an insect.

Figure 2.39 (middle right). The harvest ant, an insect.

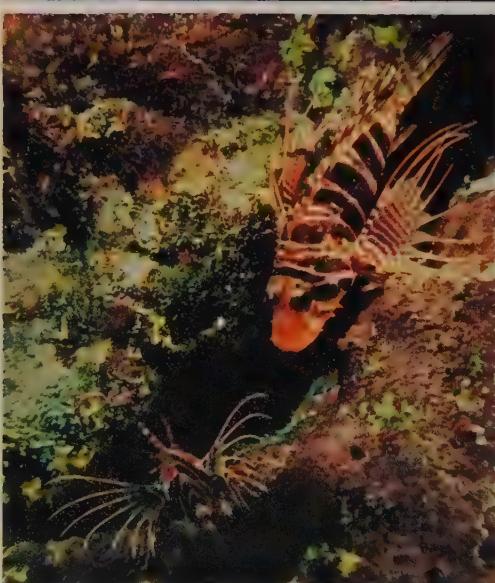


Figure 2.40 (lower left). A woolly aphid, also an insect.

Chordate members include:

Figure 2.41 (lower middle). The reef-dwelling lion fish, a true bony fish.

Figure 2.42 (lower right). The amphibian tree frog.

Figure 2.43 (upper left). A tokay gecko, a reptile.

Figure 2.44 (upper right). The alligator, also a reptile.

Figure 2.45 (middle left). Rhinoceros viper, a reptile.

Figure 2.46 (center). Avocet, a shore bird.

Figure 2.47 (middle right). A blue-crowned pigeon.

Figure 2.48 (lower left). Tasmanian gray kangaroo, a marsupial mammal.

Figure 2.49 (lower middle). Opposum, also a marsupial.

Figure 2.50 (lower right). A white-footed deer mouse, a placental mammal.



Figure 2.51 (above). A hippopotamus, an even-toed hooved mammal (an artiodactyl).

Figure 2.52 (middle) Snow leopard, a carnivorous mammal.

Figure 2.53 (lower left). Hyrax, a small, herbivorous mammal that is related to the elephant.

Figure 2.54 (lower right). An orangutan, a primate.



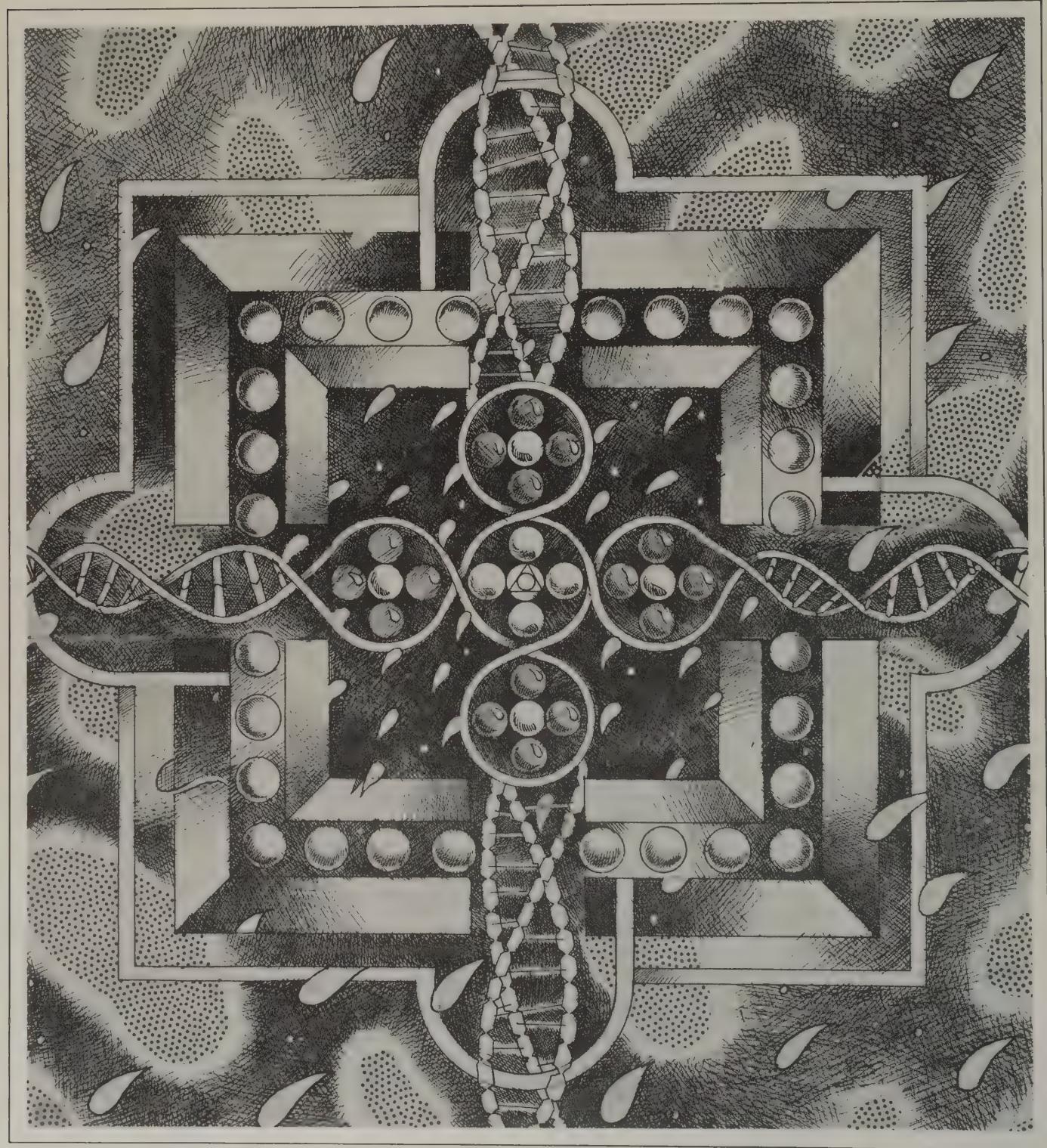


FURTHER READING

For more detailed discussion of the topics of this chapter, see Unit Seven of this book, particularly Chapters 32, 36, 37, and 38.

Dickinson (1967) describes the life and work of Linnaeus. Good general discussions of biological classification are given by Blackwelder (1967), Copeland (1956), Mayr (1942, 1969), and Savory (1963). More specific discussions of plant classification are given by Bell (1967), Bell and Woodcock (1968), Davis and Heywood (1963), and Solbrig (1970). Animal classification is discussed by Blackwelder (1963), Mayr (1963, 1969), Mayr, et al. (1953), and Simpson (1961). For detailed discussion of the rules by which species and higher taxa are named, see works by Smith and Williams (1970), and Stoll, et al. (1964). Romer (1968) gives a good overview of the diversity of life and its evolutionary significance.

For further discussions of the nature of species, see works by Dobzhansky (1937), Mayr (1957), and Simpson (1951). Alston and Turner (1963) discuss biochemical contributions to problems of classification, and Sokal (1966) describes a recent quantitative approach to classification.



The Physical Basis of Life

If the scheme of philosophy which we now rear on the scientific advances of Einstein, Bohr, Rutherford and others is doomed to fall in the next thirty years, it is not to be laid to their charge that we have gone astray. Like the systems of Euclid, of Ptolemy, of Newton, which have served their turn, so the systems of Einstein and Heisenberg may give way to some fuller realization of the world. But in each revolution of scientific thought new words are set to the old music, and that which has gone before is not destroyed but refocussed. Amid all our faulty attempts at expression the kernel of scientific truth steadily grows; and of this truth it may be said—The more it changes, the more it remains the same.

— Eddington (1927)

3

Atoms and Bonds



The study of chemistry has become essential for an understanding of life sciences. Without the dramatic applications of chemical principles, biology today would still be a descriptive science supporting a good deal of theory but lacking experimental foundation. The physical sciences have given a firm scientific basis to biology. In turn, the synthesis of disciplines called biochemistry has led to vast improvements in medicine. For example, the developments of anesthesia, antibiotics, and other drug therapy have been crucial steps in the successful application of biology to the practice of medicine. The doctor has come a very long way from the days when he dosed his patient with fox fangs and bled him with leeches. Without an application of chemistry to biology and thus to medicine, life would still be nasty, brutish, and short.

Biological processes can be understood in terms of their chemical behavior and their adherence to the same physical laws that govern inanimate systems. Certain biological processes such as human memory are so complex that they have not yet been described in chemical terms, but there is every expectation that they will be so explained. Much of the excitement of biological research stems from the ever-present question: How can molecules comprised of only a few elements be constructed so as to carry out the function of such an intricate organ as the human brain? As biology has been reinforced with chemistry, it has become obvious that, at the molecular level, all living organisms are remarkably alike.

The study of the interaction of light with atoms and molecules has led to an understanding of how plants transfer energy from the sun to highly ordered molecules. The concept of chemical charge can explain how an organism converts the stored energy of fats and carbohydrates into useful work. Bonding between atoms provides the basis for the three-dimensional structure of complex arrays of molecules. Thus, chemistry is central to biology because the chemical structure and function of molecules are interdependent. It is the sum of the function of all the molecules in a living cell that determines the development and proliferation of that cell and distinguishes it from its neighbors.

ATOMIC THEORY

As early as the fifth century B.C., the idea that all things are made up of tiny atoms was suggested by the Greek philosopher Leucippus and his student Democritus. For centuries there was little support for such a view, but in the early years of the nineteenth century John Dalton, an English chemist, found that he could best explain his experimental results by using the idea that the various substances are composed of minute "atoms." His data indicated that the atoms combined with each other in a number of fixed ratios to form compounds.

From Dalton's theory comes the basis of modern chemistry. Before plunging into a discussion of that chemistry—particularly in relation to the living world—one other aspect of Dalton's theory should be made clear.

Neither Democritus nor Dalton ever saw an atom. Nor has anyone else, for although the term "atom" is used in describing chemical reactions and living processes, the atom is a *model*. It is a model in the sense that it is a representation of reality—as good a representation as can be developed from available information—but nevertheless an abstraction.

Dalton's was a very simple atomic model. As more information has been gathered, the atomic model has become increasingly complex in

Figure 3.1a (left). Dalton's table of atomic weights that he derived in 1803.

Figure 3.1b (right). John Dalton, an English chemist and physicist, who presented the first clear statement of atomic theory.

ELEMENTS			
Hydrogen	1	Sulfur	46
Azotum	7	Diphosphorus	68
Carbon	12	Lime	50
Oxygen	16	Zinc	50
Potash	18	Copper	50
Soda	20	Lead	70
Lime	22	Silver	70
Soda	28	Gold	190
Potash	42	Platina	190
		Mercury	167

detail and comprehensive in its ability to explain actual phenomena. In the following discussions, a model of the atom is used that is complex enough to account for most important chemical properties, yet one considerably simplified from the complete modern understandings of atomic structures.

Dalton postulated that all substances are made up of small, indivisible particles called atoms and that each element contains only a single kind of atom. Atoms can combine to form molecules, which are the basic particles of chemical compounds. Thus, Dalton pictured the molecule of carbon dioxide (then called "fixed air") as a combination of an atom of carbon and two atoms of oxygen. Chemists later devised a shorthand notation for describing atoms and molecules, using the first one or two letters of the names of the elements. In many cases, the abbreviation is based upon the Latin or German name of the element. For example, "Na," the abbreviation for sodium, is derived from the Latin term "natrium." Subscript numerals are used to indicate the numbers of each kind of atom in a molecule; thus, carbon dioxide is written as CO_2 .

Dalton knew from chemical analyses that about 3 grams of carbon combine with each 8 grams of oxygen when carbon dioxide is formed. Because each molecule of CO_2 contains one carbon atom and two oxygen atoms, any sample of CO_2 must contain twice as many oxygen atoms as it does carbon atoms. Therefore, if a certain number of carbon atoms weighs 3 grams, the same number of oxygen atoms must weigh 4 grams. In other words, each oxygen atom is $4/3$ as heavy as a carbon atom.

In this fashion, Dalton was able to deduce the relative weights of the atoms of the 30 or so elements known at the time. The hydrogen atom

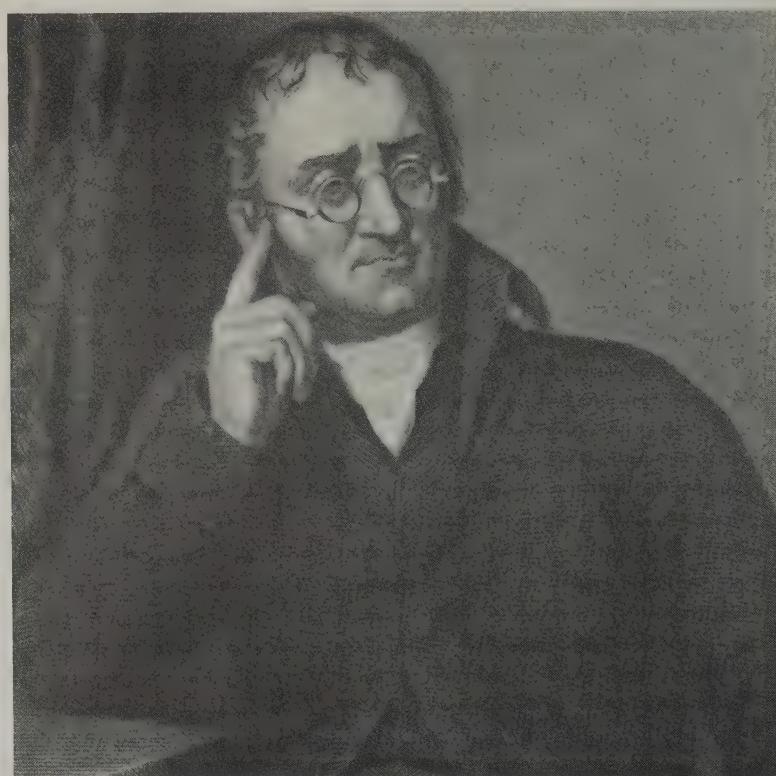


Figure 3.2 (left). A model of the nuclear atom. The classic model of the atom is a dense nucleus of neutrons and protons surrounded by electrons. Shown here is an oxygen atom with 8 protons (orange), 8 neutrons (yellow), and 8 electrons.

Figure 3.3 (right). The electron cloud representation of the atom. The negatively charged electrons are distributed diffusely around a positively charged nucleus. Note that the atom does not have a sharp boundary.

proved to be the lightest, and therefore Dalton arbitrarily set its weight equal to 1. Some of Dalton's relative atomic weights later proved to be erroneous because he had made incorrect assumptions about the number of atoms in certain molecules. For example, he assumed that the formula for water is HO; later studies showed that it is in fact H₂O. Within a few decades after Dalton's work, however, complete tables of atomic weights were available. For various reasons, the values of atomic weights were later calculated by giving oxygen a weight of 16.00. On this scale, hydrogen has an atomic weight of 1.008 instead of exactly 1.00. Recently, the standard has been revised again, but the values of the atomic weights have been changed only slightly by these redefinitions.

The Nuclear Atom

Only in the most simplified models can an atom be regarded as a simple particle. It is clear from many kinds of evidence that almost all of the mass of an atom is concentrated in a tiny fraction of total volume. This *nucleus* carries a positive electrical charge; the remaining volume of the atom has an equal negative electrical charge but very little mass.

The nucleus contains two kinds of particles: protons and neutrons. The *proton* carries the smallest amount of positive electrical charge yet observed, which can thus be called one unit of positive charge (+1). The *neutron* has nearly the same mass as the proton but carries no electrical charge.

The region surrounding the nucleus is occupied by particles called electrons. The *electron* carries an electrical charge equal in strength to that of

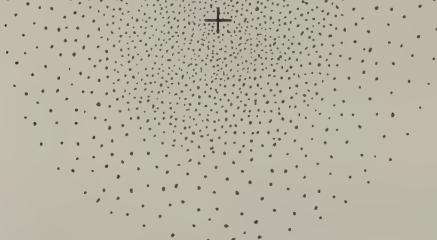
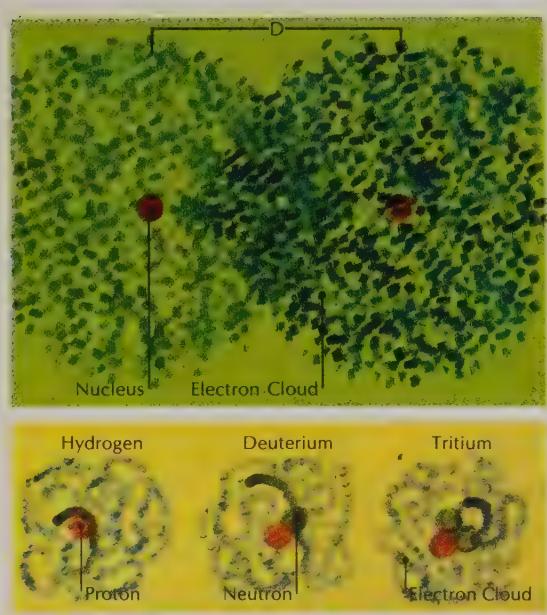


Figure 3.4 (above). The hydrogen molecule. D refers to the distance between the two nuclei in H_2 .

Figure 3.5 (below). The isotopes of hydrogen.



the proton but of negative sign—in other words, one unit of negative charge (-1). The mass of an electron is only $1/1840$ of the mass of a proton or neutron.

Other subatomic particles have been observed to exist briefly during high-energy nuclear reactions, such as those that occur in atomic bomb explosions and in the sun. In order to explain the observed properties of nuclear reactions, physicists are currently developing models in which the proton and neutron are themselves regarded as composed of yet smaller units. However, a simplified model that pictures the atom as composed of the three basic particles—protons, neutrons, and electrons—is sufficient to explain chemical reactions.

The lightest and simplest atom is that of hydrogen. Its nucleus is composed of a single proton, and a single electron occupies the space around the nucleus. The diameter of the nucleus is about 10^{-13} centimeter (0.000000000001 cm). The diameter of the entire atom is more difficult to define because it has no sharp boundary. It is possible, however, to measure the distance between the nuclei of two atoms joined together in a molecule. Half of this distance may be considered as a convenient representation of the radius of the atom. Such measurements indicate that the diameter of the hydrogen atom is about 10^{-8} cm.

As a way of visualizing the spatial relationships within the atom, suppose that it were enlarged to the size of a football stadium. The nucleus could be represented by a gum drop resting on the center of the field. The electron may be represented by a fly buzzing about the stadium, usually to be found somewhere inside the stadium but occasionally moving outside its boundaries as well. The picture serves as a reminder that most of the volume of an atom is empty space; it is also important to recall that nearly all of the mass of the atom is concentrated in the nucleus.

The hydrogen atom is distinguished from all other elements by the single proton in its nucleus. An atom of helium contains two protons in its nucleus; an atom of lithium contains three protons. In each case, the positive electrical charge of the protons is balanced by the negative charge of an equal number of electrons in the space surrounding the nucleus. Each element is made up of atoms whose nuclei contain a particular number of protons; this number is called the *atomic number* of the element. Carbon, with six protons in its nucleus, has an atomic number of 6, and the atomic number of oxygen is 8.

Although all atoms of a particular element have the same number of protons in the nucleus, not all have the same mass. About 0.016 percent of all hydrogen atoms, for example, have a mass about twice as great as that of a proton. These atoms contain one proton and one neutron in the nucleus. Such an atom is called hydrogen-2 (2H) and is said to have a mass number of 2. The *mass number* is equal to the sum of the number of protons and the number of neutrons in the nucleus. The two kinds of hydrogen atoms are called *isotopes* of hydrogen. All of the isotopes of a particular element have very similar chemical properties; they differ only in the number of neutrons in the nucleus of the atom.

Most chemical elements are made up of mixtures of isotopes. Oxygen, for example, has three stable isotopes. About 99.8 percent of natural oxygen is composed of oxygen-16 (with 8 protons and 8 neutrons in the nucleus). The remaining atoms are those of oxygen-17 (with 8 protons and 9 neutrons) and oxygen-18 (with 8 protons and 10 neutrons).

Although all three of these oxygen isotopes are stable, isotopes of other

elements do have unstable nuclei. These isotopes are said to be radioactive, and their nuclei undergo radioactive decomposition with the characteristic emission of such high-energy fragments as γ -rays (very high-energy light) or β -particles (high-energy electrons). Each radioactive isotope decomposes distinctively at a characteristic rate, the number of decomposition products depending upon the number of unstable nuclei present. With an appropriate detector, scientists can determine the concentration and nature of a particular radioactive isotope by observing the quantity and quality of its decomposition products.

The use of unusual isotopes of elements has had a sweeping impact in biology. Radioisotopes of carbon and hydrogen, discovered only a few decades ago, have allowed biologists to describe just how plants convert carbon dioxide, water, and sunlight into sugar and how humans convert that sugar back into usable energy, water, and carbon dioxide. Radioisotopes of sulfur and phosphorus were employed to prove that the carrier of genetic information was deoxyribonucleic acid (DNA). Today, radioisotopes such as iron, iodine, chromium, and cobalt are used routinely in

Table 3.1
Radioisotopes That Are Useful to Biologists

ISOTOPE	Atomic Number	Natural Abundance	Halflife	Mode of Decay
H ³	1	synthetic	12.3 years	β^-
C ¹⁴	6	synthetic	5730 years	β^-
O ¹⁸	8	.204 %	stable	none
P ³²	15	synthetic	14.3 days	β^-
I ¹³¹	.53	synthetic	8.1 days	β^-

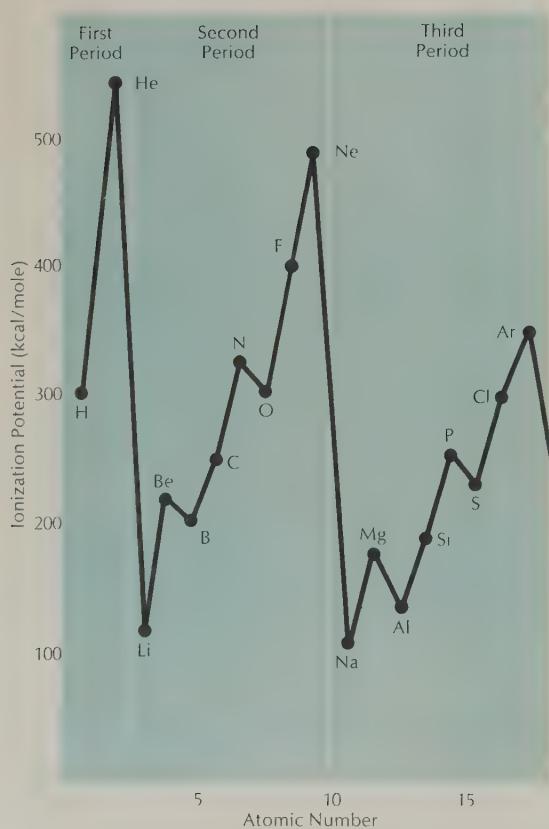
medicine, and many other isotopes are frequently used in the laboratory to uncover a vast array of biological processes. Table 3.1 shows the properties of a number of isotopes useful to biologists.

An atom is electrically neutral because the positive charge of the protons in the nucleus is balanced by the negative charge of an equal number of electrons around the nucleus. However, electrons can be removed from or added to an atom, giving it a net electrical charge. Such an electrically charged atom is called an *ion*. (Positively charged ions are called *cations*; negatively charged ions are *anions*.) For example, the electron may be removed from an atom of hydrogen, leaving only the nucleus with a charge of +1. Chemists use the symbol H⁺ to represent this hydrogen ion. Because there is an electrical force of attraction between the proton and the electron in the hydrogen atom, energy is required to remove the electron and form the hydrogen ion.

The amount of energy needed to remove an electron from the atom is called the *first ionization energy* (I_1) of the element; the energy needed to remove a second electron is called the *second ionization energy* (I_2), and so on. Table 3.2 shows the ionization energies for some of the lighter elements. Note the variations in the ionization energies of the elements; some elements lose electrons and form ions much more easily than do others. The first ionization energy may be regarded as a measure of the strength with which the atom holds its “loosest” electron. This property determines the chemical behavior of the elements.

The energies are measured in kilocalories per mole—a mole is 6.02 ×

Figure 3.6. The first ionization potentials for the first three periods of the periodic table.



10^{23} atoms, a number calculated such that the weight of a mole of atoms equals the atomic weight in grams. The same holds true for molecules—that is, 6.02×10^{23} molecules has the same molecular weight in grams. When discussing biological reactions, scientists speak of fractions of a mole because the amount of materials involved are often millionths or billionths

Table 3.2
Ionization Energies of Some Elements (kcal/mole)

ELEMENT	Chemical Symbol	Atomic Number	I_1	I_2	I_3	I_4
Hydrogen	H	1	313.5			
Helium	He	2	566.9	1254		
Lithium	Li	3	124.3	1744	2823	
Beryllium	Be	4	214.9	419.9	3548	5020
Boron	B	5	191.3	580.0	874.5	5980
Carbon	C	6	259.6	562.2	1104	1487
Nitrogen	N	7	335.1	682.8	1094	1786
Oxygen	O	8	314.0	810.6	1267	1785
Fluorine	F	9	401.8	806.7	1445	2012
Neon	Ne	10	497.2	947.2	1500	2241
Sodium	Na	11	118.5	1091	1652	2280
Magnesium	Mg	12	176.3	346.6	1848	2521
Aluminum	Al	13	138.0	434.1	655.9	2767
Silicon	Si	14	187.9	376.8	771.7	1041
Phosphorus	P	15	254	453.2	695.5	1184
Sulfur	S	16	238.9	540	807	1091
Chlorine	Cl	17	300.0	548.9	920.2	1230
Argon	Ar	18	363.4	637.0	943.3	1379
Potassium	K	19	100.1	733.6	1100	1405
Calcium	Ca	20	140.9	273.8	1181	1550

Source: B. H. Mahan, *University Chemistry*, 2nd ed. (Reading, Mass.: Addison-Wesley, 1969), p. 444.

of a mole (micromoles or nanomoles, respectively). Another unit of energy is the kilocalorie. A kilocalorie is 1,000 calories and is often replaced in discussion of food energy by the synonymous term “Calorie” (note the capital C). One calorie (small c) is defined as the amount of energy required to raise the temperature of 1 gram of water by 1° Centigrade. A kilocalorie is the amount of energy required to keep a 100 watt light bulb lit for 40 seconds. Human beings require about 2,000 Calories of food energy per day to sustain normal activity—slightly less than the continuous burning of a 100 watt bulb.

Electrons and Chemical Properties

When the first ionization energies of Table 3.2 are plotted on a graph (Figure 3.6), a regular pattern is visible. The first ionization energies of the elements helium, neon, argon, krypton, and xenon are much higher than those of the neighboring elements, whereas the first ionization energies of lithium, sodium, potassium, rubidium, and cesium are extremely low.

These regularities correspond to chemical properties of the elements. Lithium, sodium, potassium, rubidium, and cesium belong to a group of elements called *alkali metals*. They form metallic solids and react readily with many other elements, releasing large amounts of energy in most of these reactions. The alkali metals all tend to form positive ions, such as Li^+ ,

Figure 3.7. The relative atomic sizes of the inert gases.

Na^+ , and K^+ . These properties appear to be due to the ease with which an electron can be removed from the alkali atom.

Helium, neon, argon, krypton, and xenon are strikingly different from the alkali metals in their chemical properties. These elements belong to a group called the *inert gases*, and they are distinguished by their almost total lack of chemical activity. Under normal conditions, the inert gases do not take part in any chemical reactions. Again, these properties are consistent with the fact that it is extremely difficult to remove an electron from the atom of an inert gas.

The elements that have an atomic number one greater than the alkali metals form a group called the *alkaline-earth metals*: beryllium, magnesium, calcium, strontium, barium, and radium. These elements are also chemically active, but the atom of an alkaline-earth metal tends to lose two electrons in chemical reactions, forming an ion such as Be^{++} , Mg^{++} , or Sr^{++} . The tendency to lose two electrons can be readily explained on the basis of the information in Table 3.1. Lithium, for example, easily loses its first electron, but a much greater amount of energy is needed to remove a second electron. Beryllium, on the other hand, loses its first and second electrons relatively easily but strongly resists the removal of a third electron.

Both the lithium ion (Li^+) and the beryllium ion (Be^{++}) are left with two electrons, the same number of electrons as are present in the neutral atom of the inert gas helium. In fact, there is a general tendency for atoms to gain or lose electrons in such a number that the remaining electron structure will be similar to that of an inert gas. It appears that the electron structure of the inert gases is an extremely stable one. The *halogens* (fluorine, chlorine, bromine, and iodine) hold their electrons almost as tightly as do the inert gases. However, these elements tend to gain an electron in chemical reactions, forming ions such as F^- and Cl^- and thus attaining electron populations similar to those of inert gases.

The electron populations of the inert gases are 2, 10, 18, 36, 54, and 86—a series that shows a certain elusive regularity. (Note the differences between successive numbers in the series.) The regularity is best shown in the *periodic table* (Figure 3.8), in which the elements are listed in order of increasing atomic number. Each vertical column of the table includes elements of similar chemical properties.

The periodic table was developed in 1869 by the Russian chemist Dmitri Mendeléev on the basis of the 63 elements then known. During the following decades, chemists discovered many new elements, guided in their search by the blank spaces in Mendeléev's table. Mendeléev had arranged the elements in order of increasing atomic weight, although he had to make a few exceptions to this rule in order to keep elements of similar chemical properties in the same column. Although the table proved extremely useful in organizing the elements, chemists had no theory to account for the regularities that it revealed.

In the early years of the twentieth century, a number of new discoveries helped to make sense of the table. The discovery of the subatomic particles (electrons and protons) led to Ernest Rutherford's model of the nuclear atom and to Henry Moseley's measurements of atomic numbers. With this information, it became clear that the regularities in the periodic table reflect the tendency for atoms to achieve certain stable electron populations. However, there was still no explanation for the tendency to seek these particular electron populations.

The clues that led toward an explanation came—as so often happens in



Figure 3.8 (above). Periodic table of the elements, first developed by Dmitri Mendeléev in 1869.

Figure 3.9 (below). The energy of a light wave, or photon, is given by the relative $E = h\nu$ (Planck's constant). As ν increases, the energy of the photon increases. Note that the wavelength (λ) becomes smaller as the energy increases. Thus, x-rays or ultraviolet rays that have short wavelengths are more energetic than visible or infrared light rays, which have longer wavelengths.

	Group I		II																										
	Period																												
1	H 1												He 2																
2	Li 3	Be 4											B 5	C 6	N 7	O 8													
3	Na 11	Mg 12	Transition Elements										Al 13	Si 14	P 15	S 16													
4	K 19	Ca 20	Sc 21	Ti 22	V 23	Cr 24	Mn 25	Fe 26	Co 27	Ni 28	Cu 29	Zn 30	Ga 31	Ge 32	As 33	Br 35	Kr 36												
5	Rb 37	Sr 38	Y 39	Zr 40	Nb 41	Mo 42	Tc 43	Ru 44	Rh 45	Pd 46	Ag 47	Cd 48	In 49	Sn 50	Sb 51	I 53	Xe 54												
6	Cs 55	Ba 56	*	Hf 72	Ta 73	W 74	Re 75	Os 76	Ir 77	Pt 78	Au 79	Hg 80	Tl 81	Pb 82	Bi 83	At 85	Rn 86												
7	Fr 87	Ra 88	*											* La 57	Ce 58	Pr 59	Nd 60	Pm 61	Sm 62	Eu 63	Gd 64	Tb 65	Dy 66	Ho 67	Er 68	Tm 69	Yb 70	Lu 71	
	*	Ac 89	Th 90	Pa 91	U 92	Np 93	Pu 94	Am 95	Cm 96	Bk 97	Cf 98	Es 99	Fm 100	Md 101	(Ts 102)														

science—from studies that seemed completely unrelated. The German physicist Max Planck concluded from his studies of energy radiated by heated objects that electromagnetic energy (such as light or radio waves) is not continuous but, like matter, comes in small, discrete packages. Planck called these packages of energy quanta, and he showed that the energy of a single quantum of radiation, or photon, is related to the frequency of the radiation by the simple equation

$$E = h\nu$$

where E = energy of a single quantum,
 h = a number called Planck's constant, (3.1)
and $\nu(\text{nu})$ = the frequency of the radiation.

Planck's quantum theory, published in 1900, represented such an extreme departure from the accepted laws of physics that physicists were hesitant to accept it. It proved so useful, however, that its publication is today considered to mark the dividing line between classical and modern physics.

One of the most impressive successes of the quantum theory was its application to the structure of the atom. It had been known for half a century that gaseous elements produce radiation of certain characteristic frequencies when the gas is burned or when an electrical discharge is passed through it. A solid heated to high temperatures produces radiation of a wide range of frequencies, the frequency of greatest radiation depending upon the temperature of the solid. When light from such an incandescent solid is analyzed in the device called a spectroscope (Figure 3.10), a continuous spectrum is produced. In contrast, the radiation from a gaseous element produces a series of bright lines; each element produces its own character-

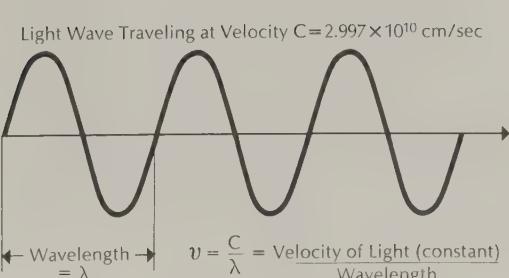


Figure 3.10. A continuous spectrum (upper portion) is generated by the hot filament of an incandescent bulb. A line spectrum (lower portion) is produced by the excitation of electronic energy levels in the atoms of an element heated by a flame. (From General Chemistry, by Linus Pauling, Third edition. W. H. Freeman and Company. © 1970)

istic line spectrum. These lines are not randomly placed but occur in series with very regular patterns. Table 3.3, for example, shows the line spectrum of hydrogen gas. There are two groups of lines—one in the visible part of

Table 3.3
Lines in the Spectrum of Hydrogen

LINE	Frequency (cycles/second)	Wavelength (centimeters)	Energy of Photons (kcal/mole)
<i>Visible Group</i>			
1	4.57×10^{14}	6.57×10^{-5}	43.6
2	6.17×10^{14}	4.86×10^{-5}	58.8
3	6.91×10^{14}	4.34×10^{-5}	65.9
4	7.31×10^{14}	4.10×10^{-5}	69.7
5	7.55×10^{14}	3.97×10^{-5}	72.1
6	7.71×10^{14}	3.89×10^{-5}	73.6
<i>Ultraviolet Group</i>			
1	24.65×10^{14}	1.22×10^{-5}	235.2
2	29.22×10^{14}	1.03×10^{-5}	278.8
3	30.81×10^{14}	0.97×10^{-5}	294.0
4	31.49×10^{14}	0.95×10^{-5}	301.1
5	31.96×10^{14}	0.94×10^{-5}	304.9

Source: Adapted from Chemical Education Material Study, *Chemistry: An Experimental Science* (San Francisco: Freeman, 1963), p. 255.

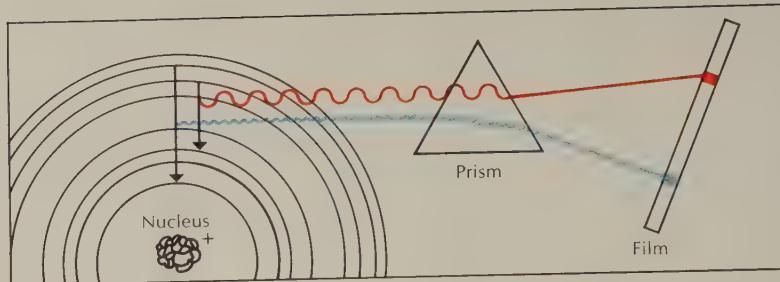
the spectrum, the other in the ultraviolet part. Each group shows a regular decrease in the spacing between lines with increasing frequency.

Although physicists had developed mathematical expressions to summarize the regularities in line spectra, they had no explanation for their existence. One of Rutherford's students, Niels Bohr, felt that spectra must be related to the structure of the nuclear atom proposed by Rutherford. Bohr felt that a combination of Planck's quantum theory and Rutherford's



Figure 3.11 (above). Origin of the line spectra of elements. Electrons in excited energy levels fall into levels of lower energy. During this process, they emit a photon of light whose energy is equal to the energy difference between the initial and final energy level of the electron.

Figure 3.12 (below). Diagram showing the ultraviolet energy level of hydrogen. When an electron excited to the third energy level in hydrogen drops to the second energy level, it emits a photon equal in energy to the difference between the two levels.



nuclear atom model might produce a better explanation of the line spectra. Bohr's quantized model of atomic structure did indeed prove useful and became the basis of modern models.

Using Planck's equation (Formula 3.1), one can translate the frequency of the lines in the hydrogen spectrum into terms of energy. The righthand column of Table 3.3 shows the energy per mole of photons (quanta of light) for each of the hydrogen spectral lines. Bohr suggested that an electron does not move randomly about the nucleus but can exist only at certain levels of energy. When an electron moves from one energy level to a lower one, a photon is produced with energy just equal to the difference between the two energy levels. When the hydrogen is heated or subjected to electrical discharge, electrons are excited—that is, they obtain energy and move to the higher energy levels. As they drop back to the more stable, lower energy levels, electrons emit photons of just those frequencies corresponding to the energy differences between the possible levels.

Because the lines in the ultraviolet group are produced by photons of greater energy than those producing the visible lines, it seems logical to suppose that the ultraviolet lines are produced by electrons falling from higher energy levels to the lowest possible energy level. Using this assumption and assigning an arbitrary energy value of 0.0 kcal/mole to the lowest energy level, one can obtain the set of energy levels shown in Figure 3.12. The series of lines in the ultraviolet group does not end with the sixth line but continues with an apparently infinite number of lines spaced ever more closely. The limit approached by the lines of highest energy is 313.5 kcal/mole. Presumably, this number corresponds to the amount of energy released as a free electron is captured by the atom and moves into the lowest possible energy level.

What would happen if an electron moved from the third to the second energy level? The energy of the electron would drop from 278.8 to 235.2 kcal/mole, and thus a photon with energy of 43.6 kcal/mole should be emitted. Such photons are emitted, producing the first line of the visible group. Similarly, all of the visible lines can be explained by movements of electrons from higher levels to the second energy level. Similar series of lines are formed in the infrared (low-energy) photons, just as would be predicted for electrons falling to the third, fourth, and higher levels.

This model indicates that 313.5 kcal/mole of energy are released as a free electron falls into the first energy level. Therefore, 313.5 kcal/mole of energy should be needed to remove an electron from the atom, and this number is indeed the ionization energy of hydrogen (Table 3.2).

Bohr's model provided an explanation of the relationship between the various groups of lines in the hydrogen spectrum. It was based, however, upon the assumption (unjustified by the laws of physics developed through study of macroscopic objects) that electrons could exist only at certain discrete energy levels within the atom. Furthermore, Bohr's model did not provide quantitatively correct explanations of the spectra of elements other than hydrogen. For these reasons, physicists were reluctant to accept Bohr's model. The contradictions and problems were eventually resolved in the theories of quantum mechanics, developed during the 1920s.

In the modern model of atomic structure, the motion of each electron in an atom is described by an orbital, a description of the space in which the electron is most likely to be found. According to the theories of quantum mechanics, it is not possible to describe an exact path for an electron. All

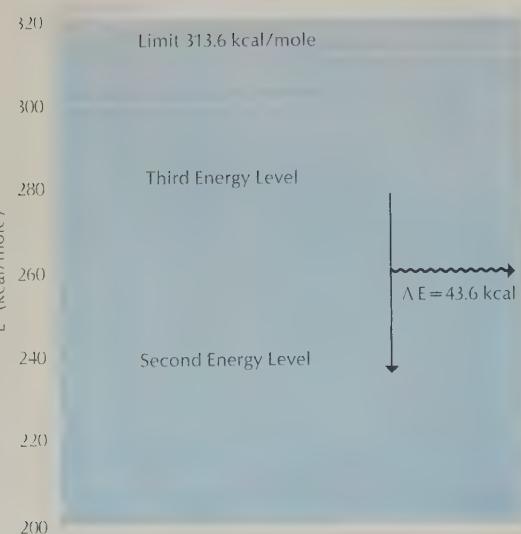


Figure 3.13. The Bohr model of a hydrogen atom. This model postulates an atom in which an electron travels in one of several orbits. When the electron falls from a higher energy orbit to a lower energy level, it emits light of a certain wavelength. Each group of arrows represents energy transitions that give rise to spectral lines. These energy transitions are shown in the spectral lines below.

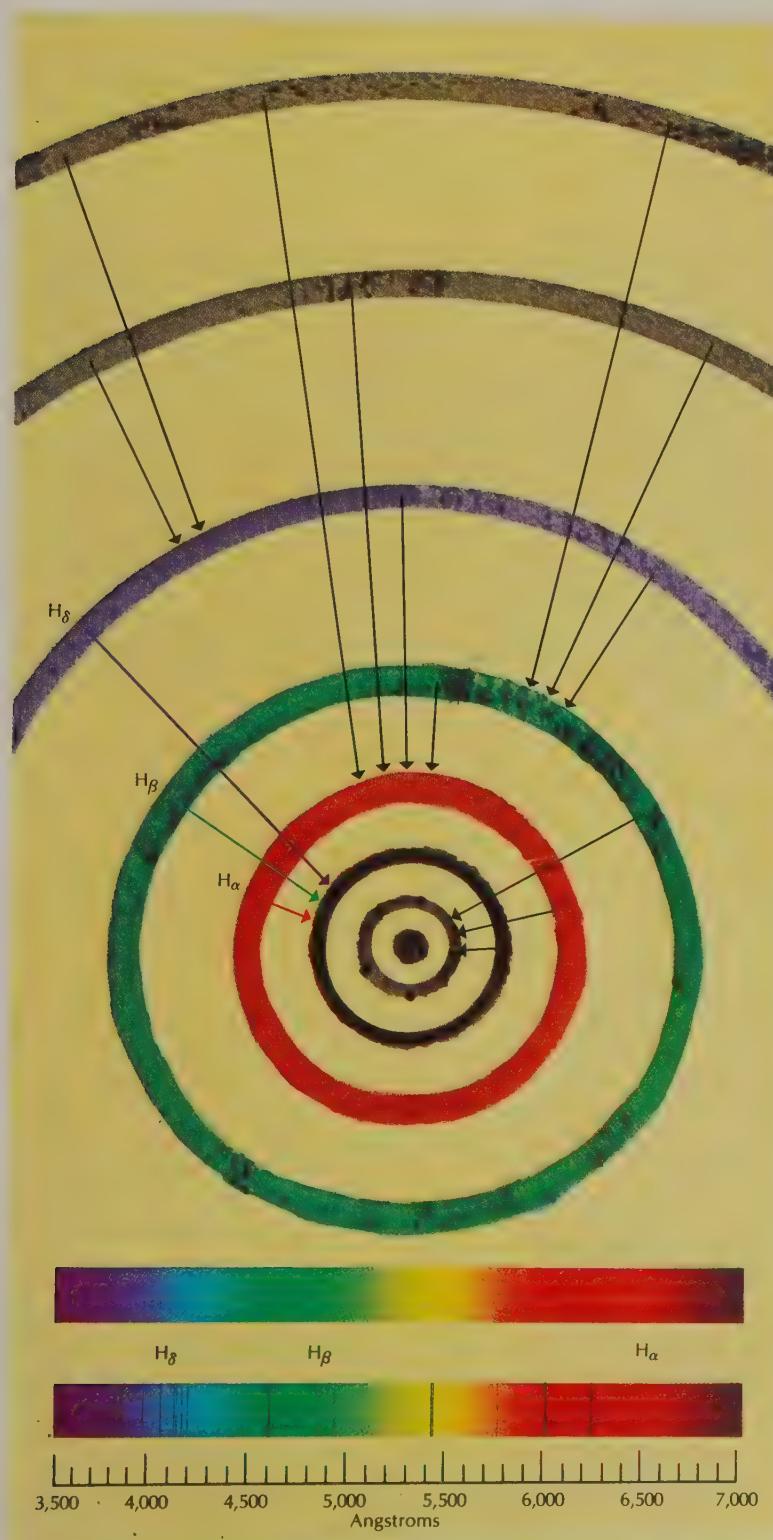
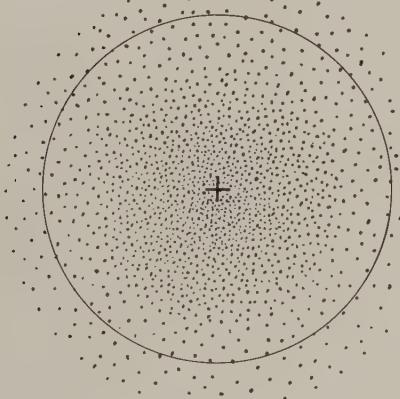


Figure 3.14. Cross section of an atom. Although there is a probability that the electrons of an atom may be found anywhere in space, a sphere may be defined so that the probability of finding the electrons inside is nearly 100 percent.



that can be done is to predict the probability of finding the electron at any given point in space around the nucleus. Each pair of electrons in an atom moves in a distinct orbital of a particular energy level, and the shapes of the orbitals help to explain the positions of atoms within a molecule.

Because the energy levels of some orbitals are nearly identical, it is possible to describe most of the chemical properties of elements in terms of groups of orbitals, or *electron shells*. Each of the inert gases has a full outer electron shell, and other elements tend to gain or lose electrons in order to achieve a full outer shell. The first shell can hold only 2 electrons; the inert gas helium, for example, fills this shell with its 2 electrons. The second shell can hold 8 electrons; the 10 electrons of neon fill both the first and second shells. The third shell also can hold 8 electrons; the 18 electrons of argon fill the first, second, and third shells.

In the next row of the periodic table, potassium and calcium show the properties to be expected if they contain, respectively, 1 and 2 electrons in the fourth shell. However, the next 10 elements—scandium through zinc—behave chemically as if they also have only 2 electrons in the outer shell. Apparently, after 2 electrons have been placed in the fourth shell, the third shell is able to accept another 10 electrons. The 10 elements (zinc through scandium) are called transition metals and are somewhat similar in their chemical and physical properties. After the third shell has been expanded to 18 electrons, the filling of the fourth shell resumes; the properties of gallium show that it has 3 electrons in the fourth shell. The fourth shell is completed with 8 electrons in the krypton atom.

The number of electrons in each shell for each atom is indicated in the periodic table. The elements of atomic numbers 57 through 71 differ only in the number of electrons in the third from the outermost shell. Because chemical properties are determined primarily by the outer electrons, these elements are very similar chemically—so similar, in fact, that it is difficult to separate them by chemical methods.

This picture of electron structure provides a model that accounts for the regularities summarized in the periodic table. Each element in a particular column has a similar population of electrons in its outer shells; each tends, through the gain or loss of electrons, to achieve a full outer shell.

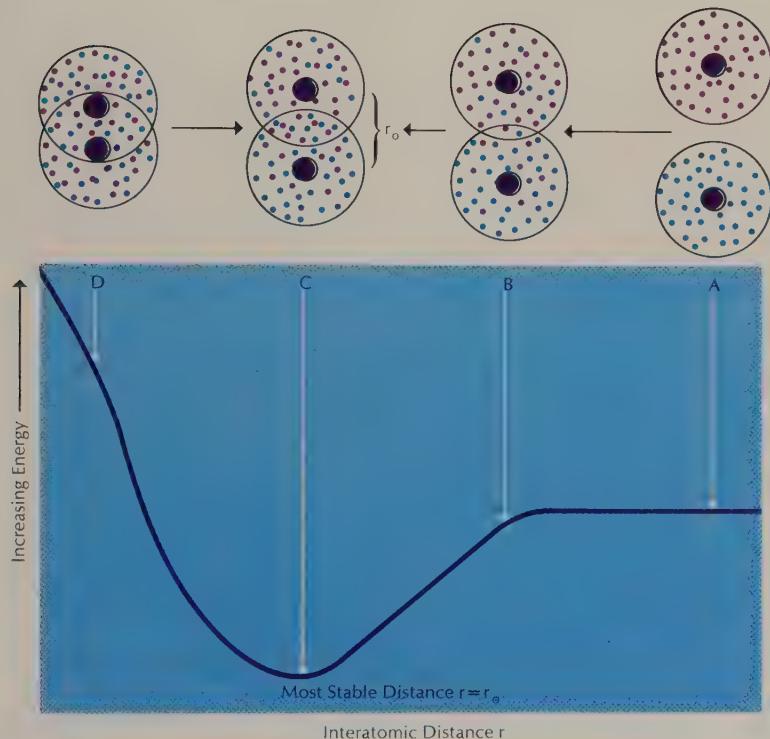
The ionization energies shown in Table 3.2 can be explained in terms of this model. The ionization energy represents the energy needed to take an outer electron from its normal energy level and move it to a level so high that it is free from the nucleus. In the lithium atom, it is relatively easy to remove the single electron from the second shell, but a much greater amount of energy is needed to remove the remaining electrons from the lower energy level of the first shell. The beryllium atom has two electrons in the second shell that can be removed relatively easily.

CHEMICAL BONDS

The helium atom, with two electrons filling its first shell, is very stable. The hydrogen atom has only one electron in this shell and is much more reactive chemically. It readily becomes bonded to other atoms to form molecules. For example, hydrogen gas at normal temperatures is made up not of individual hydrogen atoms but of diatomic (two-atom) molecules composed of two hydrogen atoms bound together. Chemists symbolize this molecule as H_2 .

The formation of this bond can be understood by visualizing what happens as two hydrogen atoms approach each other. When the atoms are

Figure 3.15. Formation of the chemical bond. From right to left, A depicts two free atoms that are moving in space. In B, the atoms are starting to interact, and the energy of the pair is decreasing as they share their electrons. The bond is fully formed in C, and the energy of the pair is minimized at its most stable internuclear distance, r_0 . In D, any attempt to push the atoms closer together causes the energy of the system to rise rapidly because of internuclear repulsion. This most stable distance, r_e , is the band length.



close together, there is electrical attraction between the nucleus of each atom and the electron of the other. There is also electrical repulsion between the two nuclei and between the two electrons. The attractive forces will tend to hold the two atoms together, and the repulsive forces will tend to push them apart. Because it is known that the two atoms do tend to remain together at some characteristic internuclear distance, the attractive forces must be stronger than the repulsive forces.

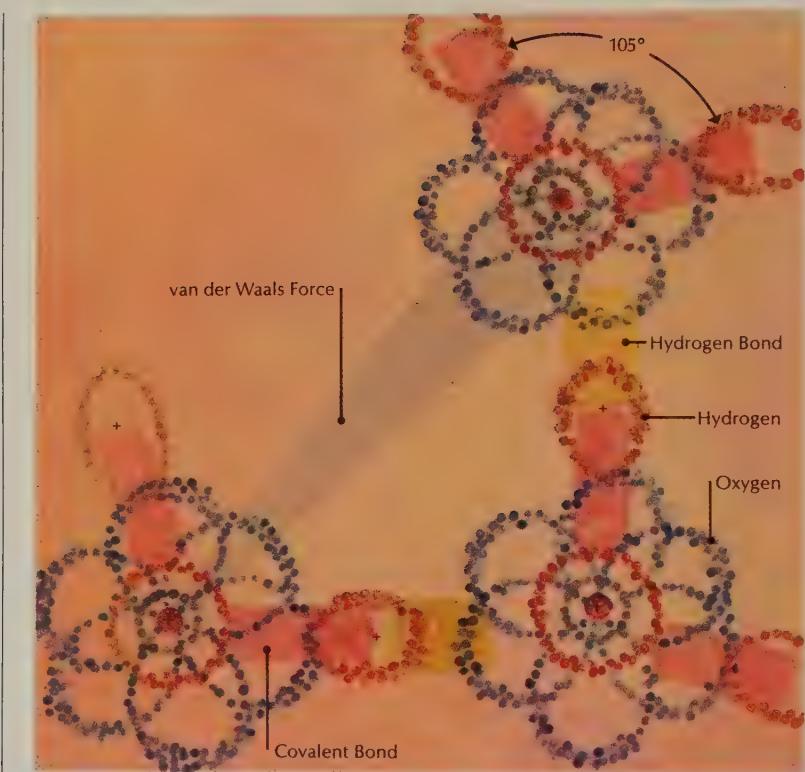
Each hydrogen atom has a “vacancy” in its outer shell. When the two atoms are close together, the unfilled orbitals overlap. In the region of overlap, each electron can be shared by both nuclei. Because each electron is attracted to both nuclei and is able to move within a stable orbital, there are strong attractive forces holding the two atoms together. In fact, it appears that the two electrons correlate their movements in such a way that they stay far apart from each other, minimizing the repulsive forces between the two electrons and helping to hold the molecule together.

In the H—H bond, electrons are shared equally by the two nuclei. In this way, each atom has approximated the stable electron population of a full outermost shell. Such a bond, in which electrons are shared between nuclei, is called a *covalent bond*. Electron-dot formulae are used to symbolize the electrons of the outermost shell involved in chemical bonds. Each hydrogen atom, with its single electron, may be represented as $\text{H}\cdot$, and the hydrogen molecule with its shared pair of electrons may be written as $\text{H}:\text{H}$.

A helium atom has two electrons filling its outer energy level ($\text{He}::$). If two helium atoms approach each other, little overlap of orbitals can occur because each outer shell is fully occupied. The helium atoms therefore do not form bonds but remain as independent atoms.

The oxygen atom has six electrons in its outer shell, with room for two

Figure 3.16. Types of chemical bonds. Shown here in the water molecule are covalent bonds, which bind atoms through shared electrons; hydrogen bonds, which are strong electrical links; and van der Waals forces, which are weaker electrical links between the oxygen nucleus and electrons of an oxygen atom.



more. Therefore, oxygen would be expected to form covalent bonds, sharing two electrons with other atoms. For example, each of the unpaired electrons could be shared with a hydrogen atom, forming a molecule that could be represented as H—O—H or as H_2O , the water molecule.



The oxygen nucleus has a charge of 8^+ , whereas the hydrogen nucleus has a charge of only 1^+ . Thus, the oxygen nucleus exerts a much stronger attractive force on the shared electrons than does the hydrogen nucleus, and the shared electrons are most likely to be found nearer the oxygen nucleus. As a result, the portion of the molecule near the oxygen nucleus has an excess of negative charge, whereas the portions near the hydrogen nuclei have an excess of positive charge. A bond such as the O—H bond, in which the two nuclei do not equally share the electrons, is a polar covalent bond and is said to have partial ionic character. In a completely *ionic bond*, the bonding electrons are not shared at all but are held entirely by one of the nuclei; the two atoms are held together solely by the electrical attraction between the cation (the atom that has lost an electron) and the anion (the atom that has gained an electron).

Such ionic bonds are formed in table salt (sodium chloride, NaCl). Within a crystal of salt, there is a regular latticelike arrangement of sodium (Na^+) and chloride (Cl^-) ions, with each ion attracted to neighboring ions of the

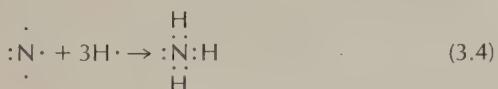
opposite charge. Because no covalent bonds are formed, there is really no molecule of NaCl. Table salt dissolves readily in water because the Na^+ and Cl^- ions separate readily under the electrical attractions of the positive and negative ends of the water molecule.

Oxygen, like hydrogen, forms a diatomic gas (O_2 molecules). In this case, two pairs of electrons must be shared between the two oxygen atoms:



Such a *double bond* is stronger than a single bond (where only one pair of electrons is shared) and draws the two nuclei closer together. In a simple diagram, the double bond may be represented by the symbol $\text{O}=\text{O}$.

Nitrogen has only five electrons in its second shell and therefore can share three pairs of electrons with atoms such as hydrogen, forming the molecule of ammonia (NH_3).



Like hydrogen and oxygen, nitrogen exists as a diatomic gas (N_2). Here, the two atoms share three pairs of electrons, forming a triple bond that may be represented as $\text{N}::\text{N}$ or as $\text{N}\equiv\text{N}$.

Organic Chemistry

Carbon has four electrons in its outer shell and therefore can share four pairs of electrons with hydrogen atoms to form methane (CH_4).



The importance of carbon to the chemistry of living systems arises from its exceptional tendency to form strong, stable covalent bonds with other carbon atoms. Thus, long chains of carbon atoms may be formed, with various other atoms bonded to the remaining unpaired electrons of the carbon atoms. Because the carbon chains can branch or form rings, millions of carbon compounds can be formed.

Other elements such as silicon or boron form strongly bonded chains in the elementary state, but carbon is unique in that it forms strong chains even when various other atoms are bonded to the remaining unpaired electrons of the carbon atoms. When chains of carbon atoms branch or reunite to form rings, additional structures are available. With the endless possibilities of bonding among carbon atoms, it is not surprising that the study of carbon compounds is an entire field in itself, a field known as organic chemistry. The name denotes the central importance of carbon-containing molecules (or organic compounds) to living organisms. Much of the recent intense search for extraterrestrial life has focused on looking for organic molecules on the various planets and in specimens of rock and dust retrieved from the moon. The existence of past or present life would be

Figure 3.17. Sodium chloride (NaCl) – a cubic ionic crystal. The brown spheres are sodium ions and the gray spheres chloride ions.

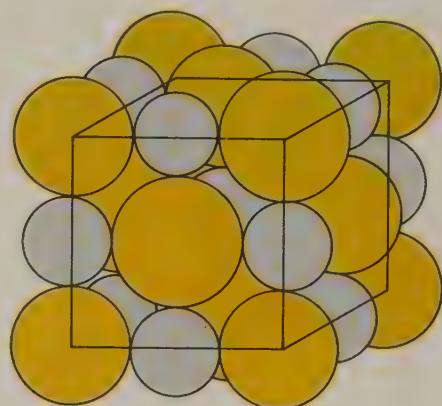
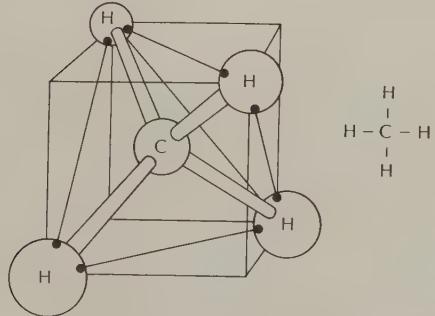


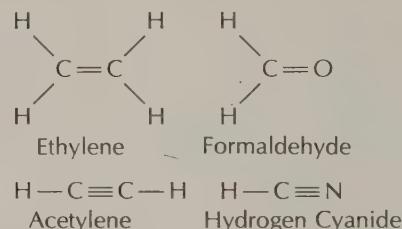
Figure 3.18. The tetrahedral structure of methane. Note that the tetrahedral arrangement of electron pairs minimizes interelectronic repulsion.



indicated if scientists found organic compounds. Thus far, the search has proved fruitless.

A most important feature of organic chemistry is the carbon atom bound to four other atoms (commonly termed tetravalent carbon). The covalent carbon-hydrogen bonds in such a compound as methane are called *single bonds*. In such tetravalent carbon compounds, the four bonds of the central atom are directed toward the vertices of a regular tetrahedron. Each bond forms an angle of 109.5° with its neighbors. This angle is the maximum separation of bonds attainable with four substituents bound to a central atom, and it permits great bond strength together with the least unfavorable interactions between nonbonded atoms. The remarkable strength of diamonds is accounted for by the fact that they consist of a three-dimensional lattice of tetrahedral carbon atoms.

Carbon can enter into multiple bond formation with itself or other atoms. In a compound such as ethylene or formaldehyde, two electrons from a carbon atom are shared with two from a second atom to make a four-electron bond, called a *double bond*. When three electrons from carbon are shared with three from a second atom, as in the case of acetylene or hydrogen cyanide, a six-electron bond, or *triple bond*, is formed.



All the above compounds are used extensively in the manufacture of plastics. In addition, acetylene is used as a fuel in welding; hydrogen cyanide is a well-known poison; and formaldehyde, due to its usefulness as a preservative, is widely used for embalming.

Although stronger than a single bond, a double bond is not fully twice as strong; similarly, a triple bond is not 3/2 as strong as a double bond. Thus, organic molecules containing multiple bonds often tend to undergo reactions in which single bonds are created at the expense of destroying double or triple bonds. Many reactions involving carbon compounds in living systems depend on the reactivity of a few of these multibonded functional

groups of atoms, such as the *C=O* bond (carbonyl group) depicted in formaldehyde. Another rather special type of multiple bonding in organic chemistry is exemplified by benzene. This compound and others with related structures are called aromatic substances — presumably because many that are volatile have a pleasing aroma. The additional stability of these compounds is a unique property of certain organic ring compounds containing alternating double bonds. Many such aromatic structures are found in molecules essential for living organisms. Like the substituents attached to double and triple bonds, the substituents attached to aromatic rings lie in a single plane.

Organic molecules typically contain tens or hundreds of atoms. Compounds containing only carbon and hydrogen are called *hydrocarbons*.

Figure 3.19 (above). The structural formula of polymeric polyethylene. Each molecule is composed of thousands of "monomer" CH_2 units.

Figure 3.20. Space-filling model (lower left) and structural formula (lower right) of glucose, a crystalline sugar.

Gasoline is a mixture of hydrocarbons, each containing about eight carbon atoms. Paraffin is a hydrocarbon containing about 20 carbon atoms. Polyethylene is a hydrocarbon containing thousands of carbon atoms linked together in a simple chain (Figure 3.19).

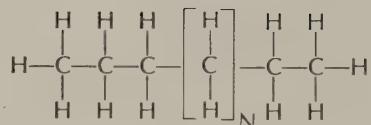
Polyethylene can be considered as composed of the basic— CH_2 —unit repeated over and over again. Molecules that are built up by joining together simpler units (*monomers*) are called *polymers*. Polyethylene is a simple polymer because it contains the same monomer repeated over and over again (a homopolymer). It is possible to construct polymers in which a number of different monomers are joined together. Such polymers, called heteropolymers, have interesting properties and play important roles in living systems. Both proteins and nucleic acids are heteropolymers.

Thus, carbon forms the backbone of most organic molecules, and the strongly bonded chains of carbon atoms can be considered a framework to which other atoms are bonded. The other three major elements of organic compounds—hydrogen, oxygen, and nitrogen—provide a range of bonding capabilities, forming one, two, and three covalent bonds, respectively. With only these four elements, it is possible to build an almost infinite range of molecules with almost any desired structure or chemical property. From these possibilities, the variety of living systems is constructed.

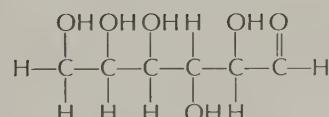
Other Reactive Groups

There is a bewildering variety of organic compounds. Some degree of simplicity can be obtained because there are a few important *reactive groups* of atoms that appear in many different kinds of molecules. Hydrocarbon molecules in which one or more hydrogen atoms are replaced by hydroxyl (—OH) groups are called *alcohols*. More than one group may replace hydrogen atoms in a hydrocarbon chain. Crystalline sugar consists of a hydrocarbon chain with alcohol groups and an aldehyde or carbonyl group replacing hydrogens attached to each carbon in the chain (Figure 3.20). The sensation of sweetness is associated with the aldehyde or carbonyl group; sugars taste sweet and perfumes have a pleasant smell because of the presence of one of these groups.

Like many biochemicals, sugars can be polymerized to form long chains. The sugar chains, or polysaccharides, include such substances as starch and cellulose. Sugars can react with other kinds of molecules to form nucleosides (a sugar combined with a nitrogenous base), nucleotides



The Hydrocarbon Polyethylene



The Sugar Glucose

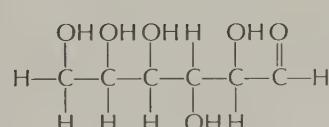
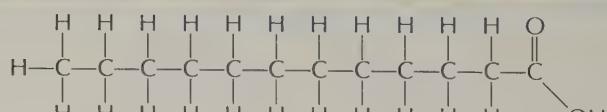
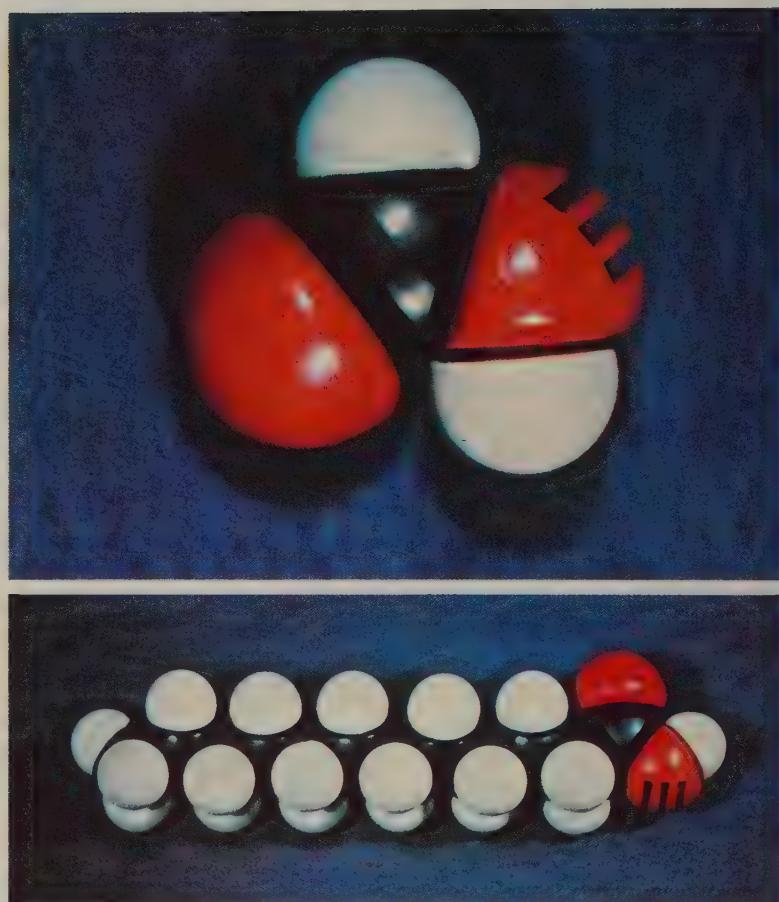
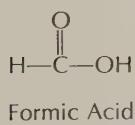


Figure 3.21. Structural formula (upper left) and space-filling model (upper right) of formic acid, the simplest organic acid.

Figure 3.22. Space-filling model (middle right) and structural formula (lower right) of lauric acid, a fatty acid.



Lauric Acid—A Fatty Acid

(sugar, nitrogenous base, and phosphoric acid), glycoproteins (sugar polymer and protein), and other complex associations.

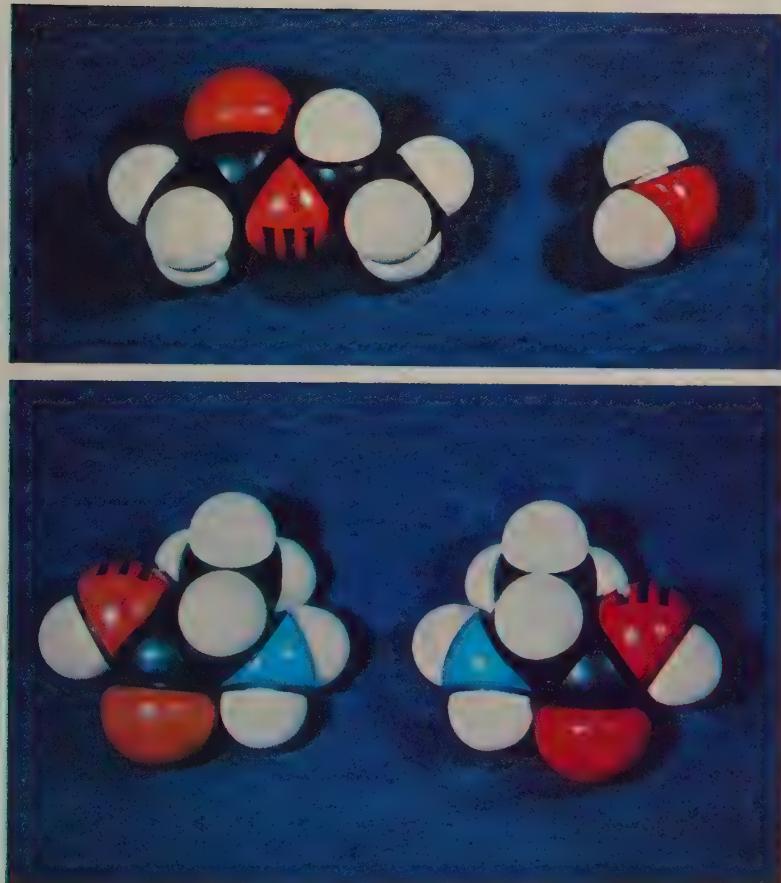
The carboxyl group (—COOH) is characteristic of the *organic acids*. The simplest organic acid is formic acid (Figure 3.21), which causes ant bites to be irritating. Another simple organic acid, acetic acid, gives vinegar its sour taste. The *fatty acids* are long hydrocarbon chains with a carboxyl group on the end (Figure 3.22).

An acid may react with an alcohol, eliminating a water molecule and forming an ester (Figure 3.23). This reaction is somewhat similar to the acid-base neutralization reaction that occurs in inorganic chemistry. However, alcohols do not act like bases; they do not ionize in water to give hydroxide ions. In fact, alcohols are nonelectrolytes.

One of the principal constituents of cell membranes is a *lipid* formed by the reactions of fatty acids, such as stearic acid, with an alcohol called glyc-

Figure 3.23. Space-filling model (upper left) and structural formula (upper right) of the ester ethyl acetate. The water molecule to the right of the ester is the other product of the reaction of ethyl alcohol and acetic acid, which formed the ester.

Figure 3.24 (middle right). Structural formula of a lipid molecule.



erol (Figure 3.24). Lipids are composed of an organic alcohol linked to organic acids through ester bonds.

The *amino acids* are among the most important small organic molecules. In these molecules, two reactive groups are found—a carboxyl group on one end and a basic configuration, the amino group ($-\text{NH}_2$), on the next carbon in the chain. There are about 20 commonly occurring amino acids in nature. All of them have the basic structure shown in Figure 3.25, in which R may represent any of various reactive groups.

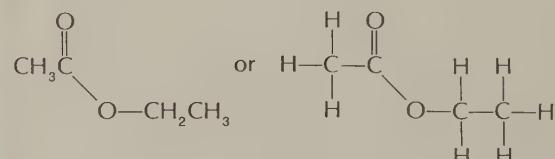
Electronegativity and Hydrogen Bonding

The electron-pulling ability of a nucleus involved in a covalent bond is called the *electronegativity* of that atom. The relative electronegativities of a few elements will serve as an example, where the symbol $>$ means greater than and \approx means equivalent to:

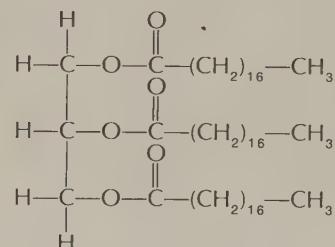
$$\text{F} > \text{O} > \text{N} \approx \text{Cl} > \text{Br} > \text{C} \approx \text{S} > \text{P} \approx \text{H} \quad (3.6)$$

The greater the electronegativity, the greater the tendency for the nucleus to draw the shared electron pair. The greater the difference in the electronegativity of two atoms, the more ionic will be the character of a bond between them. A bond of partial ionic character is often called a polar

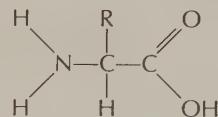
Figure 3.25. Space-filling models of amino acids (lower left) and structural formula (lower right) of the amino acid alanine. Amino acids may exist in two forms, L and D, which are related to each other by a mirror plane. Only L amino acids (the left model) are found in nature.



The Ester Ethyl Acetate

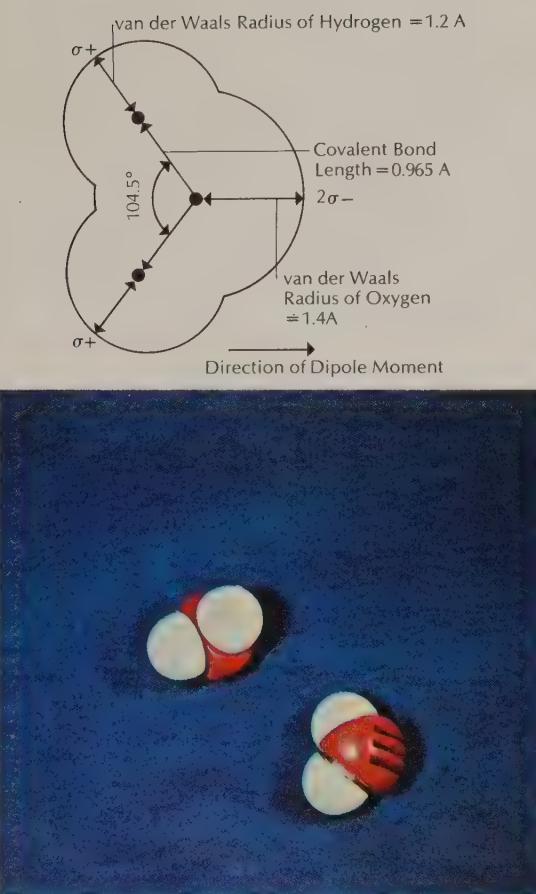


A Lipid Molecule



An Amino Acid

Figure 3.26. Crystallographic model (above) and space-filling model (below) of a water molecule, showing the bond angle and length.



bond because it possesses positively and negatively charged poles. It can be seen from the ordering given above that hydrogen fluoride (HF) is more polar than hydrogen chloride (HCl), which is more polar than hydrogen bromide (HBr). Water (H_2O) is more polar than ammonia (NH_3). The H—C bonds in methane are not very polar because the electronegativities of hydrogen and carbon are not very different.

There is an entire spectrum of bonds of varying polarity, ranging from completely covalent bonds such as H—H at one extreme to completely ionic bonds such as Na^+Cl^- at the other extreme. Most bonds in organic molecules are near the covalent end of this spectrum. These strong covalent bonds give organic materials much of their stability and structure.

The existence of polar bonds makes possible another form of bonding called *hydrogen bonding*. To emphasize the polar nature of the water molecule, its electron-dot formula can be written as



In this representation, the symbol δ represents a fraction of a unit charge.

If another water molecule approaches, there is a weak attraction between the positive hydrogen ends of one water molecule and the negative oxygen end of the other. This attraction creates something like a weak ionic bond between the two molecules. It is this type of bonding that produces the rigid structure of ice, and similar bonds give some semicrystalline structure to liquid water. Such bonds are called hydrogen bonds.

In organic molecules, hydrogen bonds occur most often when a polar bond containing a hydrogen atom occurs near a polar bond containing either an oxygen atom or a nitrogen atom. The hydrogen bonds play important roles in determining the three-dimensional structures of protein and nucleic acid molecules.

Water

All the complex substances in living organisms make up only a small fraction of the weight of living tissues. The principal component is water, which accounts for more than 75 percent of the weight of most tissues. Some exceptions are hair, horn, solid bone, spores, seeds, and so on, all of which are metabolically relatively inert. When spores (dormant bacteria) are transformed into cells showing active metabolism, an increase in water content always takes place.

To a chemist, water is an unusual substance possessing remarkable properties. It is a compound of extreme stability. It is a remarkable solvent. It does not mix with most organic substances but is strongly attracted by most inorganic substances, including itself. When frozen into a solid, it expands (unlike most other substances, which contract upon solidification). But because it is such an abundant substance and because of its paramount importance for living systems, water is often taken for granted without explicit discussion of its properties.

All of water's oddities can be understood in terms of its molecular structure (Figure 3.26). In the water molecule, there are eight valence electrons. Four are involved in covalent bonds between the oxygen atom and

Figure 3.27 (above). The tetrahedral structure of water.

the hydrogen atoms. The remaining four electrons are in nonbonding orbitals on the oxygen atom. The shape of the water molecules is an isosceles triangle, and the H—O—H bond angle is approximately 105°. The electronegativity of the oxygen atom makes the O—H bonds polar, with a net positive character at the hydrogen atoms and a net negative character at the oxygen atom. If a tetrahedron is described with the oxygen atom at its center and the hydrogen atoms at two of its corners, then the regions of highest density of the nonbonding electrons are directed toward the remaining two corners of the tetrahedron (Figure 3.27). This symmetry minimizes the electrical repulsions between the eight valence electrons.

The water molecule is thus an electrically polar structure. In a group of water molecules clustered together, a positively charged region on one molecule tends to be attracted toward a negative region on another molecule. There are two positive regions on each molecule—the hydrogen atoms—and two regions with negative character—the nonbonding electron densities. Thus, each water molecule can have four nearest neighbors. Each of the nonbonding pairs of electrons attracts a positive hydrogen atom on a neighboring water molecule, and each of the hydrogens attracts the oxygen ends of neighboring molecules. Thus, each oxygen atom is at the center of a tetrahedron of four other oxygen atoms. Equivalently, water molecules can be regarded as spheres, each with four nearest neighbors (Figure 3.28). This ordered structure represents the molecular arrangement in ice.

When ice melts, the higher coordinated structure of the crystal breaks in many places, but not all of the hydrogen bonds are broken. The molecules tend to pack closer together so as to fill up some of the vacant space. This packing results in the greater density of liquid water. When ice is converted to water at 0°C, only about 15 percent of the hydrogen bonds are broken. Cold water contains interconnected groups of water molecules whose structures are based on the same pattern as in ice, only with some broken bonds. As the temperature continues to rise, increasing thermal motion of the molecules tends to make the liquid expand again. The aggregates of water molecules are in dynamic equilibrium, constantly breaking up and re-forming, so that there are no permanent crystalline structures. As the temperature rises, more of the hydrogen bonds are broken, but a considerable amount of this *crystalline* structure remains even at 100°C.

It requires a remarkably large amount of energy to melt ice, to raise the temperature of liquid water, or to vaporize water. All of these phenomena (heat of fusion, heat capacity, and heat of vaporization, respectively) can be explained in terms of the highly ordered structure of water and are essential in maintaining a relatively constant temperature for living systems.

When the surface area of a liquid is increased, molecules that were formerly in the interior, surrounded by and attracted to neighboring molecules, must be brought to the surface. Work must then be done against the attractive forces operating between molecules in the interior. These forces are exactly those against which work must be done in vaporizing the liquid. In vaporization, however, the molecules are removed completely from the intermolecular forces, whereas for molecules on the surface the intermolecular forces remain except in the direction pointing away from the liquid phase. The result is that, at the surface, a molecule is in a state of higher energy than when it was in the interior. In accord with the tendency of all systems to attain a state of minimum energy, the surface of the fluid will

Figure 3.28 (below). Water molecules arranged in ice form. In the model shown, the molecules of ice have been pulled apart to show their configuration more clearly, whereas in an actual structure, the molecules would be closer together. In a similar model showing water in liquid form, the molecules would be more loosely organized, and they would be further apart and joined by more hydrogen bonds.

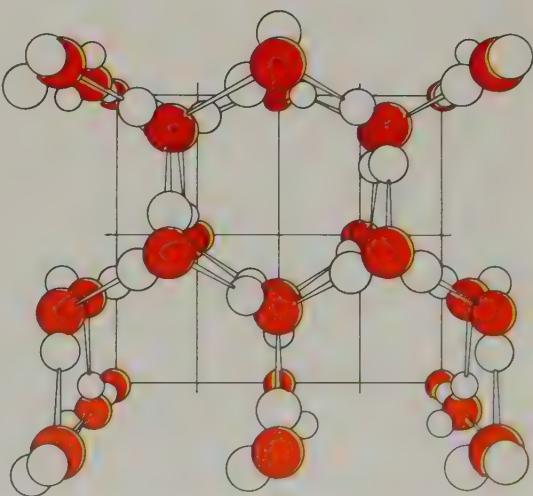
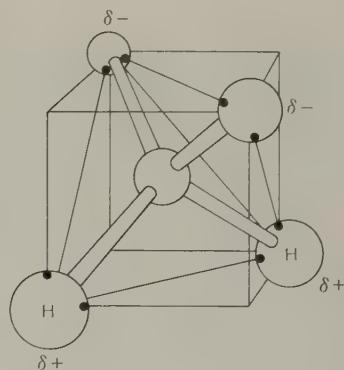


Figure 3.29. A water skipper feeding on water. This insect is able to walk on the surface of the pond because the water has such a high surface tension.



Figure 3.30. Salt dissolving in water. Polar water molecules cluster around each sodium and chloride ion as the salt dissolves.

assume a shape that minimizes the surface area, so as to maximize the number of molecules in the interior and minimize the *surface energy* of the system. Any tendency to deform the surface from this minimum area will be counteracted by the tendency of the molecules to remain in the interior.

It is therefore not surprising that substances with high heats of vaporization have high surface tensions. Water has the highest surface tension of any known liquid, with the possible exception of certain metals in the liquid state and some molten salts. This high surface tension accounts for the ability of water to rise in the capillary spaces of soil and plants. The surface tension is responsible for the ability of water skippers and other insects to walk on the surface of a pond.

Water is an excellent solvent for any polar substance, whether ionic or covalent. In the case of ionic substances (salts), the electrical attractions operating between ions in the crystalline state are overcome by electrical attractions between the ions and the oppositely charged moieties of the water molecules. For example, solid sodium chloride can be dissolved in water, even though the crystal itself is an extremely stable configuration, because the process of dissolution lowers the energy of the system still further. The sodium ions will be surrounded by water molecules with their oxygen atoms oriented toward the sodium ion; the chloride ion will be surrounded by water molecules with their hydrogen atoms oriented toward the Na^+ . If this system was not more stable than the initial state consisting of $\text{solid}_{\text{NaCl}}$ and water, then dissolution would not occur.

Numerous nonionic compounds also dissolve in water. Notable examples are molecules containing the polar amino, hydroxyl, carboxyl, and keto groups, all of which can form hydrogen bonds with the water molecules. On the other hand, substances containing large hydrocarbon moieties and other nonpolar groups are generally only slightly soluble, if at all, in water. Such substances tend to be concentrated in droplets that coalesce at the surface in films because they are not attracted by the water molecules.

Bond Energies

Table 3.4 shows average bond energies for some of the bonds that are important in organic compounds. The average bond energy represents the approximate amount of energy needed to break that bond in any compound in which it occurs. The actual bond energy in a particular molecule will vary somewhat from these average values, depending upon the other atoms that are attached to the atoms involved in the bond. The values in this table provide a reasonable estimate of the strength of various bonds involved in organic molecules and of the energy that can be released as such a bond is formed.

IONS, ACIDS, AND BASES

Because most biochemical reactions take place in solution, the study of the nature of molecules in the dissolved state is essential. When ionic substances are dissolved in water, the polar water molecules cluster around the ions, separating them and yielding a solution of positively charged and negatively charged ions scattered throughout the water molecules. The substance being dissolved is called the *solute*, and the substance in which it is dissolved is called the *solvent*. If an electric voltage is applied across the solution, the positive ions move toward the negative terminal, and the negative ions move toward the positive terminal. This flow of ions produces an

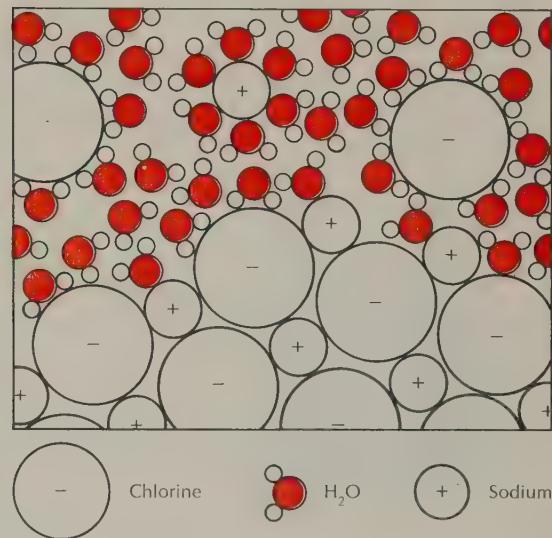
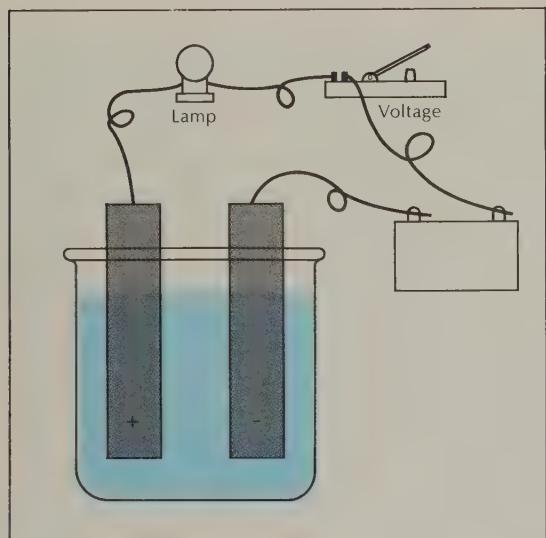


Table 3.4
Average Bond Energies

BOND	Average Bond Energy (kcal/mole)
$\text{C}\equiv\text{C}$	199.6
$\text{C}=\text{O}$	178
$\text{C}=\text{C}$	145.8
$\text{H}-\text{F}$	134.6
$\text{Si}-\text{F}$	129.3
$\text{O}-\text{H}$	110.6
$\text{C}-\text{F}$	about 110
$\text{H}-\text{H}$	104.2
$\text{H}-\text{Cl}$	103.2
$\text{C}-\text{H}$	98.7
$\text{N}-\text{H}$	93.4
$\text{Si}-\text{O}$	88.2
$\text{H}-\text{Br}$	87.5
$\text{C}-\text{O}$	85
$\text{C}-\text{C}$	82.6
$\text{S}-\text{H}$	81.1
$\text{C}-\text{Cl}$	80
$\text{C}-\text{N}$	80
$\text{C}-\text{S}$	62.0
$\text{Si}-\text{Si}$	42.2
$\text{O}-\text{O}$	33.2

Source: Linus Pauling, *The Nature of the Chemical Bond* (Ithaca, N.Y.: Cornell, 1960), p. 85; B. H. Mahan, *University Chemistry*, 2nd ed. (Reading, Mass.: Addison-Wesley, 1969), p. 458.

Figure 3.31. Experimental apparatus used to determine the conductivity of a solution.



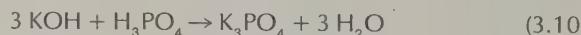
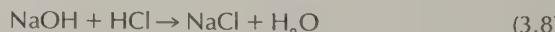
electric current within the solution, and the solution conducts an electric current. The magnitude of the current is proportional to the number of ions in the solution. The simple apparatus pictured in Figure 3.31 can be used to determine the current flow and hence the number of ions. If the bulb burns brightly, the solute is a *strong electrolyte*; if the bulb burns dimly, the solute is a *weak electrolyte*; if the bulb does not light up at all, the solute is a *nonelectrolyte*.

Compounds that are nearly completely ionic, such as NaCl, NaOH, CaCl₂, and KBr, dissolve in water to become strong electrolytes. Compounds with polar bonds, such as HgCl₂, PbBr₂, and CuCl₂, are weak electrolytes. Compounds with nonpolar covalent bonds, such as CH₄, N₂, and most biomolecules, are nonelectrolytes. It is difficult, if not impossible, to know from the chemical formula whether a compound is a strong or weak electrolyte or a nonelectrolyte. One must either be knowledgeable in chemistry or perform a test such as that shown in Figure 3.31.

Two particular ions are of great importance in chemistry—the hydrogen ion (H⁺) and the hydroxide ion (OH⁻). A compound that releases hydrogen ions when dissolved in water is called an *acid*, and one that releases hydroxide ions or accepts hydrogen ions is called a *base*. Acids and bases are especially important because many chemical reactions are accelerated in acidic or basic solutions. Acids are characterized by their ability to dissolve many metals with the simultaneous release of hydrogen gas, a characteristic sour taste (the sour taste of lemons and vinegar is due to the presence of weak acids), and by the color changes they produce in certain dyes (such as litmus paper). Bases taste bitter, feel slippery (soap is slippery because it contains small amounts of base), and change the colors of the indicator dyes in a fashion opposite to that of acids.

Some of the more common acids are nitric acid (HNO₃), hydrochloric acid (HCl), sulfuric acid (H₂SO₄), and phosphoric acid (H₃PO₄). Some common inorganic bases are sodium hydroxide (NaOH), calcium hydroxide [Ca(OH)₂], and ammonium hydroxide (NH₄OH).

If mixed in the proper proportions, acids and bases exactly neutralize each other to give a solution that has the properties of neither an acid nor a base. The products of this *neutralization* reaction are an ionic compound, or *salt*, and water. The following equations are examples of neutralization reactions:



The concentration of hydrogen ions in solution is so important to most chemical reactions that chemists use a special notation when discussing it. They have devised a *pH scale*, which varies between 1 and 14, to specify the acidic or basic character of a solution (Interleaf 3.1). A pH of 7.0 means that the solution is perfectly neutral—that is, the concentrations of H⁺ and OH⁻ in the solution are equal and the solution is neither acidic nor basic. A pH lower than 7.0 means that the solution is acidic; the lower the pH, the more H⁺ ions are free in solution and the more acidic the solution is. A pH greater than 7.0 means that the solution is basic; the greater the

The pH is defined as being equal to the negative logarithm (to the base 10) of the hydrogen ion concentration in moles per liter of solution:

$$\text{pH} + -\log_{10} [\text{H}+] = +\log_{10} \frac{1}{[\text{H}+]}$$

Analysis shows that there is 10^{-7} gram (g) of hydrogen ions present in a liter of pure water. For hydrogen ions, 10^{-7} g is equal to 10^{-7} moles (because H has an atomic weight of 1). Therefore, the pH of pure water is $\log_{10} \frac{1}{.0000001}$ of 7. Pure water is chemically neutral, that is to say it is neither acid nor base, and the concentration of hydrogen ions must equal the concentration of hydroxide ions (the only base that can be present in pure water).

Table 3.5
pH Values for Various Solutions

SOLUTION	pH
Pure gastric juice	about 0.9
Orange juice	2.6–4.4
Vinegar	3.0
Grapefruit juice	3.2
Tomato juice	4.3
Urine	4.8–7.5
Saliva	6.6
Milk	6.6–6.9
Distilled water	7.0
Intestinal juice	7.0–8.0
Blood serum	7.4
Tears	7.4
Pancreatic juice	7.5–8.0
Egg white	8.0
Sea water	8.0

Source: E. S. West and W. R. Todd, *Textbook of Biochemistry* (New York: Macmillan, 1951), p. 49.

Interleaf 31

THE pH SCALE

pH, the fewer H^+ ions and the more basic the solution. The pH of a number of solutions is given in Table 3.5.

VALENCE AND OXIDATION NUMBER

The electrons in the outer shell that can be shared with other atoms are called the *valence electrons*. The *valence* of an element is a number representing the number of electron pairs that can be shared in covalent bonds or the number of electrons that are gained or lost in the formation of ions. Thus, hydrogen has a valence of 1, oxygen has a valence of 2, nitrogen has a valence of 3, and carbon has a valence of 4. The concept of valence was part of early attempts to explain the regularities summarized in the periodic table and has largely been abandoned since more complete explanations of bonding capability have arisen.

However, a related concept—*oxidation number*—is of considerable importance in modern chemistry. For simple atoms and *ions*, the oxidation number is equal to the net charge on the atom or ion. Thus, the oxidation number of H^+ is +1, that of Be^{++} is +2, that of Cl^- is -1, and that of He is 0. In *molecules*, however, the oxidation number is assigned on a more arbitrary basis: each shared pair of electrons is assigned to the nucleus that attracts it most strongly. The oxidation number is then calculated as the charge that the atom would bear if the electrons were actually completely held by those nuclei. For example, in H_2O the shared electrons of the O—H bonds are attracted more strongly by the oxygen than by the hydrogen. Thus, all the shared electrons are assigned to the oxygen atom, giving it a net charge of -2. (Its own eight electrons are balanced in charge by the eight protons in its nucleus; the two electrons contributed by the hydrogen atoms provide an excess of negative charge.) The hydrogen atoms each lose one electron, giving each hydrogen atom a net charge of +1. Thus, in H_2O hydrogen is assigned an oxidation number of +1 and oxygen is assigned an oxidation number of -2. The assignment of electrons in calculating oxidation numbers is an arbitrary mathematical operation and does not represent the actual distribution of electrons in the molecule. In a bond where electrons are shared equally, the shared electron pair is split between the two atoms; thus, in H_2 the oxidation number of hydrogen is 0.

In the O—H bond, oxygen attracts the electrons more strongly than does hydrogen (Formula 3.6); thus, the electron pairs in H_2O are assigned to the oxygen atom, giving oxidation numbers of +1 for hydrogen and -2 for oxygen. In the covalent molecular compound HCl, chlorine attracts the electrons more strongly than does hydrogen; the electron pair is assigned to the chlorine atom, giving oxidation numbers of +1 for hydrogen and -1 for chlorine.

In many reactions, the oxidation numbers of elements are changed. For example, water can be formed from hydrogen and oxygen gases:



In this reaction, the oxidation number of hydrogen is changed from 0 (in H_2) to +1 (in H_2O). In such a case where the oxidation number is increased, the element is said to have been *oxidized*. This process of oxidation may be described as a loss of electrons; the molecule that accepts the

electrons (in this case, oxygen) is called the *oxidizing agent*, or *oxidant*. In the same reaction, the oxidation number of oxygen is changed from 0 (in O_2) to -2 (in H_2O). Such a decrease in oxidation number is described by saying that the oxygen has been *reduced*. Reduction is a gain of electrons; the molecule that donates the electrons (in this case, hydrogen) is called the *reducing agent*, or *reductant*.

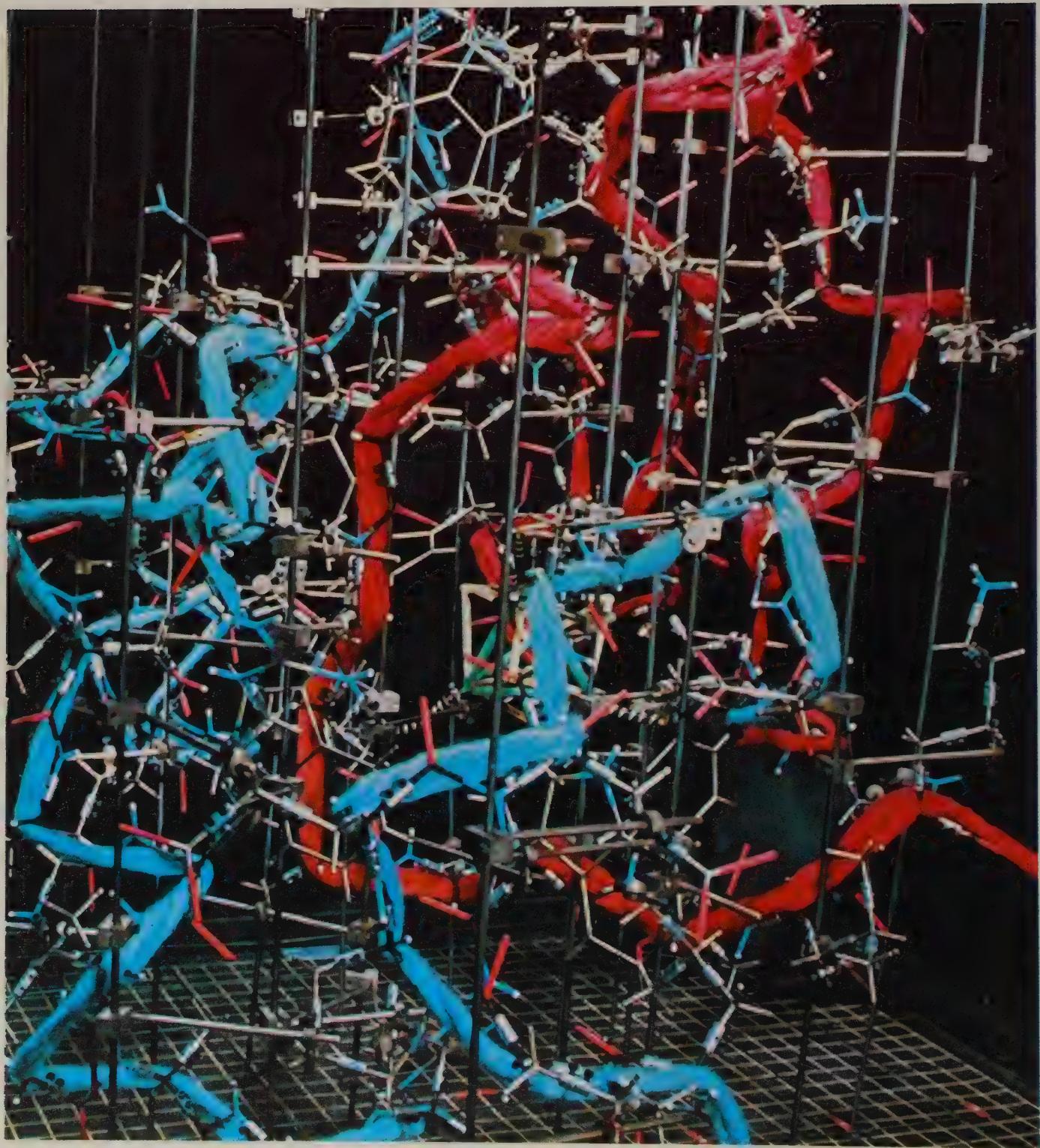
A reaction in which oxidation numbers are changed is called an *oxidation-reduction reaction*, or *redox reaction*. In any such reaction, a reductant donates electrons and is oxidized, while an oxidant accepts the electrons and is reduced. In many cases, the electrons are not actually transferred from one molecule to the other but are shared in a polar covalent bond. Nevertheless, it is useful to think of such reactions as processes of electron transfer in which a certain amount of energy is absorbed or released as the electrons are transferred. This way of looking at oxidation-reduction reactions helps to clarify many of the chemical processes involved in living systems.

FURTHER READING

The Chemical Education Material Study (1963) presents an exceptionally clear introduction to basic ideas of chemistry. An excellent paperback treatment of many of the topics of this chapter is given by Herz (1963). A particularly current and clear college chemistry text is the one by Mahan (1969). Pauling (1960) clarifies modern ideas about bonding and molecular structure.

4

Chemistry of Life



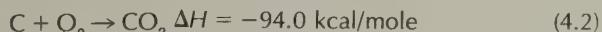
Energy transfers within living systems are accomplished through changes involving matter—that is, through chemical changes. These chemical reactions are of almost endless variety, taking place in structures of all degrees of complexity. Throughout biology, such reactions are encountered repeatedly, for they underlie most of the phenomena observed on a macroscopic scale.

When a chemical reaction occurs, bonds within the original molecules (the reactants) are broken, and new bonds are formed to create molecules of the products. One result of these changes is the release or the absorption of energy. For example, consider the chemical reaction that occurs when coal is burned. Coal is composed primarily of carbon, which combines with oxygen gas in the air to form the gas carbon dioxide. The reaction can be summarized as follows:



In this reaction, the double bond in the oxygen molecule is broken, and two new double bonds are formed in the carbon dioxide molecule (which could be written as $O=C=O$). The formation of the $C=O$ bonds releases far more energy than is consumed in breaking the $O=O$ bond; in other words, the products are at a lower energy state than the reactants. Thus, for each mole of carbon that is burned, a mole of CO_2 is formed, and 94.0 kilocalories of energy are released. When coal is burned, this energy is given off as heat and light.

Each molecule has a certain energy content, or heat content, H . The heat released during the reaction represents the difference between the heat content of the reactants and that of the products. Thus, chemists symbolize the *heat of reaction* as ΔH (ΔH is the difference between the two values of H) and define it as the heat content of the products minus the heat content of the reactants. When coal is burned, the reactants have a greater heat content than the products; thus, ΔH for this reaction has a negative sign. In general, a negative ΔH indicates a reaction releasing heat, an *exothermic* reaction. The reaction is customarily written as follows:



Once a fire has been started, coal continues to burn until all the available carbon or oxygen is consumed. Just as electrons tend to move spontaneously to the lowest available energy level (releasing photons of light energy as they do so), molecules tend to move to the lowest available energy state (releasing heat energy as they do so).

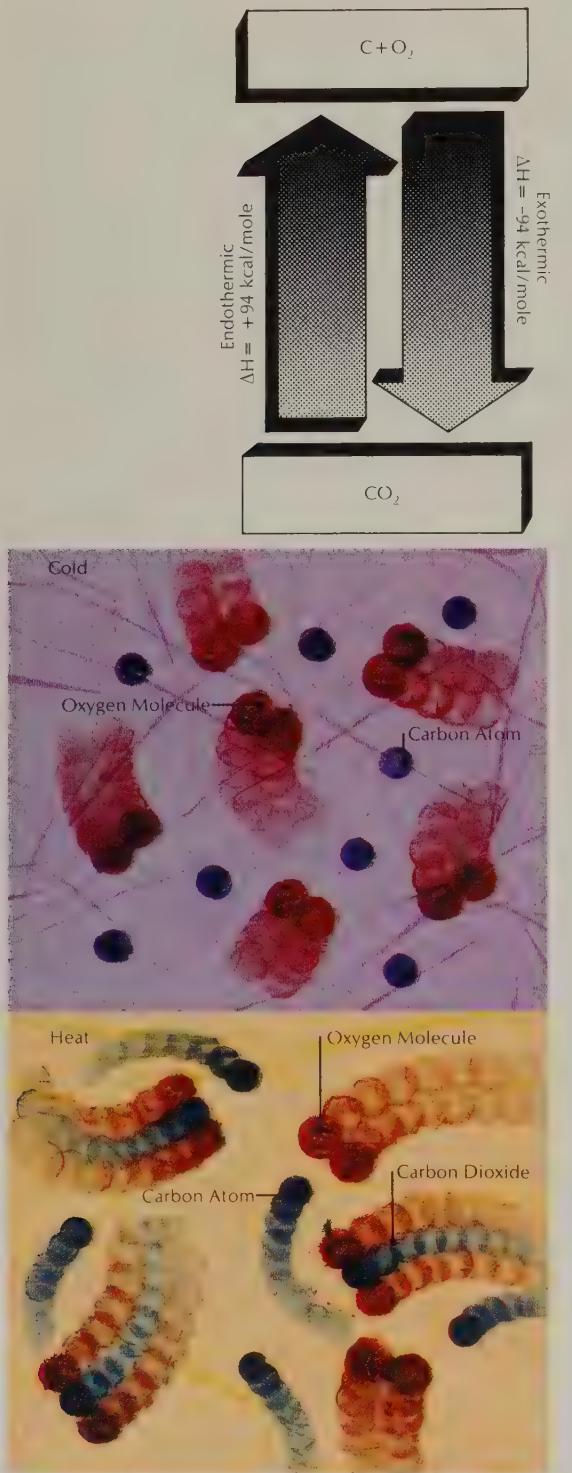
However, coal can be stored in the presence of oxygen for years without noticeable change. The reaction occurs only when coal is heated to a high temperature (the flash point) to get the reaction started. Why must energy be supplied to begin the reaction? The molecular model provides a simple explanation.

THE COLLISION THEORY

The chemical reaction can occur only when a molecule of oxygen and an atom of carbon approach each other closely. In fact, it seems logical to suppose that chemical reactions involve collisions between molecules, atoms, or ions of the reactants. This *collision theory* provides a simple picture

Figure 4.1 (above). Enthalpy changes in the combustion of coal in the presence of oxygen to form carbon dioxide.

Figure 4.2 (below). The collision theory.



of the mechanism for the reaction. An oxygen molecule collides with a carbon atom. The force of the collision depends on the speed of the molecules involved, and speed is a function of temperature. At a low temperature, the molecule moves slowly, and the energy of the collision is not sufficient to break the double bond in the oxygen molecule. The carbon atom and the oxygen molecule simply bounce apart; no chemical change occurs. At a high temperature, however, the molecules are moving rapidly and collision occurs with enough energy to break the O—O bond. For an instant, some sort of intermediate grouping of the atoms exists. This activated complex has a high heat content and is unstable. It quickly readjusts into the CO_2 molecule as the two C—O bonds form, releasing energy.

The energy relationships during the reaction can be graphed as in Figure 4.4. The high energy content of the activated complex forms a barrier to the progress of the reaction. Until energy is available to form the complex, the reaction cannot occur. Once the reaction has begun, the energy released is sufficient to push other molecules across the energy barrier and to keep the reaction going; once coal has ignited, the fire keeps burning without further heat supplied to it.

The collision theory implies that chemical reactions occur more rapidly if the concentrations of the reactants are increased, because collisions then occur more frequently—as is the case for most chemical reactions. The theory also implies that reactions proceed more rapidly at higher temperatures, because molecules move more rapidly at elevated temperatures, tend to collide more frequently, and more of the collisions are energetic enough to pass the energy barrier of the activated complex. These anticipated effects seem to occur in most chemical reactions.

Chemical reactions are reversible. If a molecule of CO_2 acquires enough energy during a collision with another molecule, it can form the activated complex, which can break apart to form carbon and oxygen. However, the energy barrier to be crossed in this direction is much higher (moving from right to left in Figure 4.4). More-energetic collisions are needed to cross the barrier in this direction; thus, at any given temperature, the rate at which this reverse reaction occurs is lower than the rate at which the forward reaction occurs. Yet unless the temperature is so low that no CO_2 molecules have enough energy to cross the barrier, a small amount of CO_2 will continually be breaking down to form C and O_2 .

Equilibrium

As the reaction proceeds and the reactants are used up, the frequency of collisions between C and O_2 decreases. At the same time, as more CO_2 is formed, the frequency of collisions leading to the reverse reaction increases. An equilibrium is reached when the rates of the forward and reverse reactions are exactly equal. In most cases, however, the amounts of products and reactants are not equal when equilibrium is reached. At equilibrium, no further macroscopic changes are detectable; the amounts of C, O_2 , and CO_2 in the system remain constant, despite the fact that individual molecules are still undergoing the forward and reverse reactions.

It is essential to separate the two distinct features controlling the course of chemical reactions. On the one hand, the energy charge accompanying a reaction dictates whether or not the formation of products is a favorable process. On the other hand, the activation energy—the energy required to push the reactants to the stage of the activated complex—dictates how fast the reaction will occur. The magnitude of the energy change is indicative of

Figure 4.3 (above). Burning of nitrogen in the air to produce nitrous oxides. This type of reaction is one of those responsible for the production of photochemical smog from the exhaust products of internal combustion engines. Only the lower reaction goes to completion because it is the only one of the three reactions with sufficient energy and the proper geometry to form the activated complex that goes to products. Increasing the temperature and pressure of the reaction causes more collisions to take place and thus increases the likelihood of product formation.

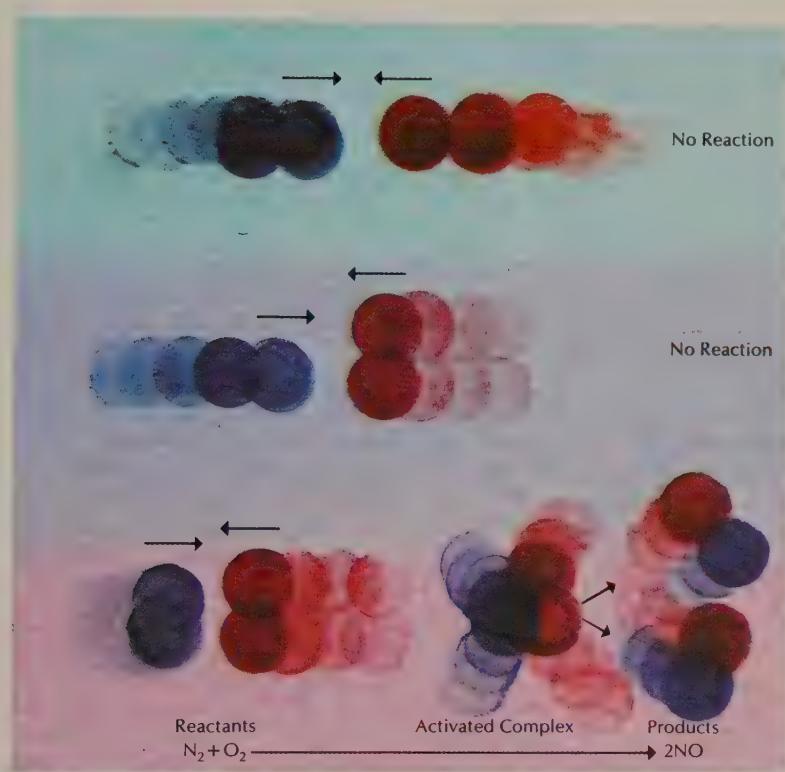


Figure 4.4 (below). A reaction coordinate diagram for the burning of coal in air (ΔE = energy of activation). Energy must be applied initially so the reactants will gain sufficient energy (ΔE^* FORWARD) to reach the activated state. Once this energy is supplied (as in the form of a lighted match), it is sufficient to cause the reaction to continue until equilibrium is reached. In this case, almost all of the reactants will end up as CO_2 because the products have lower energy. If the reaction were run backward, the ΔE^* REVERSE would be much greater than the ΔE^* FORWARD.

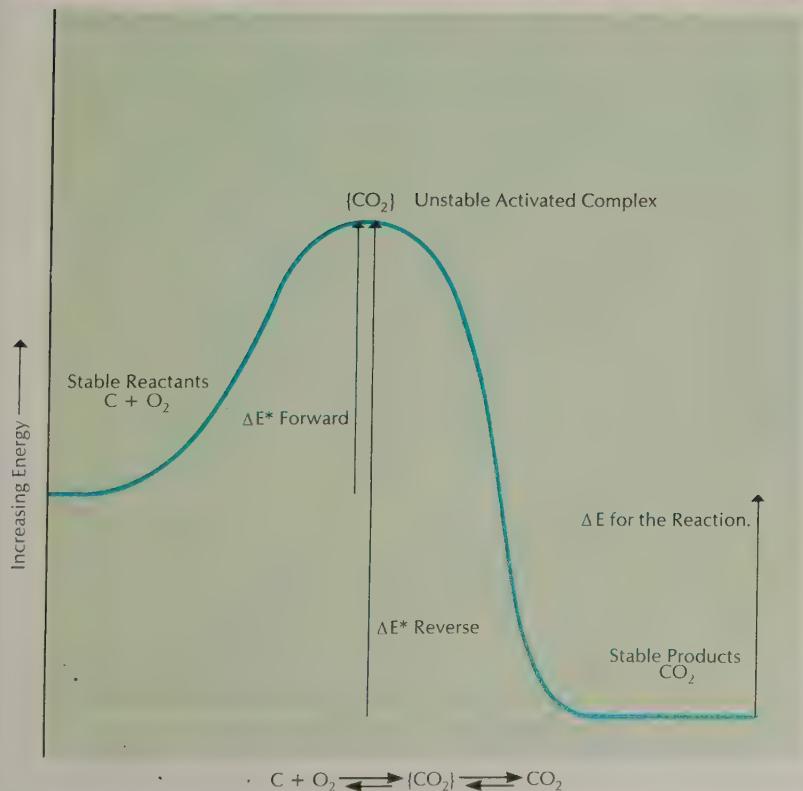
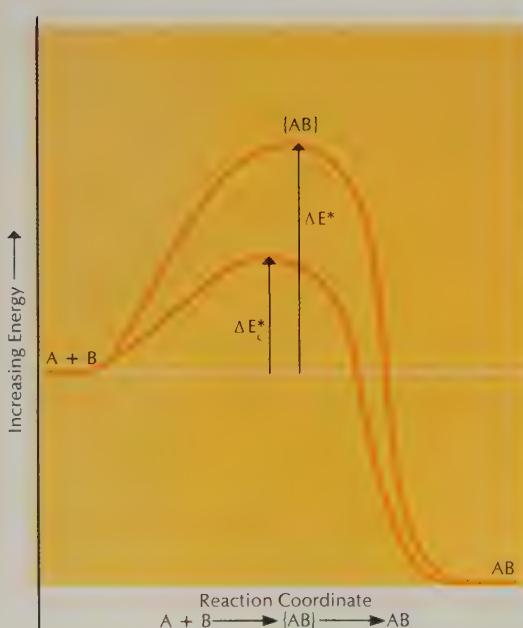


Figure 4.5. Action of a catalyst portrayed graphically. A catalyst (ΔE^*) lowers the energy barrier over which the reactants (A and B) must pass in order to form products (AB). The amount of energy released in the overall reaction is not altered by the catalyst, nor is the final equilibrium, which is governed solely by the difference in energy between reactants and products. The catalyzed reaction reaches equilibrium more rapidly, because on the average more molecules have enough energy to get over the barrier in a given period of time than in an uncatalyzed reaction.



what the equilibrium concentration of reactants and products will be, and the amount of activation energy required controls the rate of reaction — how long it takes to reach this equilibrium.

Most chemical reactions occur slowly at moderate temperatures, because few collisions are energetic enough to produce the activated complex. Although the final equilibrium may heavily favor the products over the reactants, at low temperatures it may take hours, days, even months to reach equilibrium. Yet most living systems operate at temperatures of less than 100°F — relatively low temperatures in chemical terms. How does the organism manage to carry out the vast number of reactions needed for life processes at rapid rates?

Catalysts

A reaction can be speeded up if the concentrations of the reactants are increased; organisms use many different mechanisms to bring the reactants together in high concentrations. For example, reactants may migrate near each other and be assembled on cell membrane surfaces. An even more favorable increase in reaction rate can be obtained if the energy barrier to the reaction is lowered; then more collisions will be successful in forming the products. For most chemical reactions, a lower energy barrier can be produced by the addition of a suitable catalyst, a substance that is not consumed in the reaction but provides an alternate mechanism for the reaction. In the presence of the molecules of the catalyst, a sequence of activated complexes can be formed to carry out the reaction, with each of the new activated complexes having a lower heat content than the uncatalyzed complex (Figure 4.5). Although the rate of reaction along the old path is not altered, a much higher rate of reaction is made possible along the new path. Because the energy barriers for the forward and reverse reactions are lowered by the same amount, the catalyst speeds up both the forward and reverse reactions equally. Thus, the same equilibrium ratio of products to reactants is reached, but it is reached more rapidly than in the uncatalyzed reaction.

ENZYMES

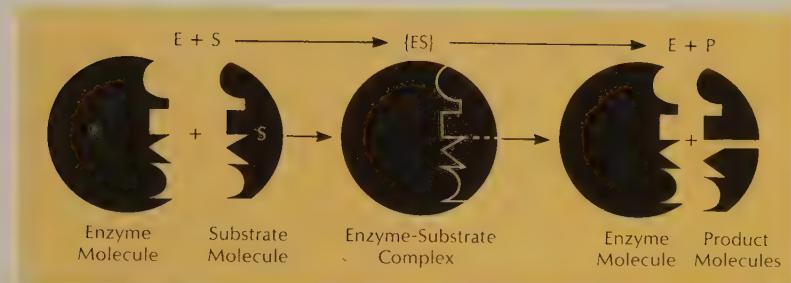
Almost every one of the myriad chemical reactions that occur in living systems is catalyzed by an enzyme, a complex, three-dimensional protein. Each enzyme catalyzes only a particular chemical reaction but is an extremely efficient catalyst for that reaction.

Because of their specificity, enzymes play an important role in determining which reactions are carried out in the living organism. Suppose that a certain compound A can be converted in the living system either to compound B or to compound C. Both reactions, $A \rightarrow B$ and $A \rightarrow C$, proceed very slowly at the temperature of the living system. If there were present an enzyme E_{AB} that catalyzed the reaction $A \rightarrow B$ and no corresponding enzyme E_{AC} were present to speed up reaction $A \rightarrow C$, then A would be converted almost entirely to B and very little of C would be formed.

If both of the enzymes, E_{AB} and E_{AC} , could be created by the living system but each was created only under certain conditions, then the production of B or C could be regulated to meet varying circumstances. The organism would be able to produce E_{AB} only under those conditions where B was needed and to produce E_{AC} only when C was needed. The creation of these enzymes occurs in chemical reactions regulated by still other enzymes. The

Figure 4.6 (above). Generalized schematic diagram showing an enzyme catalyzing the conversion of a complementary shaped substrate molecule to products. The enzyme molecule remains unchanged during the reaction.

Figure 4.7 (below). Competitive inhibition of enzymatic catalysis by a molecule that reversibly binds at the catalytic site of the enzyme, thereby competing with the substrate molecule.



information needed to direct the formation of this complex network of enzymes comes ultimately from the genetic mechanism.

It is thought that the reactant molecule, the *substrate* of the enzyme, attaches to a particular active site on the enzyme in such a position that the molecule is activated to react with another molecule. After the reaction occurs, the newly formed product molecule leaves the site, making room for another substrate molecule. Apparently, the substrate molecule fits into the active site of the enzyme, rather in the manner of a key fitting into a particular lock (Figure 4.6).

Because the fit must be exact, an enzyme is highly specific and, in most cases, accepts only a few kinds of structurally similar substrate molecules. Nevertheless, because one enzyme molecule can service substrate molecules over and over again, only a few enzyme molecules are needed to catalyze the reaction of many millions of substrate molecules. The shape of the enzyme molecule probably changes slightly as it attaches to the substrate molecule. This slight change is thought to make the substrate molecule somewhat unstable and thus hasten the reaction.

In some cases, a particular molecule may resemble the substrate sufficiently closely to become attracted to the active site but be sufficiently different that no reaction occurs. When such a molecule occupies the active site, the enzyme is blocked, or *inhibited*. No substrate molecules can then attach to the active site, and the inhibited enzyme ceases to catalyze the reaction. If the inhibiting molecule becomes covalently bonded to the active site or permanently alters it, the enzyme is *irreversibly inhibited*.

If the inhibiting molecule binds reversibly to the active site, then those enzyme molecules bound to inhibitors are no longer free to catalyze the conversion of substrate to product, and the overall rate of reaction diminishes. An example of this phenomenon is provided by the familiar human protein hemoglobin, which catalyzes the transportation of oxygen from the living tissues to those cells where the oxygen is used. This process is inhibited by carbon monoxide, which binds to the active site of hemoglobin some 200 times as avidly as oxygen. Because the atmosphere is about one-fifth oxygen, it would require a concentration of only 0.1 percent carbon monoxide to inhibit fully one-half of all oxygen transport. Such a process, in which the substrate and the inhibitor compete reversibly for the same active site, is called *competitive inhibition*. That this process is reversible is illustrated by the practice of Tokyo traffic policemen who are subjected to very high concentrations of carbon monoxide. They periodically are relieved of duty and revive themselves by breathing pure oxygen.

Inhibition of selected reactions could provide a means of controlling the chemical mechanism of an organism. Many common drugs, such as penicillin, are selective inhibitors, inhibiting some of the important reactions in

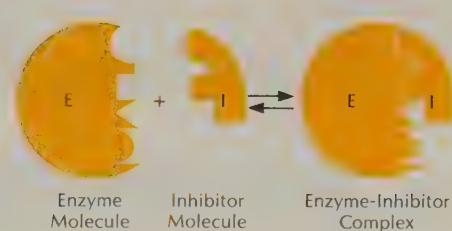
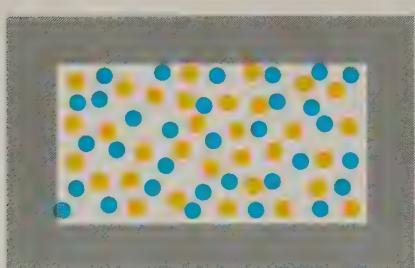
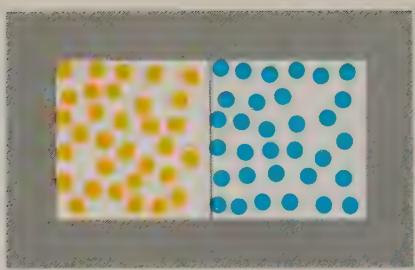


Figure 4.8 (above). J. Willard Gibbs, who derived the second law of thermodynamics.

Figure 4.9a (below). Diagram illustrating the principle of entropy. Two metal blocks are placed in an insulated enclosure that prevents heat from entering or leaving the system of the two blocks. Initially, the block on the left is hot, and the one on the right is cool. After a time, however, the heat flows into the cool block and the temperature becomes uniform throughout the two blocks, an example of the increasing entropy of the

system. At this point, it is impossible for the system to return to its original state without the expenditure of some energy from an outside source.



the infecting organism but not inhibiting important reactions in the host organism. The search for a drug that cures cancer is essentially a search for some reaction occurring in the tumor cells that could be inhibited by a molecule that would not inhibit any important reactions in normal cells.

AVAILABLE ENERGY AND ENTROPY

The energy available for useful work is usually discussed in terms of the second law of thermodynamics, which states that energy-releasing processes occur to increase entropy. Entropy (the Greek *trope* means to turn or change) is a broad measure of the amount of disorder or randomness of a system. As the disorder of a system increases, the entropy increases; completely random distribution of all the molecules of a system would represent a condition of maximum entropy. The production of energy in a system is generally accompanied by the loss of a portion of that energy to the surroundings. The lost energy serves to heat the surroundings, but it is not available for useful work within the system. When the random motion of molecules is increased, this wasted heat serves to increase the entropy of the surroundings. A number of familiar processes are marked by an increase in entropy—heat flows from warm objects to cool ones, compressed gas when released escapes to the atmosphere, and corroding metal washes away in the rain ultimately to be diluted in the sea. None of the reverse of these spontaneous processes will occur unless energy is expended; all of them are marked by an increase in randomness—an increase in entropy.

Living systems are marked by their high degree of order. Plants maintain this low entropy content by consuming the energy of the sun to achieve photosynthesis—a process that involves the conversion of numerous small molecules in the surroundings to highly organized energy-rich macromolecules within the plant (Chapter 5). On the other hand, animals maintain their low entropy content at the expense of increasing the entropy of their environment. Man, for example, consumes the highly organized molecules produced by plants or lower animals and excretes a number of very much smaller, less organized molecules—primarily carbon dioxide—that are energy poor.

When discussing the chemical reactions that occur in living systems, biologists prefer to define entropy as that portion of the energy change from converting reactants to products that is unavailable for useful work. Chemists find it useful to define a new expression of energy change that takes into account both the change in heat content (ΔH) and the entropy change. This expression is the free energy change (ΔG) and is a measure of the energy that is actually available for useful work. A reaction with a negative ΔG (release of free energy) is called an *exergonic* reaction; one with a positive ΔG (consumption of free energy) is called *endergonic*.

METABOLISM: TRANSFER OF ENERGY

The formation of CO_2 from C and O_2 is a strongly exergonic reaction. The $\text{C}=\text{O}$ bonds in CO_2 form readily with the release of large amounts of energy; conversely, these bonds can be broken only if large amounts of energy are supplied. In general, if a bond is formed only by the input of energy (an endergonic reaction), energy will be released when it is broken (an exergonic reaction). *High-energy bonds*—those that release at least 5 kcal/mole when broken—are of great importance in the transfer of energy within the living system. High-energy bonds involving phosphorus and

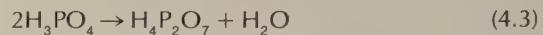
Figure 4.9b. An interpretation of the principle of entropy. Fuels that provide heat—the stars, the sun, fossil fuels, and so on—all result in an increase in the randomness of molecular motions, or entropy, which has also been described as the “general running down” of the universe.



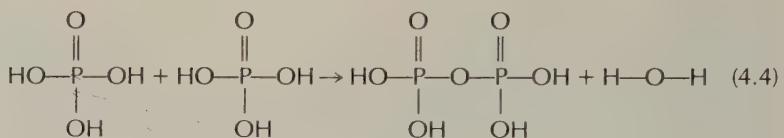
Figure 4.10. A model of coupled reactions. Living cells must carry out many reactions that are energetically unfavorable. The reaction C → D is an example of an endergonic reaction requiring the expenditure of free energy for its completion. Reactions of this type are coupled to exergonic reactions such as A → B. This coupling is done through enzymatic cofactors that store and transfer the energy obtained in the first reaction (A → B) for use in the reaction C → D. In many instances, the cell utilizes ATP as the cofactor for coupling endergonic and exergonic reactions.

oxygen play major roles in metabolic processes of all living organisms because energy is shuttled from exergonic reactions to endergonic ones through this mechanism of energy storage.

Pyrophosphoric acid ($H_4P_2O_7$) can be formed from orthophosphoric acid (H_3PO_4) in the following reaction:



or

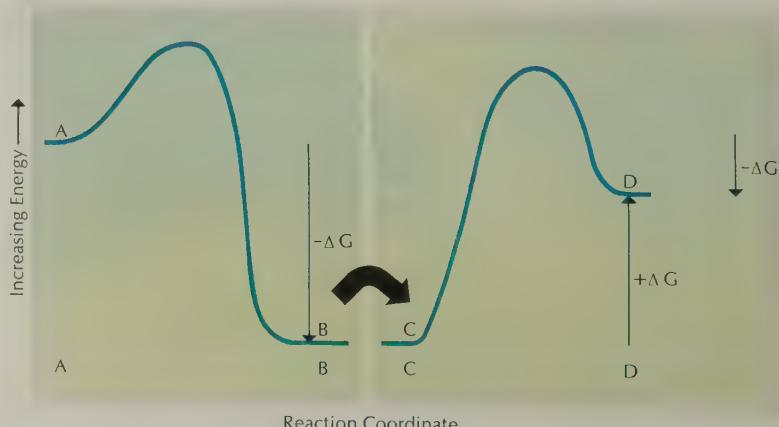


This reaction is endergonic ($\Delta H = +6.1$ kcal/mole). The pyrophosphate linkage (P—O—P) is formed only through the input of energy. The reverse reaction is exergonic and proceeds spontaneously with the release of energy. In this reverse reaction, pyrophosphoric acid is broken down in a reaction with water (called *hydrolysis*) to form orthophosphoric acid and energy:



The chemist who wishes to prepare pyrophosphoric acid simply heats orthophosphoric acid, thus supplying the energy needed to carry out the endergonic reaction. At the higher temperature, the equilibrium is shifted toward the pyrophosphoric acid, although a considerable amount of orthophosphoric acid remains when equilibrium is reached. However, an organism operates at a relatively low temperature. Can it possibly create a compound such as pyrophosphoric acid?

An enzyme will not shift the equilibrium (as does the heating); it will merely increase the rate at which the equilibrium is reached. In this case, an enzyme would allow pyrophosphoric acid to be more rapidly hydrolyzed at low temperatures. The organism solves this sort of problem by transferring energy in high-energy bonds from exergonic reactions to carry



out endergonic reactions. The molecules that can shuttle energy in high-energy bonds are called *cofactors*. An exergonic and an endergonic reaction coupled by cofactors are called *coupled reactions*.

Figure 4.11 shows the structural formula of a molecule of a substance called adenosine triphosphate (ATP). Note that it has two pyrophosphate linkages. These high-energy bonds, like those in pyrophosphoric acid, can easily be broken by hydrolysis (the addition of water). An example of the hydrolysis of ATP is shown in Figure 4.12; the reaction can be summarized as follows:



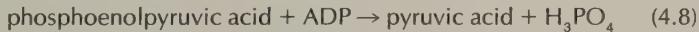
As would be expected, the formation of ADP (adenosine diphosphate) from ATP is quite exergonic. Conversely, the formation of ATP from ADP and H_3PO_4 (the reverse reaction) is quite endergonic.

In the living system, energy that is released as ATP is being hydrolyzed to form ADP can be used as a source of energy for such endergonic reactions as the formation of pyrophosphoric acid. Suitable enzymes are required to carry out both reactions at moderate temperatures, and most strongly energetic reactions in living systems involve at least four groups of molecules: reactants, enzymes, cofactors, and products.

A similar coupling of exergonic and endergonic reactions is involved in the formation of the ATP itself. The following reaction, involving the hydrolysis of phosphoenolpyruvic acid (another phosphorus-containing compound), is even more exergonic than the conversion of ATP to ADP:



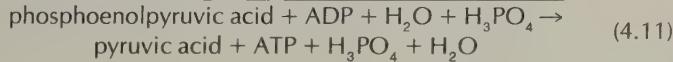
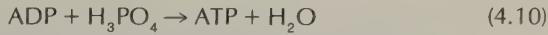
In the organism, the following reaction takes place:



This reaction can be regarded as the coupling of the two reactions that have already been discussed:



and



The amount of H_2O and of H_3PO_4 is the same before and after the reaction; therefore, the overall reaction can be summarized as:

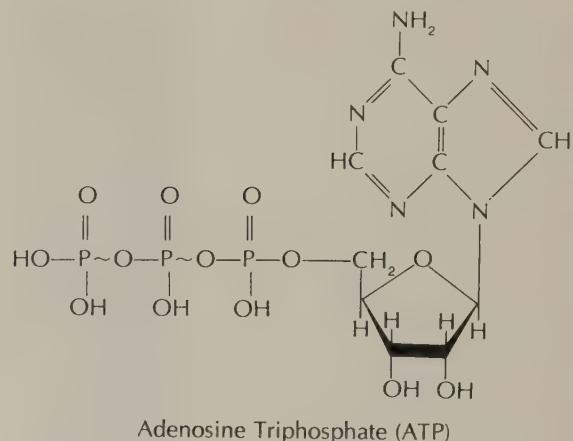


In the living system, this reaction is facilitated by a particular enzyme that catalyzes the transfer of the phosphate group (PO_4) from the phosphoenolpyruvic acid to ADP.

The first reaction (Formula 4.9) is strongly exergonic; the second (Formula 4.10) is strongly endergonic. However, the total reaction is

Figure 4.11 (above). The structural formula of adenosine triphosphate (ATP).

Figure 4.12 (below). The hydrolysis of adenosine triphosphate to adenosine diphosphate and inorganic phosphate.



Adenosine Triphosphate (ATP)

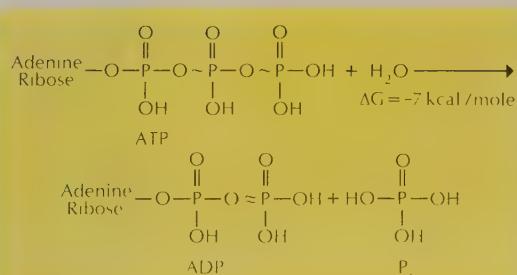
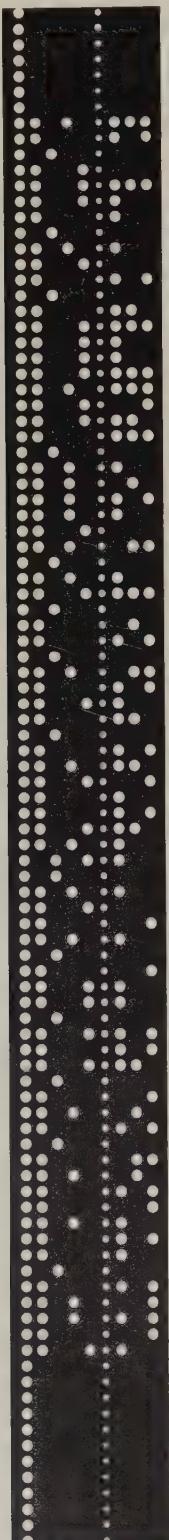


Figure 4.13. An example of a machine-punched code on paper tape. The tape bears the message: "We hold these Truths to be self-evident, that all Men are created equal. . . ."



moderately exergonic, because the positive ΔG of the second reaction is more than balanced by the negative ΔG of the first reaction. Thus, the energy obtained by hydrolyzing phosphoenolpyruvic acid is used to convert ADP to ATP. Energy has been stored in the high-energy pyrophosphate linkage of the ATP. This energy can now be used to carry out some other endergonic reaction, which can be coupled with the hydrolysis of ATP.

ATP is the negotiable currency of the energy exchange in the organism. Because the breakage of the pyrophosphate linkage in ATP is so exergonic, when the appropriate catalysts are present, the energy stored in the ATP can be spent for many purposes. The use of the energy is accomplished by coupling the breakdown of the ATP with an endergonic reaction. Because most of the reactions involved in growth—that is, in building the complex molecules of the living system—are endergonic, this energy source is very important to the system. It is noteworthy that, under the conditions of the living cell, ATP is a relatively stable compound, requiring enzymatic catalysis in order to undergo rapid reactions. It should be emphasized that the stability of a compound is reflected by its reluctance to decompose and is not determined by the energy change occurring on decomposition.

GENETICS: TRANSFER OF INFORMATION

What sort of system exists to transfer the information about this complex chemical mechanism to the next generation of organisms? What would be the requirements of such an information system? An exploration of these questions must begin with a look at the nature of information itself.

Information, in essence, is a message—a readable message. The message may be in various forms and may be read in various ways. It may be stored in one form and delivered in another. Nevertheless, certain characteristics are indispensable. Information implies structure. Consider a message composed of paragraphs, which are made up of sentences, words, and letters. Understanding of the message is made possible by recognition of the structure of the letters.

But not all messages are written. In spoken messages, the structure of the sounds is important. Mathematical equations, musical notation, sculpture, a passage of orchestral music—each of these forms of information has a structure. Alter the structure and the message may be altered; alter the structure sufficiently and the message may be lost entirely.

Messages set forth in a linear form, such as writing, have an important characteristic in their sequence. RUN does not carry the same message as does URN, and RNU is nonsense in English. Furthermore, the message must be read in a particular direction; RUN read backward becomes NUR, meaningless in English but meaningful in German. Other message forms, such as music, are also linear. Beethoven's Fifth Symphony played backward does not carry the message that Beethoven intended; if a tape recording of the music is cut up and spliced in a new sequence, an interesting tape collage may result, but the message will again be altered.

How could these characteristics be used to design a system to carry the information (the "blueprint," so to speak) needed for the construction of a molecule of the enzyme E_{AB} ? Because the information is to be used in reactions among molecules, it is reasonable to suppose that the information itself will be stored in some sort of molecular structure.

The kind of molecule needed to store this information depends upon the complexity of the information to be stored. The structure of a skyscraper requires a more elaborate set of blueprints than does the structure of a

homemade coffee table. Similarly, the complexity of the blueprint for enzyme E_{AB} depends upon the complexity of the enzyme molecule itself.

Enzyme E_{AB} , like every other enzyme, is a protein, a particular type of heteropolymer. A blueprint for the construction of a homopolymer could be relatively simple. It must specify the monomer to be used, the way in which the monomers are to be linked together, and the number of monomers in the polymer. Because the monomers are identical, sequence is irrelevant. However, for a heteropolymer such as an enzyme, the blueprint must also specify the sequence in which the various monomers are to occur in the chain.

Because the protein to be constructed is a linear molecule, the blueprint can be readily encoded in another linear molecule. The length of the molecule must be sufficient to include all of the information needed to specify fully the structure of the protein; because the protein is a macromolecule, the message must also be stored in a macromolecule. Finally, the message—when read in the proper direction—should specify the sequence of monomers in the protein chain.

From this reasoning, it seems that the information should be stored in a linear macromolecule. This information molecule must be a heteropolymer, for a homopolymeric message could not carry the information needed to construct the proper sequence of different monomers in the heteropolymeric protein. Proteins contain some 20 different monomers, but the information molecule would not necessarily have to contain this many different monomers. Just as 26 letters can be used to encode many different words, so groups of a few different monomers can be used to encode information about the 20 monomers found in proteins.

The message molecule used by organisms has just the characteristics deduced above. It is a heteropolymer called *ribonucleic acid* (RNA), a linear macromolecule made up of four different kinds of monomers. In certain viruses (RNA viruses), RNA is the ultimate repository of genetic information. In other viruses and all other organisms, RNA is the working genetic information—in a sense it is the copy of the blueprint taken to the construction site rather than the master copy kept in the office. The master copy is *deoxyribonucleic acid* (DNA). DNA is also a linear polymer composed of four different kinds of monomers, differing slightly in structure but similar to the same four kinds as in RNA.

Biochemists have succeeded in determining the basic structure of the nucleic acids, they have built some simple information molecules from scratch, and they are beginning to develop the ability to design and to build RNA and DNA molecules that will carry any desired bit of genetic information.

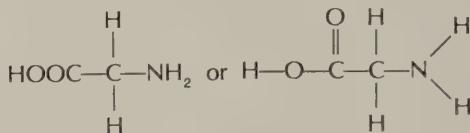
Direct observation of a molecule is sometimes quite valuable, particularly with such powerful techniques as x-ray diffraction. However, certain kinds of information can be obtained only by taking apart a substance such as RNA. The first problem is to obtain a sample of pure RNA that can be studied. In one common approach, the molecule is hydrolyzed; as in the case of the pyrophosphate linkage in ATP, many complex biochemicals can be broken apart through reaction with water. Hydrolysis may be carried out under mild conditions with the aid of an enzyme, or it may be done under harsher conditions in the presence of hot acid or alkali.

The pieces are separated and studied in the hope of understanding their structure. It may be necessary to separate these pieces still further before a simple molecule is obtained that can be recognized by its properties. Then

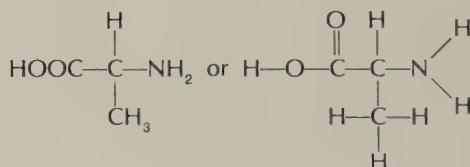
the biochemist must figure out how the pieces were linked together in the original molecule and how that molecule functions. This sort of endeavor, carried out over the past 120 years with increasingly sophisticated techniques, has yielded an enormous amount of information about the chemistry of living systems. In particular, it has permitted rapid progress toward an understanding of the transfer of information within the organism. This understanding begins with a closer look at the structure of the proteins, which are constructed according to the information stored in DNA and RNA.

Proteins

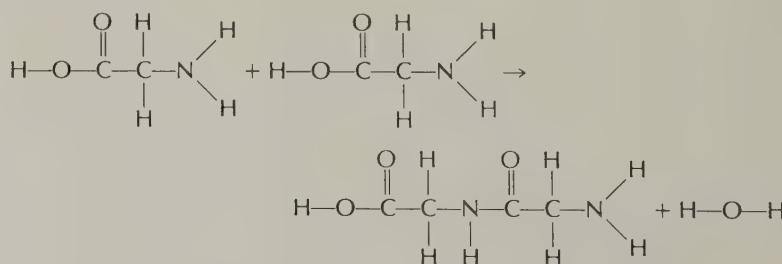
The monomers that make up the protein polymer are *amino acids*. The simplest amino acid, glycine, consists of a carbon atom bonded to a carboxyl group (COOH), an amine group (NH_2), and two hydrogen atoms:



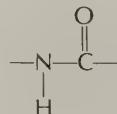
The other 20 or so amino acids are similar, but one of the hydrogen atoms attached to the central (alpha) carbon is replaced by a more complex group. In alanine, for example, one of the hydrogens is replaced by a methyl (CH_3) group:



An amino acid can form linkages to other amino acids at either end. The reaction proceeds through several intermediate stages, but the net result is to free a water molecule and to attach the nitrogen in the amine group of one amino acid directly to the carbon in the carboxyl group of the other amino acid. As an example, the combination of two glycine molecules could occur in this fashion:



The linkage formed shown as:



is called a peptide linkage, and the resulting compound is called a *peptide*. In this case, because there are two amino acids in the peptide, it is a dipeptide. However, more amino acids could be added to either end of the chain. In fact, *polypeptides* exist with many thousands of amino acids joined together. One of the smallest of the proteins is salmine, a polypeptide containing 58 amino acids joined by peptide linkages.

The many proteins needed by an organism are synthesized from the amino acids. Some amino acids can be synthesized directly by the organism from simple nitrogen-containing inorganic compounds. Animal organisms must obtain other amino acids by breaking apart protein molecules in food. The human organism, for example, requires a supply of 8 essential *amino acids* in the proteins of the diet; the other 12 amino acids used in the synthesis of proteins can be built by the human organism if sufficient nitrogen is supplied in the diet. The dietary requirements vary from species to species. For example, humans can synthesize glycine, whereas young chicks must have this amino acid supplied in their diets. Rats can synthesize glycine but must be supplied histidine, which is not an essential amino acid for humans. Thus, an organism not only requires a certain amount of protein in its diet but requires certain kinds of protein that supply its essential amino acids—the amino acids that it cannot synthesize.

Each of the amino acids except glycine exists in two isomeric forms, which are identical in chemical properties but can be distinguished by optical properties. The alpha carbon atom of the amino acid is bonded to four different groups of atoms (except in glycine, where two of the bonds are to hydrogen atoms). The spatial arrangement of these bonds can be represented by placing the alpha carbon in the center of a tetrahedron with the four groups at the corners (Figure 4.14). Note that there are two possible configurations of the four groups. These two arrays are the mirror image of one another and are called optical isomers. They possess a kind of "handedness" and cannot be superimposed upon one another. In this three-dimensional representation, it is clear that the *D* isomer cannot be converted into the *L* isomer by any simple rotation of the molecule; bonds must be broken and re-formed to convert one isomer into the other. All of the amino acids found in the proteins of living systems are of the *L* form, and living systems are unable to make use of *D* isomers in building proteins. There is no adequate chemical explanation for this preference; proteins made of *D* amino acids can be synthesized in the laboratory. Apparently, the information

Figure 4.14. Optical isomers of the amino acid alanine. Only the L-form is found in nature.

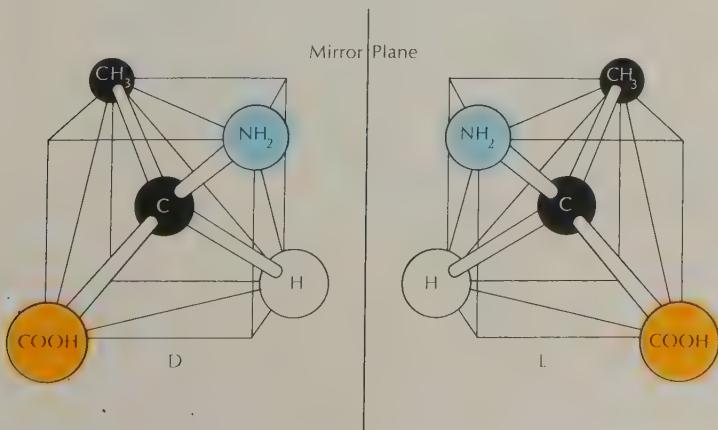


Figure 4.15. Schematic model of a right-handed α -helix. There are three amino acids in one hydrogen-bonded loop. Note that all —CO and —NH groups form hydrogen bonds.

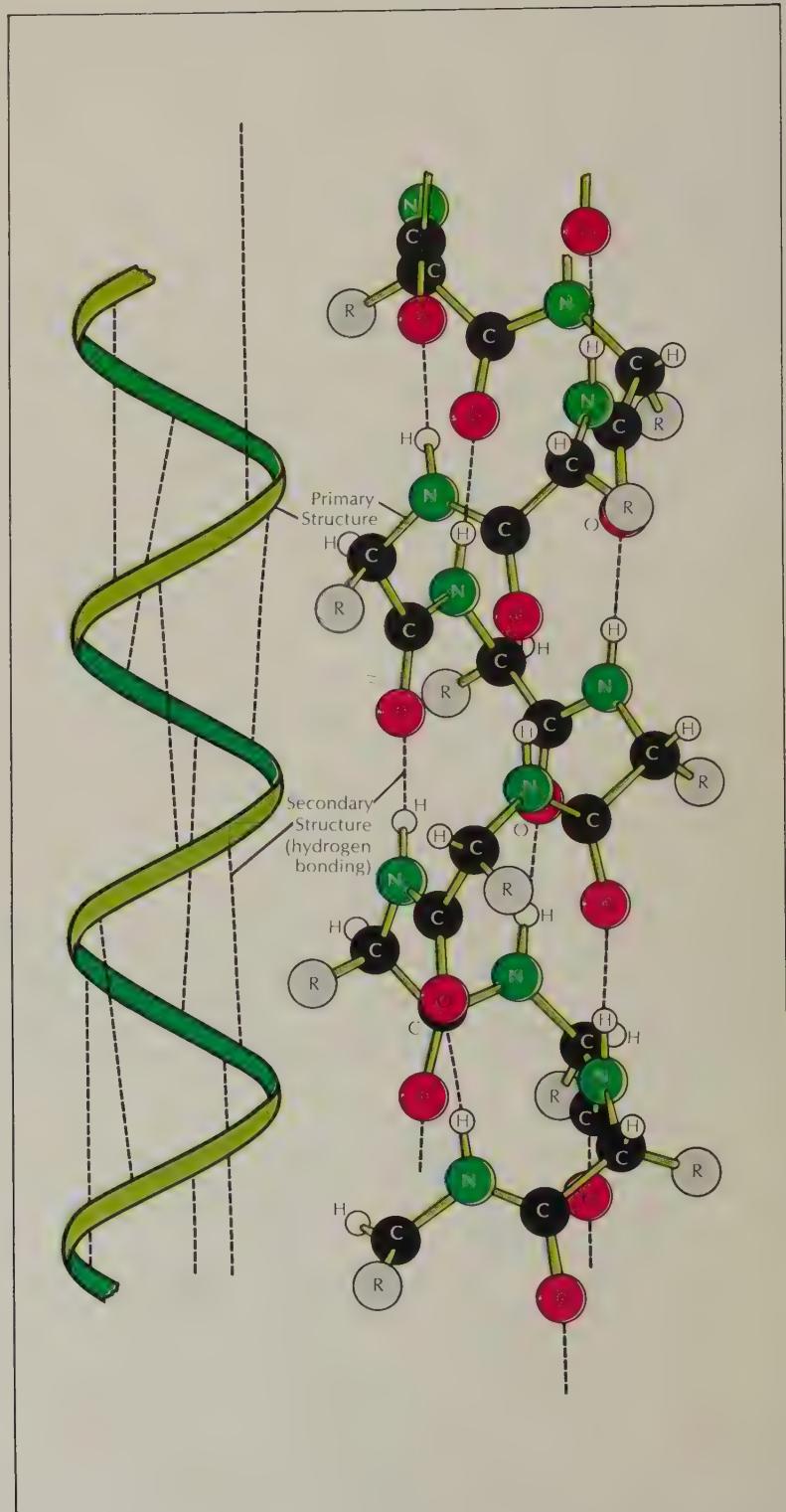


Figure 4.16. Schematic model of a protein molecule, comprised primarily of α -helical structures.

encoded in the nucleic acids specifies the use of *L* amino acids, rather than the *D* form, for protein synthesis.

Thus far, a protein molecule has been described as a long, straight polypeptide chain. In many cases, however, the chain winds into a coil of one sort or another. This coil may be a rather random twisting, or it may be a very regular winding, resulting in a structure like that of a helical spring. One such helical structure that has been identified is the right-handed alpha helix (Figure 4.15). In some proteins—called *globular* proteins—the coiled structure is bent, folded (often several times), and twisted until the overall shape of the molecule is spherical or ellipsoidal. Those proteins with a more-or-less linear structure are called *fibrous* proteins. Most enzymes are globular proteins. Most of the proteins making up structural elements of organisms are fibrous.

The globular protein molecule is held in its twisted position by bonds other than those in the polypeptide backbone (Figure 4.15). The coils of the alpha helix are held together by hydrogen bonds (shown as H—O).

Still other bonds account for the folding and bending of the coil. In the portion of a protein molecule shown in Figure 4.16, a *disulfide bridge* (—S—S—) has formed between two amino acids, both cysteine, some

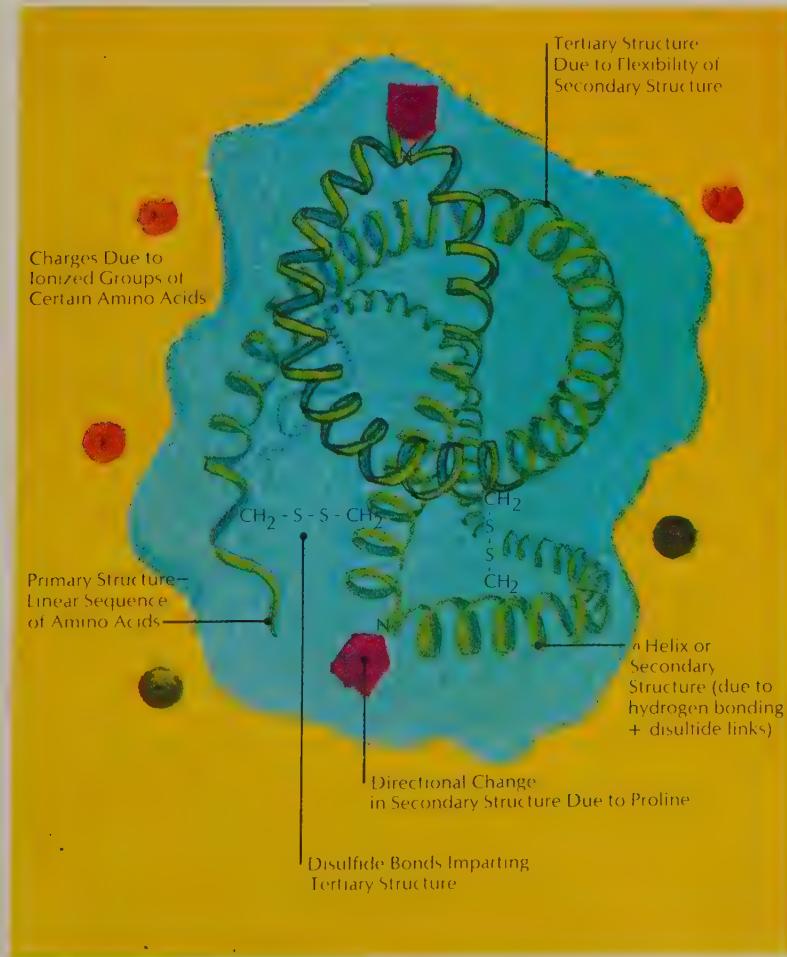
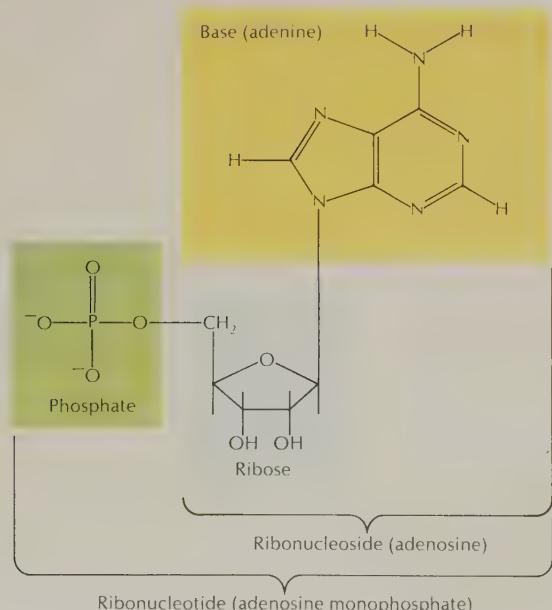
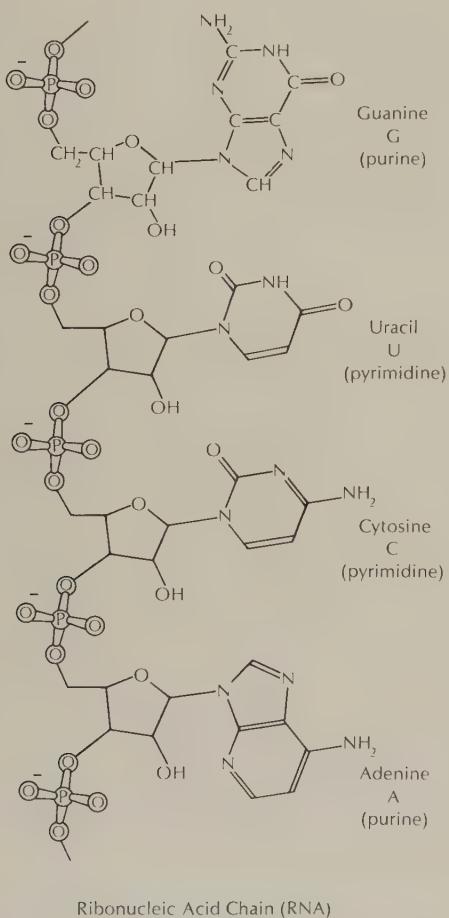


Figure 4.17 (above). Structural formula for a section of ribonucleic acid (RNA). One of each of the nucleotide bases found in RNA is shown.

Figure 4.18 (below). Nomenclature for the chemical subunits comprising the building blocks of RNA—the ribonucleotide bases.



distance apart on the chain. Cysteine has a CH_2SH group attached to the alpha carbon. The hydrogen atoms have been lost, and a covalent bond has been formed between the sulfur atoms of the two cysteine molecules. The disulfide bridge holds this portion of the protein molecule in a looped shape. Breakage of such bonds may greatly change the shape and therefore the properties of a protein molecule, even though the polypeptide backbone itself remains intact. Thus, the biochemical properties of many proteins may be altered by conditions not extreme enough to break apart the polypeptide chains. For example, many proteins are denatured (so altered in structure that they lose their useful properties) by slight heating, gentle stirring, or treatment with weak acids.

Many of the globular protein molecules are made up of several independent polypeptide chains associated by mutual attractive forces or joined by disulfide bonds. Hemoglobin is made up of four such chains or subunits. In some of the fibrous protein molecules, independent polypeptide chains are similarly bonded together and, in some cases, even twisted together into multiple coils or helices.

The amount of information needed to describe the twisting, folding, and cross-linkages in large protein molecules is tremendous. It would seem that the RNA molecule would have to be many times as large as the protein molecule whose "blueprint" it contains. However, biochemists now think that this information need not be supplied separately. When a sequence of amino acids is strung together in the proper order, the twisting and folding seem to occur automatically as a result of the nonbonded interactions between regions of the chain. Thus, the complex structure of the protein is a result of the sequence of amino acids in its backbone and need not be specified separately.

RNA

The information molecule, RNA, is also a polymer, but it is not a protein. RNA contains four kinds of nucleotide monomers (Figure 4.17). Each of the nucleotides is made up of a sugar, a phosphate group, and an organic base. In each of the four nucleotides the sugar is ribose. They are therefore called *ribonucleotides*, and the polymeric structure is known as ribonucleic acid (RNA). The phosphate group is also identical in the four ribonucleotides, which differ only in the base attached to each. The four bases are adenine, guanine, uracil, and cytosine (Figure 4.18). Because each of these bases contains nitrogen, they are sometimes called the nitrogenous bases.

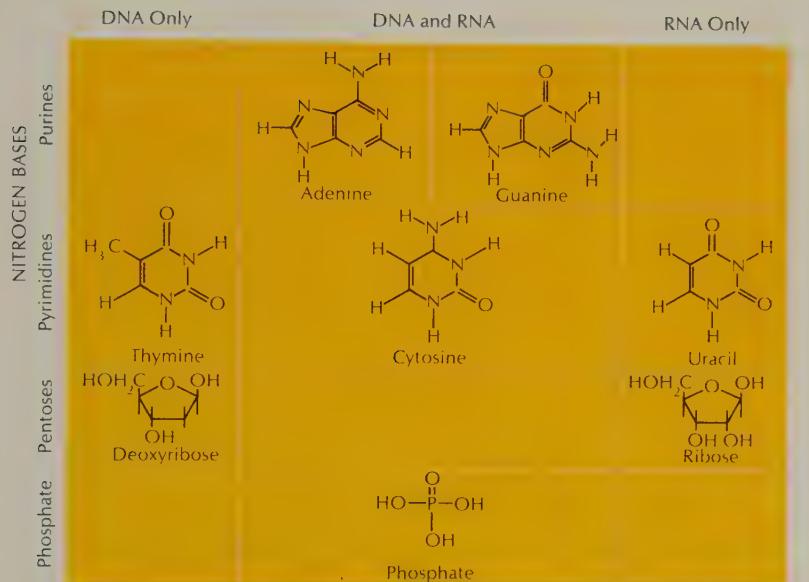
The polynucleotide RNA is formed by linkages between the sugar of one nucleotide and the phosphate group of the next. Thus, the backbone of the polymer is a series that could be represented as — sugar — phosphate — sugar — phosphate —. The bases extend off to the side of this backbone.

Just as there are many proteins, each with its own sequence of amino acids, so are there many RNAs, each with its own sequence of nucleotides. This multiplicity makes it possible for each RNA to carry the instructions for the building of a particular protein. However, there are 20 different amino acids used in building proteins and only 4 nucleotides available in RNA to encode the sequence of amino acids for building the protein. If each nucleotide represented one amino acid in the code, RNA could not carry complete and unambiguous instructions for the construction of proteins.

However, four different symbols are quite adequate for spelling out any message if the symbols are grouped to form words. If the symbols A, G, U, and C are used to represent the four nucleotides, 16 different "two-letter

Figure 4.19 (above). The chemical structures for the ribose sugars and nucleic acid bases found in RNA and DNA.

Figure 4.20 (below). Schematic model of the double-helix configuration of the DNA molecule.



words" can be written: AA, GA, CA, UA, AG, GG, CG, UG, AU, GU, CU, UU, AC, GC, CC, and UC. A language using only two-symbol words could not provide a unique word for each of the 20 amino acids. However, a little work with pencil and paper shows that these 4 symbols can make up 64 different three-letter words. This number is more than sufficient to provide a different word for each amino acid. In fact, many of the amino acids could be represented by two or more different words (synonyms, if the analogy to language is continued).

Recently, many lines of research have shown that RNA does use a "three-letter-word" code (the so-called *genetic code*) to represent the amino acids. The three-nucleotide groups are called *codons*, which are discussed in detail in Chapter 15. These codons are arranged in sequence along the RNA molecule without spaces or overlap. The message must be read by looking at the first three nucleotides, then the next three, and so on.

In building a protein, a process of *translation* occurs in which the sequence of ribonucleotide triplet codes is expressed as a linear sequence of the corresponding amino acids in the protein molecule. There must exist a mechanism that determines where to start reading the RNA message, recognizes each codon in sequence, adds the appropriate amino acid to the growing polypeptide chain, and ends the chain at the proper point. The deciphering of the details of this mechanism has been one of the major successes of biology in recent years (Chapter 15).

DNA

In most organisms, RNA is only a working copy of the basic genetic information, which is stored in the molecules of DNA (deoxyribonucleic acid). DNA is a polynucleotide very similar to RNA. Each of the nucleotides of DNA is also composed of a phosphate group, a sugar, and a base. In DNA the sugar is slightly different from that in RNA in that it lacks one oxygen atom (Figure 4.19). This sugar is called deoxyribose, from which is derived the name deoxyribonucleic acid. The bases found in the deoxyribonucleotides are adenine, guanine, cytosine, and thymine. Except for the presence

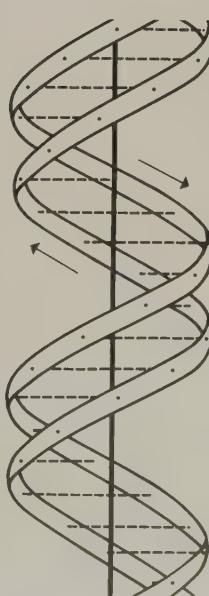
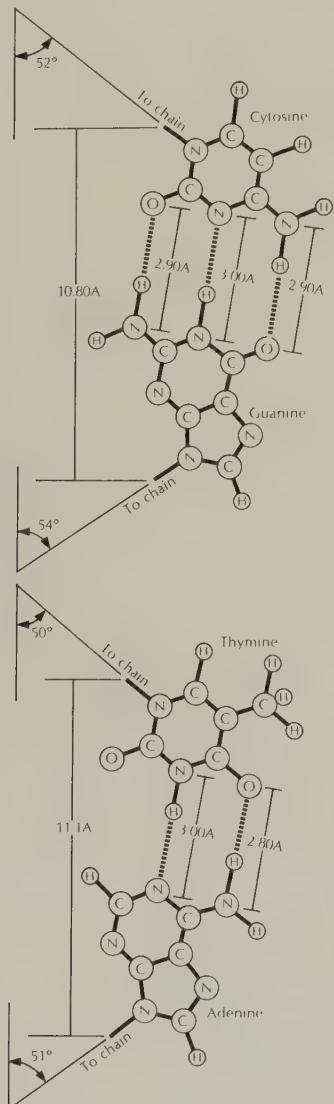


Figure 4.21. Complementary hydrogen bonding of purine and pyrimidine base pairs in DNA. Note the triple bond between cytosine and guanine and the double bond between adenine and cytosine.



of thymine instead of uracil, these bases are the same as those found in the ribonucleotides. As in RNA, the structure of the polynucleotide DNA consists of a phosphate-sugar backbone, with the bases attached along the side. However, for DNA there is one very important complication to this simple picture.

DNA must specify the sequence of ribonucleotides in RNA, which in turn specify the sequence of amino acids in proteins. What specifies the sequence of deoxyribonucleotides in DNA? The answer is other DNA. If a cell divides, the two new daughter cells must have the same genetic information that was present in the parent cell. Therefore, if DNA is the fundamental genetic material, the daughter cells must have the same kind of DNA that was present in the parent. The macromolecular structure of DNA must lend itself to the guidance of its own replication as well as the guidance of RNA synthesis.

An early clue to the nature of the DNA structure came from work by Erwin Chargaff (1955) and others who were trying to determine the base composition of DNA samples from various sources. They found, as expected, that the composition varies from species to species—bacterial DNA differs in its proportions of the four bases from human DNA, which in turn differs from the DNA of cattle. For each species, however, the proportion of adenine is always equal to that of thymine, and the proportion of guanine is always equal to that of cytosine. Another clue to the DNA structure came from x-ray diffraction measurements made by M. H. F. Wilkins, Rosalind Franklin, and others (Wilkins, Stokes, and Wilson, 1953). These measurements suggested that the DNA molecule is coiled into a helical structure.

The structure of DNA, which has since been confirmed by many kinds of evidence, was proposed by James Watson and Francis Crick (1953)—the famous double helix (Figure 4.20). According to the Watson-Crick model, discussed further in Chapter 15, the DNA molecule is composed of two polynucleotide chains intertwined in a helical spiral. The two chains are connected by hydrogen bonds between their bases; each base on one chain is weakly bound to a base on the other chain. The two sugar-phosphate chains run in opposite directions; that is, the sequence of atoms goes one way in one chain and the opposite way in the other chain.

In order for the chains to fit together in the structure indicated by the x-ray diffraction measurements, Watson and Crick proposed that a large base group on one chain must always be paired with a small base group on the other chain. In fact, because of the shapes and hydrogen bonding of the base groups, only two pairings are possible: adenine with thymine, and guanine with cytosine (Figure 4.21). Thus, wherever adenine appears in one chain, it must be paired with thymine on the other chain, and guanine on one chain must always be paired with cytosine on the other. The pairs of bases are joined by hydrogen bonds.

The two strands of the DNA molecule are not identical but are oriented in opposite directions. The bases on the one strand are complementary to the bases on the other. Suppose that the two strands could be separated, breaking the weak hydrogen bonds that hold the strands together but keeping the sugar-phosphate backbones of the two chains intact. If the environment contained deoxyribonucleotides and if a mechanism existed to put them together to form new polynucleotide strands, each of the parent strands of DNA could build upon itself a new companion strand. Because of the base pairing restrictions, each of the new companion strands would be complementary to the strand on which it was built. Therefore, the final

Figure 4.22 (above). Complementary bonding of specific purine-pyrimidine pairs results in the production of complementary DNA strands.

Figure 4.23 (middle). The DNA molecule in the process of replication according to the Watson-Crick model.

Figure 4.24 (below). Transcription of a DNA template code to an RNA molecule. The enzyme RNA polymerase attaches to the DNA molecule, opening up a short section of the double helix for transcription. As RNA polymerase moves along the DNA template, the

result would be two complementary DNA strands, each identical to the parent strand (Figure 4.22).

Actually, the mechanism is far more complex than this simple description. For example, to separate the two strands without breaking the sugar-phosphate backbones is impossible because of the intertwining of the strands. In the living system, the strands unwind gradually as complementary strands are built, and the backbones of the strands are temporarily broken and then rejoined to permit the separation of the two strands. Some organisms contain single-stranded DNA. In these organisms, the original strand presumably synthesizes a short-lived complementary strand, which then synthesizes a duplicate of the original DNA.

The Watson-Crick model fulfills the requirement for self-duplication of the information stored in the DNA molecule. No translation is involved in the DNA self-duplication; it is simply a process of *replication*. The property of mutation can be easily explained by this model. If for any reason an error should occur in the copying, that error would be passed on in future replications. Suppose, for example, that a small segment of the strand were reversed during the unwinding process. When this segment built a complementary strand, the new strand would match the reversed sequence, not the original sequence. When this new DNA molecule, in turn, separated and formed new complementary strands, the error (termed a *mutation*) would continue to be copied on every subsequent replication of the DNA.

The process of creating RNA from the information on the DNA molecule is similar to that involved in duplicating the DNA. The new strand being built is composed of ribonucleotides instead of deoxyribonucleotides, and the base uracil (instead of thymine) must be paired with the base adenine. An exact replication is not carried out, yet the copying is on a one-for-one basis, unlike the translation involved in creating a protein from the RNA molecule. The creation of RNA from DNA is called *transcription*. Because the RNA molecule is usually single stranded, only one of the DNA strands is used for transcription of the RNA. It is not always the same strand of DNA, and the mechanism by which one or the other is chosen is an area of active research. It is this phase of transcription that appears to determine how protein synthesis responds to changing environmental conditions.

LIVING CHEMICAL SYSTEMS

Although living systems contain atoms of only a few elements, the unusual properties of the carbon atom permit the formation of an incredible variety of complex molecules. The atoms and molecules themselves clearly are not alive. Yet a combination of certain complex molecules, involved in an intricate network of chemical reactions, can possess all of those properties that are ascribed to living things. Biochemists have made a striking case for the view that living things are merely very complicated mechanisms obeying the same laws of chemistry and physics as nonliving systems.

FURTHER READING

Baker and Allen (1965) discuss the chemistry of living systems. A clear and well-illustrated introduction to the chemistry of proteins is given by Dickerson and Geis (1970). Watson (1968) presents a lively and controversial account of the development of the Watson-Crick model of DNA structure.

Scientific American articles pertinent to this chapter include those by Changeux (1965), Crick (1954, 1962), and Holley (1966).

growing RNA strand peels off and attaches to a ribosome, while hydrogen bonds re-form the complementary DNA strands. Printed by permission from J. D. Watson, Molecular Biology of the Gene, © 1970, J. D. Watson



5

Photosynthesis



All biological processes dissipate energy. They convert free energy—energy available to do mechanical, chemical, or biological work—into heat. Living systems are therefore dependent upon the steady supply, transfer, and storage of free energy.

Today, the widespread occurrence of life on earth, forming an almost continuous biosphere, is made possible only because certain organisms have the ability to use photons of solar energy for the synthesis of reduced molecules containing high-energy bonds that can store chemical energy. This conversion of radiant energy to chemical energy occurs through the process called *photosynthesis*; it occurs in all green plants, in the blue-green algae, and in some bacteria. The photosynthetic process is important not only as a source of energy for the biosphere but as the source of essentially all organic molecules in the biosphere. As a vital by-product, photosynthesis produces the oxygen molecules of the atmosphere. Clearly, this process is of primary importance to the existence of life on earth, and biologists have devoted a great deal of attention to its study for the past two centuries (Interleaf 5.1).

THE PHOTOSYNTHETIC MECHANISM

In the photosynthetic process, plants use light energy to convert two extremely stable, low-energy molecules (carbon dioxide and water) into an unstable, energy-rich system consisting of organic matter and free oxygen. This energy-rich system fuels almost all other life processes. Most of the organic molecules created through photosynthesis are sugars, which plants can polymerize to form polysaccharides such as starch and cellulose. The simple sugar glucose (Figure 5.2) can be taken as typical of the organic molecules produced by photosynthesis.

The photosynthetic formation of glucose can be represented by the following chemical formula:



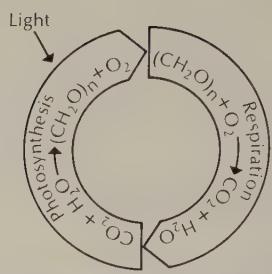
A generalized formula for photosynthesis of a single carbon unit can be written as follows:



This reaction is strongly endergonic. In the absence of catalysts, the reverse reaction (oxidation of carbohydrate to form carbon dioxide and water, with release of thermal energy) occurs only at high temperatures, as in the burning of sugar, wood, or paper. The plant is able to carry out this process at room temperature through an extremely complex system of catalyzed reactions whose sum is the overall reaction of Formula 5.2.

In photosynthesis, energy is supplied by light quanta (photons) whose energy content depends upon the frequency or wavelength of the light. In red light, each mole of photons provides about 40 kcal of energy. To provide the 114 kcal needed to combine a mole of CO_2 with a mole of H_2O , at least 3 moles of photons are needed. In other words, the reduction of a single atom of carbon (in a molecule of CO_2) by a single molecule of H_2O requires energy from at least 3 photons. There is an insignificantly small probability that 3 photons would happen to strike just as the 2 molecules

Figure 5.1. Energy cycle of the biosphere.
Photosynthetic organisms utilize light energy from the sun to synthesize large carbohydrate molecules from simpler compounds. Oxygen is produced as a by-product. Respirating organisms degrade the large carbohydrate molecules synthesized by plants, utilizing the energy obtained to sustain life functions and produce water and carbon dioxide as waste products. Because energy is constantly being lost as heat, the cycle requires constant input of light energy for its continued functioning.



It was recognized long ago that plants provide the ultimate source of food for nearly all organisms on earth. Until the seventeenth century, however, it was assumed that plants create their tissues from materials extracted out of the soil. About 1648 a Flemish alchemist, Jan van Helmont, conducted an experiment to test this theory. He grew a tree in a tub of soil, adding nothing but water for five years. In that time, the tree gained 164 pounds, but the soil weighed only 2 ounces less than it had at the beginning of the experiment. Helmont considered these results as proof of his belief that water is the basic substance of which plants (indeed, all matter, according to Helmont) are made. Some 80 years later, the English naturalist Stephan Hales published the results of his studies of plants, from which he concluded that air is also an important source of nutrients for plant growth.

In the late eighteenth century—sometimes called the age of pneumochemistry (from the Greek word for breath)—chemists were busy identifying and studying the various kinds of air (now called gases). In 1771 the most noted of the English pneumochemists, the Reverend Joseph Priestley, showed that plants “improve air” that has been “damaged” by the breathing of animals or the burning of candles. Some eight years later Jan Ingenhousz, a Dutch physician at the Imperial Court of Vienna spending a summer of research in England, found that this improvement is caused by a rapid chemical reaction induced by light in the green-colored tissues (leaves and stalks) of plants. The improvement of the air was described as “dephlogistication” until Antoine Lavoisier showed that this process could better be described as “enrichment in oxygen.”

In 1782 a Swiss pastor, Jean Senebier, discovered that the reaction now called photosynthesis occurs only in the presence of “fixed air” (now called carbon dioxide); in 1804 another learned citizen of Geneva, Théodore de Saussure, showed that water also is necessary for the reaction to take place. In 1796 Ingenhousz proclaimed that the process of photosynthesis is the main if not the only source of all organic matter in plants and thus, indirectly, also in animals feeding on plants. The early guesses of Helmont and Hales proved to be correct; plants create their tissues from carbon dioxide and water through the process of photosynthesis. In this process, oxygen is released into the atmosphere.

Some 50 years after the discovery of the chemical nature of photosynthesis, Julius Robert Mayer (a doctor of medicine famed as codiscoverer of the first law of thermodynamics) first recognized that photosynthesis converts light energy into chemical energy. Thus, by 1845 it was recognized that photosynthesizing plants represent the biggest chemical factory on the surface of the earth, as well as the ultimate power station of the biosphere.

Interleaf 5.] THE “DISCOVERY” OF PHOTOSYNTHESIS



Jan van Helmont



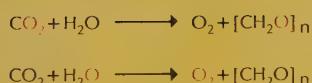
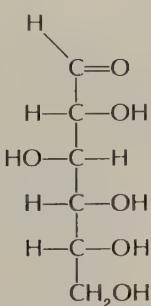
Joseph Priestly



Julius Robert Mayer

Figure 5.2 (above). The structural formula of glucose.

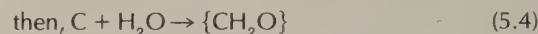
Figure 5.3 (below). Experimental evidence proving that the origin of liberated oxygen in photosynthesis is from the photolysis of water molecules. Isotope tracer experiments using oxygen-18 are shown above. Red type represents radioactive oxygen.



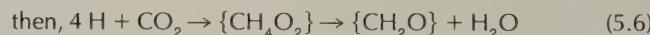
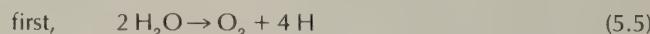
collided; the organism must possess some mechanism for gathering energy from photons and providing it at the proper time and place to convert the simple molecules to sugar.

Precise measurements indicate that, under the most favorable conditions (dim light and sufficient—but not too much—carbon dioxide), 8 photons are needed by the plant cell to consume one molecule of CO_2 and to liberate one molecule of O_2 . Three photons contain enough energy to carry out the reaction; the plant uses 8 photons. Therefore, the plant is able to use the light energy with an efficiency of about 35 percent ($3/8$), a much higher efficiency than has been achieved in the laboratory using artificial photosynthetic systems with visible light.

Originally, it was assumed that the photosynthetic process involves the removal of oxygen from carbon dioxide, followed by hydration of the remaining carbon:



According to this mechanism, the oxygen molecules are formed from oxygen atoms in the CO_2 molecules. It is possible to test this hypothesis by using isotopes of oxygen— CO_2 can be prepared with oxygen-18 rather than normal oxygen-16. When this heavy CO_2 is used in photosynthesis, the oxygen gas that is liberated is not made up of oxygen-18. However, if H_2O prepared with oxygen-18 is used in photosynthesis, the oxygen gas liberated is entirely made up of oxygen-18. It appears that the oxygen gas is formed from oxygen atoms in the H_2O . The mechanism of photosynthesis therefore must involve the *photolysis*, or splitting of water, followed by the reduction, or hydrogenation, of CO_2 :



The second part of this mechanism involves the formation of an unstable intermediate (CH_4O_2), which readily loses a water molecule to form the carbohydrate unit (CH_2O). The water thus formed is not recycled back to the first reaction but must be liberated as a waste product or used elsewhere in the plant. If this recycling did not occur, the use of heavy oxygen in H_2O ought to result in only part of the liberated oxygen being heavy.

This model of the photosynthetic mechanism indicates that 4 hydrogen atoms must be transferred from water molecules to a single molecule of CO_2 to carry out photosynthesis. Because 8 photons are needed to carry out the process, it appears that 2 photons are needed for the transfer of each hydrogen atom. Not all this energy is stored in the final product molecules; some is lost at each step in the process.

The modern tendency is to describe photosynthesis as an oxidation-reduction reaction in which 4 hydrogen atoms from water are transferred to CO_2 . Energetically, this transfer is an “uphill” process.

STAGES OF PHOTOSYNTHESIS

In the presence of adequate amounts of CO_2 and H_2O , it would be expected that the rate of photosynthesis—measured by the rate of production of

Figure 5.4 (above). Light saturation in photosynthesis. The photosynthetic rate is proportional to light intensity, but only up to a saturation point at which the rate remains constant regardless of light intensity.

Figure 5.5 (below). The light and dark stage reactions of photosynthesis. At low light intensity, the overall reaction rate is limited by the light-dependent production of intermediate compounds. At precisely the saturation point of light intensity, the light-dependent stage is producing intermediate compounds

oxygen—would be proportional to the intensity of light available (the rate at which photons are being made available to carry out the reaction). Experiments show that this proportionality is valid only up to a certain point, beyond which the rate of photosynthesis remains constant regardless of further increases in light intensity (Figure 5.4). F. F. Blackman, an English plant physiologist, suggested in 1905 that this “light saturation” occurs because photosynthesis is a two-stage process.

In the *light-limited* (or *photochemical*) stage, light energy is used to produce high-energy (chemically reactive) molecules; the rate of this process is nearly proportional to the light intensity. The light-limited stage is the energy storage phase, which includes the photochemical process. In the *dark* (or *enzymatic*) stage, the unstable intermediate molecules are converted into stable final products (oxygen and sugar) through reactions not involving light energy. The dark reactions to reduce CO_2 to sugar are catalyzed, as are all metabolic reactions, by enzymes. Cofactors also play important roles in the dark reactions.

Because enzyme molecules are very large, only a fairly small number of them can be present in the cell. Moreover, each enzyme molecule can transform only a limited number of substrate molecules each second. The enzymatic stage therefore has a maximum rate imposed by the maximum rate at which the enzyme can process substrate molecules. Many reactions and many enzymes are involved in the enzymatic stage, so that the slowest enzyme determines the ceiling rate of the entire stage. The maximum rate

at the maximum pace at which the dark stage enzymatic reactions can complete the reaction series. If more light is supplied, there is no change in the overall rate because the total reaction series cannot proceed faster than the dark stage rate.

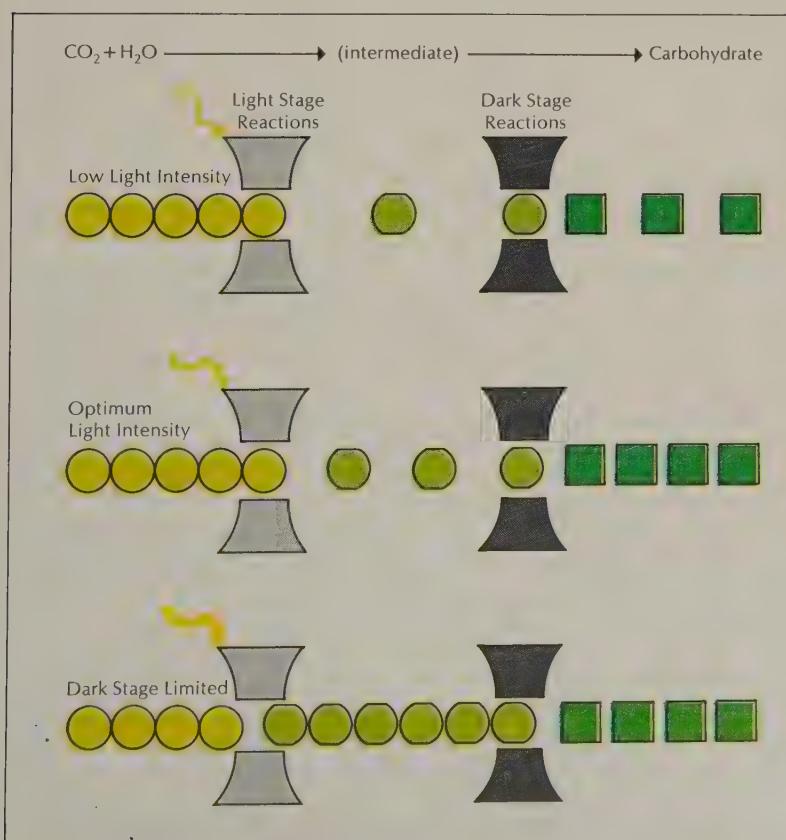
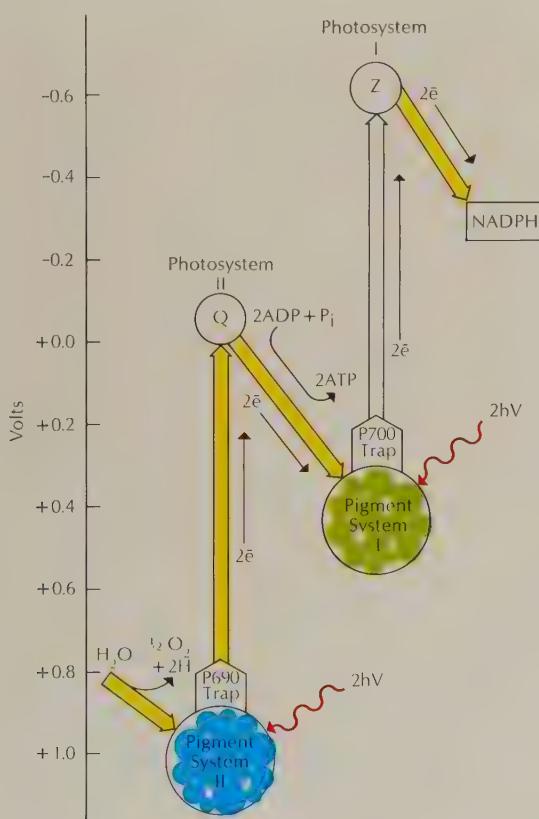


Figure 5.6. The two-step relationships of the light reactions of photosynthesis.



of this stage is achieved when all of the molecules of an enzyme involved in the process are loaded with substrate molecules and are engaged in their transformation.

Once the enzymatic stage has reached its maximum rate, further increases in the rate of the photochemical stage will simply cause an accumulation of the energy-rich intermediate molecules. These intermediates are unstable and spontaneously return to their original forms if they are not used immediately for conversion into stable end products. The instability of the intermediate products is confirmed by the fact that production of oxygen ceases almost instantly when the light supply is cut off.

The Light-Limited Stage

Because eight photons and four hydrogen atoms are needed to process each molecule of CO_2 , it appears that each hydrogen atom is pushed "up-hill" energetically in two steps, each involving a push by one photon of energy. This energetic pushing, and the trapping of the energized hydrogen, is the heart of the light-limited stage. However, many details of the two-step process in this stage remain obscure. In particular, its precise physical nature is not understood, nor is it known how the two steps are coordinated so effectively that the overall photosynthetic process can proceed with very little loss of absorbed energy quanta. Furthermore, the details of the enzymatic splitting of water to provide the hydrogen that is pushed remain unclear. In spite of the gaps, an outline of the process can be given from what is known.

Each of the two energy pushes involves a separate set of reactions. These sets are known as photochemical reaction II (PCII) and photochemical reaction I (PCI). (The Roman numerals were assigned in order of the detailed study of the steps, not in order of their occurrence in the photosynthetic process.) Hydrogen atoms are removed from water (leaving the oxygen in some unstable intermediate form) and pushed up the energy hill in PCII (Figure 5.6). The hydrogen atoms then travel down a series of redox reactions in which part of the energy is captured for the photosynthetic phosphorylation of ADP to ATP (Chapter 3). The hydrogen atoms are then given another push to an even higher energy level by PCI. Finally, the hydrogen atoms pass into another cofactor sequence, which results in the reduction of the cofactor NADP (nicotinamide adenine dinucleotide phosphate) to its reduced state, NADPH.

The net result of the light-limited stage, then, is the transfer of hydrogen atoms from water molecules to the high-energy compound NADPH, with the production of ATP molecules. Oxygen is released as a by-product of this activity. In the dark stage, NADPH acts as a reductant for the conversion of CO_2 to carbohydrate, and ATP provides chemical energy for this conversion. The nature of the intermediate molecule from which the hydrogen atoms are removed at the beginning of the light-limited stage is not yet completely known.

The Dark Stage

Several sequences of enzymatic or dark reactions are involved in the photosynthetic process. The cofactor sequence occurring between PCII and PCI and that occurring after PCI have been discussed as part of the light-limited stage. Other enzymatic reactions are involved in the formation of an intermediate molecule from the water molecule and the conversion of

the intermediate to O_2 after the removal of the hydrogen atoms at the beginning of PCII. The heart of the dark stage, and by far the best understood part of the photosynthesis, is the sequence of enzymatic reactions by which CO_2 is reduced to carbohydrate.

THE REDUCTION OF CO_2

The dark reactions involved in the reduction of CO_2 utilize the energy and reducing power trapped in the light-limited stage. Three processes are involved in this sequence of reactions: (1) incorporation of CO_2 into a carbon dioxide acceptor; (2) reduction of this complex using the ATP and NADPH produced in the light-limited stage; and (3) transformation of the reduced complex (which is probably a molecule containing three carbon atoms) into a six-carbon sugar such as glucose or fructose.

1. *Carbon-dioxide incorporation.* If the product of CO_2 incorporation is a three-carbon compound, the acceptor would be expected to be a two-carbon compound. Yet no suitable compound of this type has been identified in photosynthetic systems; it appears that two-carbon compounds are chemically too active to be tolerated by cells in substantial quantities. This puzzle was solved by Melvin Calvin and A. A. Benson, who discovered that a five-carbon sugar (a pentose, $C_5H_{10}O_5$) can bind CO_2 and then split into two three-carbon molecules in two closely associated enzymatic steps.

The five-carbon sugar involved is ribulose, and the actual CO_2 acceptor is the diphosphate of this sugar, ribulose diphosphate (RuDP). This molecule combines with CO_2 to form an unstable six-carbon compound ($\{C_6\}$), which splits through hydrolysis into two molecules of phosphoglyceric acid (PGA). The reactions may be abbreviated as follows (complete structural formulae are shown in Figure 5.7):



2. *Reduction of the intermediate.* The PGA is next reduced to a triose (three-carbon sugar) by the high-energy products resulting from the light-limited stage. In this reduction, the carbon atoms gain the appropriate number of hydrogen atoms and sufficient energy to bring them to the sugar level ($C_3H_6O_3$).

In this process, PGA first gains a high-energy phosphate group to become the much more energetic molecule diphosphoglyceric acid (DPGA).

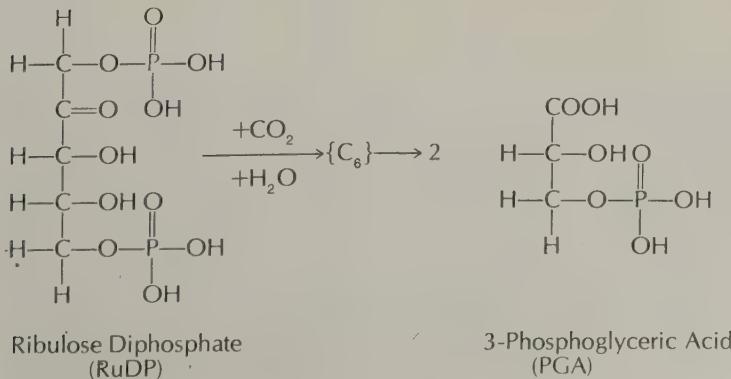
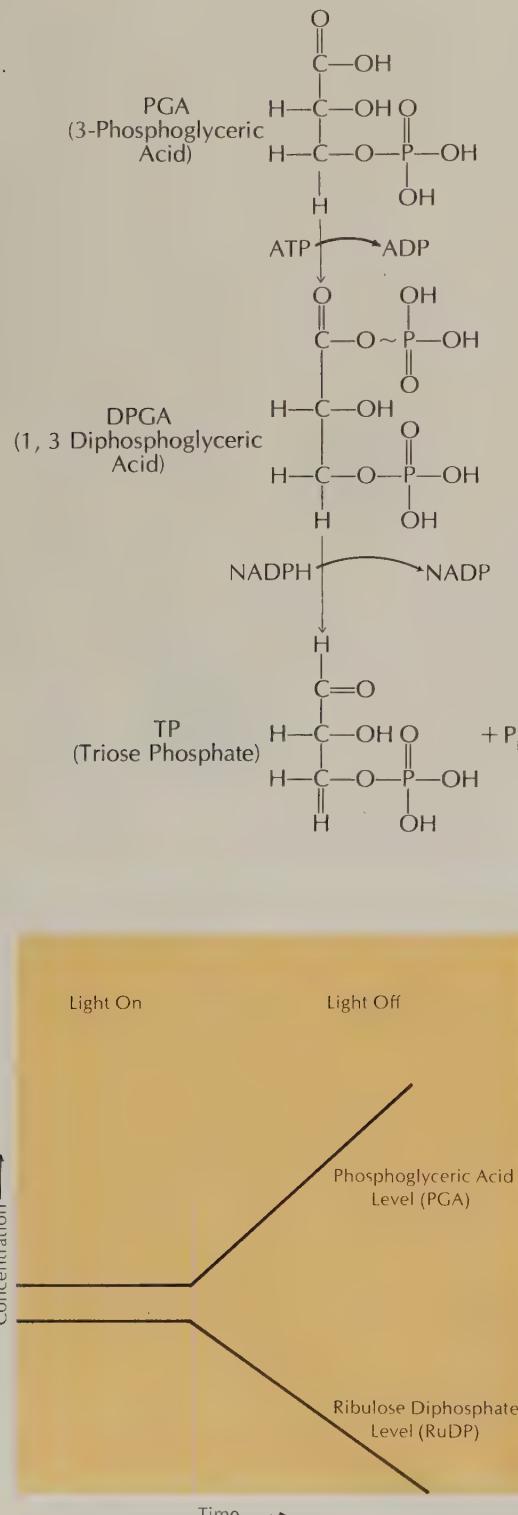


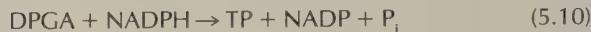
Figure 5.7. The structural formulae of ribulose diphosphate (RuDP) and 3-phosphoglyceric acid (PGA).

Figure 5.8 (above). The structural formulae of the reduction of the intermediate.

Figure 5.9 (below). An experiment illustrating the coupling of the light and dark reactions in photosynthesis.



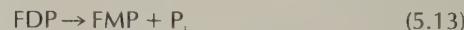
DPGA is then reduced to triose phosphate (TP) as NADPH is oxidized back to NADP (see Figure 5.8 for complete structural formulae):



Because both of the energy-rich compounds used in this step (ATP and NADPH) are produced in the light-limited stage and because ADP, P_i, and NADP are returned to the light-limited stage, the two stages of photosynthesis are intimately coupled molecularly and energetically.

If the mechanism proposed for these two steps is valid, turning off the light that falls on an actively photosynthesizing cell should lead to an immediate increase in concentration of PGA and decrease in concentration of RuDP. These changes should occur because reactions 5.7 and 5.8 can continue in the dark, whereas reactions 5.9 and 5.10 are dependent upon a supply of ATP and NADPH produced in the light-limited stage. As soon as the light is turned off, the supply of energy-rich compounds will be rapidly used up, and the transformation of PGA to TP will slow down, while production of PGA from RuDP continues. This expectation was confirmed experimentally (Figure 5.9).

3. Transformation of the carbohydrate. Finally, a sequence of enzymatic reactions combines two triose molecules to form a single six-carbon sugar (hexose). First, the TP is partially converted to another three-carbon sugar phosphate, dihydroxyacetone phosphate (DHAP). An equilibrium is reached with about 60 percent TP and 40 percent DHAP. Next, one molecule of TP and one molecule of DHAP combine to form a molecule of fructose diphosphate (FDP), which loses a phosphate group to form fructose monophosphate (FMP). The FMP is partially converted to glucose monophosphate (GMP), which loses a phosphate group to form glucose. To form 1 glucose from 6 CO₂ molecules, a total of 18 ATP and 12 NADPH will be required.



In fact, only 1/6 of the triose phosphate is converted to glucose. The other 5/6 of the TP goes through another complex sequence of reactions to produce ribulose diphosphate (RuDP), which can then act as the CO₂ acceptor to keep the cycle going. The net result of the sequence is the conversion of 10 three-carbon sugar molecules into 6 five-carbon sugars. A major role in this sequence is played by the cofactor *thiamine*, which aids in moving two-carbon units from one sugar to another. Thiamine is required in the human diet and is also known as vitamin B₁.

The complete cycle of carbon compounds involved in the dark stage includes some 13 reaction steps that are catalyzed by at least 11 separate enzymes. Melvin Calvin received the Nobel Prize in 1961 for his work in

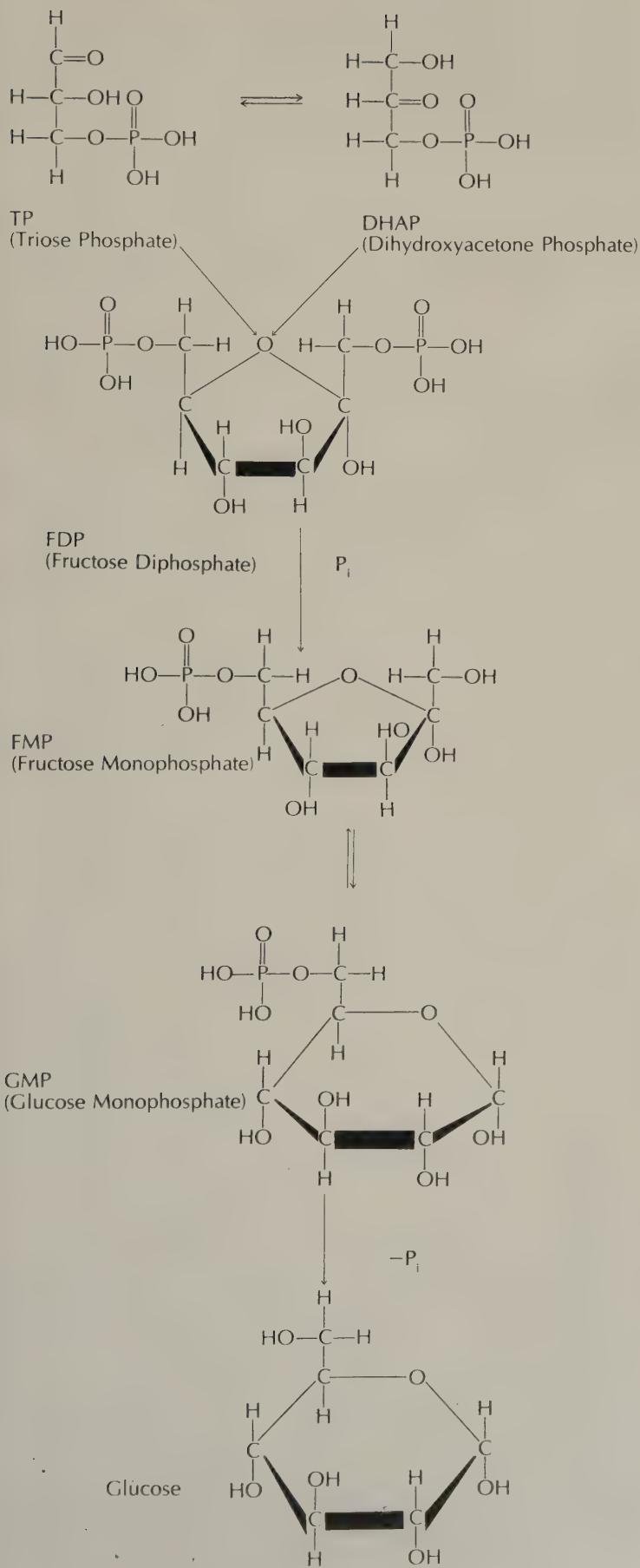
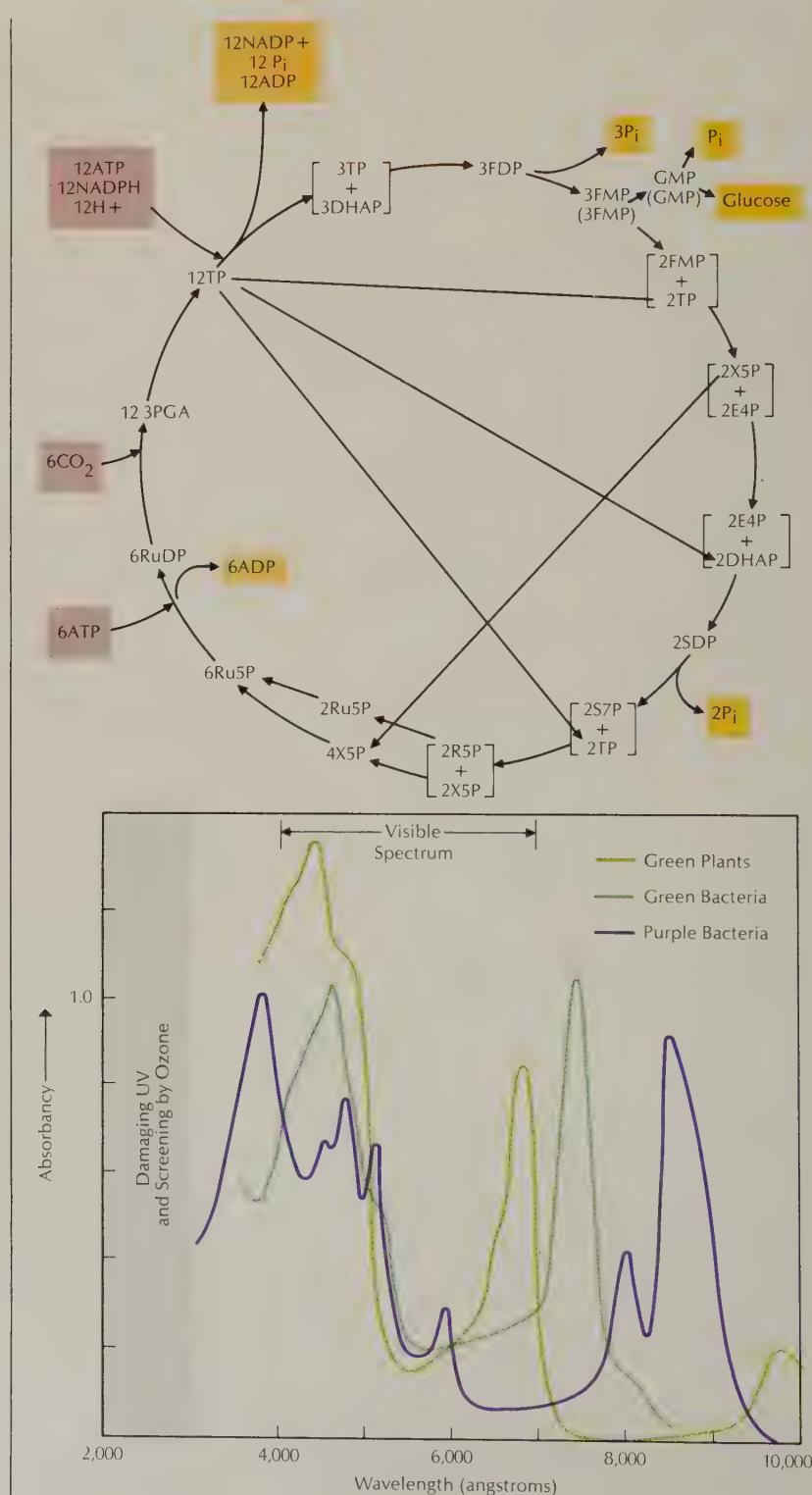


Figure 5.10. Structural formulae for the transformation of the carbohydrate.

Figure 5.11 (above). The photosynthetic formation of glucose from CO_2 via the Calvin cycle. Inputs are shaded in brown; products are in yellow. Clockwise from the left, the abbreviations designate the following terms: PGA = 3-phosphoglyceric acid; TP = glyceraldehyde 3-phosphate; DHAP = dihydroxyacetone phosphate; FDP = fructose 1,6-diphosphate; FMP = fructose 6-phosphate; GMP = glucose 6-phosphate; X5P = xylulose 5-phosphate; E4P = erythrose 4-phosphate; SDP = sedoheptulose 1,7-diphosphate; S7P = sedoheptulose 7-phosphate;

R5P = ribose 5-phosphate; Ru5P = ribulose 5-phosphate; RuDP = ribulose 1,5-diphosphate.

Figure 5.12 (below). Absorption spectra of three types of photosynthetic organisms, showing the regions of the spectrum that can be used for photosynthesis. Regions of intense absorption correspond to the absorption maxima of the respective forms of chlorophyll contained by the organisms.



clarifying the details of this cycle, which is now known as the *Calvin cycle* (Figure 5.11). Each trip around this cycle consumes 1 molecule of CO₂ and 4 hydrogen atoms and produces 1/6 of a glucose molecule.

THE LIBERATION OF OXYGEN

The details of the enzymatic reaction sequence leading from H₂O to O₂ are all but unknown. In the steps of this sequence, hydrogen atoms must be removed from the water molecule and sent into the photochemical reaction PCII. To liberate 1 oxygen molecule, 4 hydrogen atoms must be removed. This process cannot happen in a single step; rather, some intermediates must be involved, but nothing is known about their nature. It is clear, however, that these reactions are enzymatic rather than photochemical. Interestingly, the reverse process in respiration, where molecular oxygen is taken into the reaction, is also poorly understood.

Energy-Trapping Systems

The reactions of the dark stage are not very different from enzymatic reactions involved in other processes of cellular metabolism. The photochemical reactions of the light-limited stage, however, are unlike any other reactions occurring in living systems. A better understanding of these photochemical reactions is therefore of great interest to biochemists and biophysicists.

It appears from various experiments that the two photochemical reaction steps, PCII and PCl, occur in two separate reaction centers and are supplied with energy from two separate photon-trapping systems. These two energy-trapping systems absorb light of slightly different frequencies. A biologist can thus study the two reaction steps separately because light of a frequency is absorbed solely or chiefly by one of the systems. For example, in green plants and algae, far-red light (wavelengths greater than 700 millimicrons) seems to be absorbed preferentially in system I, whereas light at about 650 millimicrons is absorbed preferentially in system II. In red algae, the situation is even more striking, for green light is absorbed preferentially in system II, whereas red light is absorbed preferentially in system I.

In recent years, many studies have been made of the properties of the two systems, the reactions that each carries out, and the cooperation between the two systems. It even has been possible to separate, at least partially, the proteins that make up the two systems.

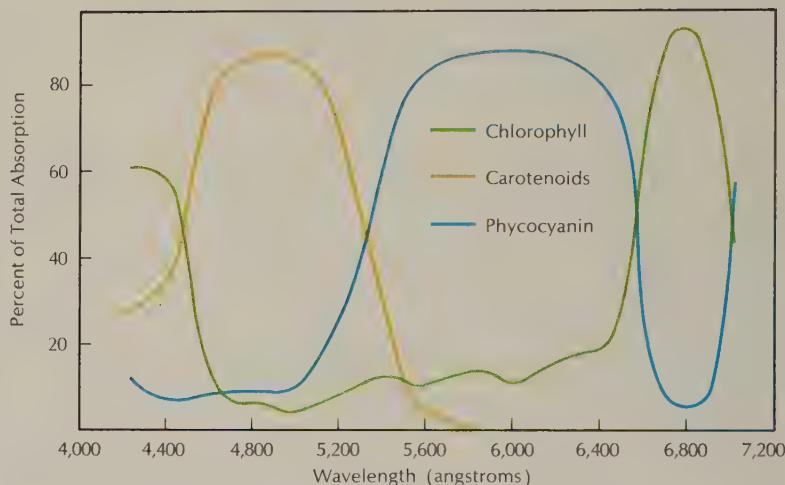
THE PHOTOSYNTHETIC UNIT

The reactions of the photochemical phase are made possible by *pigments*, unusual catalysts that are able to absorb photons. Because these molecules absorb light of particular frequencies, the color of the visible light passing through the photosynthetic cell is altered.

The involvement of a photon in a chemical reaction is a two-step process. First, the photon is absorbed by a pigment molecule; the absorbed energy raises one or more of the electrons in the pigment molecule to an energy-rich, excited state. In the excited or oxidized state, the pigment molecule becomes a catalyst, but unlike other catalysts it is able to contribute to the catalyzed reaction all or part of its stored energy. It can therefore catalyze endergonic, or uphill, reactions such as the transfer of hydrogen atoms in photosynthesis, something no ordinary catalyst can do. Thus, a chlorophyll molecule, excited by absorption of red light, can contribute

Figure 5.13 (above). Computed percentage of absorption of the different wavelengths of visible light by the chlorophyll, carotenoid, and phycocyanin pigments of Chroococcus, a blue-green alga. In the red region, absorption is due mainly to chlorophyll, and in the orange and yellow regions, it is due mainly to phycocyanin.

Figure 5.14 (below). Schematic portrayal of electron resonance in three-dimensional photosynthetic units.



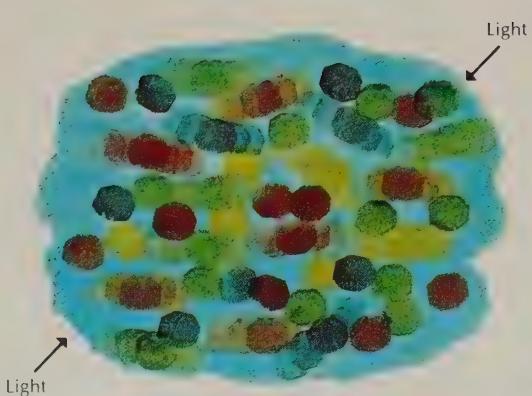
about 40 kcal/mole of energy to the reaction that it catalyzes. Its electrons then drop back to the low-energy state, and the chlorophyll molecule is ready to absorb another photon.

Not all pigment molecules present in a photosynthesizing plant cell function directly as catalysts. This function may be the responsibility of only the small proportion that are associated with certain enzymatic reaction centers. Pigment molecules are small in comparison to enzyme molecules; the molecular weight (sum of the atomic weights of all atoms in the molecule) of chlorophyll is about 1,000, whereas that of a typical enzyme is about 100,000 to 1,000,000. Thus, the cell has room for several hundred pigment molecules for each enzyme molecule.

The cell has a need for all these pigment molecules in order to capture a supply of photons. Good organic pigments, such as chlorophyll, are relatively efficient in the capture of light as compared to other molecules. Even so, a molecule of such a pigment, exposed to direct sunlight at midday, will not absorb more than about 10 photons per second. On the other hand, an ordinary enzyme molecule may transform substrate molecules at rates of tens of thousands of substrate molecules per second. Thus, the cell can use hundreds or even thousands of pigment molecules to gather enough light energy for use in a single enzymatic reaction center. The problem is how the cell gets these photons where they are needed—a process that has received a great deal of study.

It appears that pigment molecules are organized in the living organism into *photosynthetic units*. These units are three-dimensional bodies, or perhaps two-dimensional islands, within which pigment molecules can exchange energy by a process of *electron resonance*: In effect, the excitation energy is passed from molecule to molecule. Ultimately, the energy can be passed to the single enzymatic reaction center in the unit. This photophysical step of photon capture and energy transfer can be regarded as the first step of the photochemical process.

The mechanism of this migration of excitation energy has been studied in crystals and in concentrated solutions of pigments. However, the actual transfer mechanism in the living cell is far from understood. It is not known, for example, whether there are separate units for the two primary photo-



chemical systems, PCII and PCI. There is evidence that one type of unit contains about 300 chlorophyll molecules per enzymatic reaction center, but whether this unit serves PCII, PCI, or both has not yet been discovered.

Much of the information about the units has come from studies of photosynthesis during very brief flashes of light. If the flash is intense enough, each unit captures at least one photon and thus sets its enzymatic reaction center to work. The intensity of the flashes can be increased until the production of oxygen ceases to increase, indicating that "flash saturation" (each unit being busy) has been reached. At flash saturation, 1 oxygen molecule is produced for about each 2,400 chlorophyll molecules. Because 8 photons are needed to liberate 1 oxygen molecule, 8 units must be involved. Thus, it appears that each unit contains about 300 (2,000/8) pigment molecules.

The shortest dark interval between flashes that will permit a full oxygen yield on each flash is about 0.1 second at room temperature. Apparently, this amount of time is needed for the rate-limiting enzyme to process the supply of substrate molecules that it receives as a result of a single flash. The average time required for the rate-limiting enzyme to process a single substrate molecule is thought to be about 0.01 second.

The Pigments

Complete understanding of the photophysical and photochemical processes in photosynthesis has been delayed by the fact that each photosynthetic organism contains an assortment of pigments. Only one pigment is found in all plants; this pigment is chlorophyll a (Figure 5.15), which is yellowish-green in solution. It seems reasonable to suppose that this pigment alone takes part in the direct transfer of energy to the enzymatic reaction center, whereas all of the other pigments serve only as components of the photon-gathering apparatus. In fact, a large proportion of the chlorophyll a itself must be assigned to this accessory role, for each photosynthetic unit contains some 300 molecules of this pigment.

The function of the other pigments is unknown. Many plants have a second chlorophyll (blue-green chlorophyll b in green land plants and green algae, and brownish chlorophyll c in brown algae and diatoms). All plants have an assortment of yellow or orange pigments called carotenoids, and the red and blue-green algae also possess an assortment of orange-red and blue pigments of the phycobilin group.

It seems that all these pigments contribute photons with different efficiencies to the systems PCII and PCI, but the division between the two systems is not simple. Phycobilins are located primarily, but not exclusively, in

Figure 5.15 (above). The structural formula of chlorophyll. In chlorophyll a, X = —CH₃. In chlorophyll b, X = —CHO.

Figure 5.16 (below). Structural formula of β-carotene, a pigment occurring in plants.

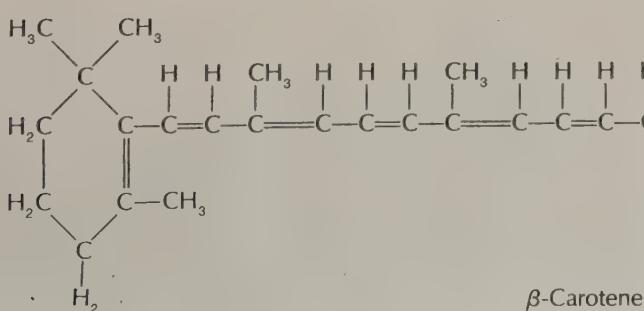
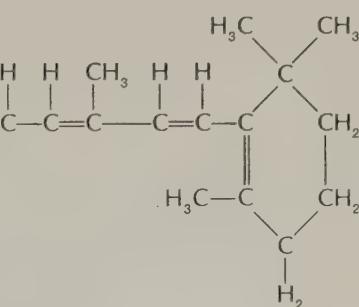
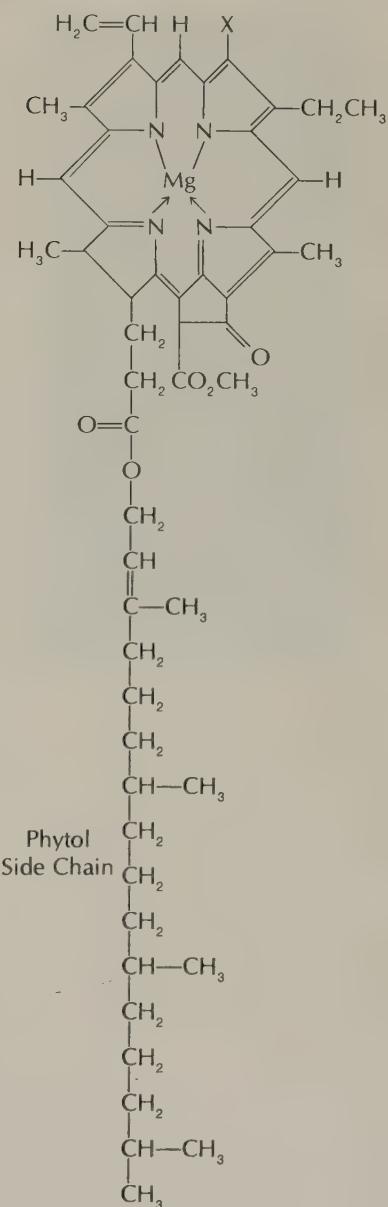
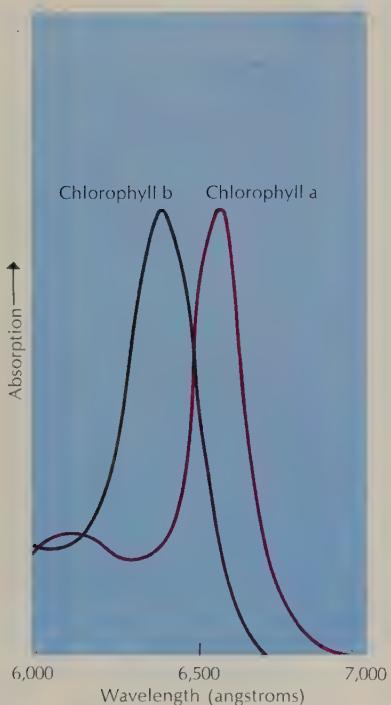


Figure 5.17 (above). Absorption bands of chlorophylls *a* and *b*. Both absorb in the red region of the spectrum and hence are colored green.

Figure 5.18 (below). The absorption spectrum of chlorophyll compared with the light energy emitted from the sun. Note that the maximum energy comes through at about 4,500 Å in the blue region. Chlorophyll usually absorbs about 6,500 Å in the red region.



PCII, as is chlorophyll *b*. Chlorophyll *a* is located primarily, but not exclusively, in PCII in red algae, but is about equally abundant in PCII and PCI in green plants and green algae. To further complicate the situation, it appears that plants contain more than one form of each pigment; these forms differ somewhat in the frequencies of light they absorb (Figure 5.17). For example, there are at least three and perhaps more forms of chlorophyll *a* in green plants. Yet when these different forms are removed from the organism and analyzed, they prove to be chemically identical. The differences in absorption spectra must arise from differences in the ways that the molecules are arranged in the cell.

It has been suggested that the function of the accessory pigments is to gather photons of frequencies not absorbed by chlorophyll and to pass the captured energy on to the chlorophyll for use in the enzymatic reactions. Much remains to be learned, however, about the photosynthetic pigments and their exact role in the photochemical process.

The chlorophylls, carotenoids, and phycobilins appear to function primarily in capturing and transferring light energy. Other pigments that are present in very small amounts appear to act directly in the redox reactions of the photochemical process. One pigment, known as P700 because its absorption peak is at 700 millimicrons, apparently acts as the "trap" in PCI, collecting the energy gathered by the photosynthetic unit and acting as the reductant (electron donor) for the second uphill push of the hydrogen atoms to NADP. One or two molecules of P700 are present for each photosynthetic unit of PCI. P700 is apparently another form of chlorophyll *a*. It has not been isolated outside of the living system. Another pigment, P690, has been identified recently as the trap that collects energy from the photosynthetic units of PCII.

Compounds called cytochromes (iron-containing proteins) have been shown to play a role as cofactors between the two photochemical reac-

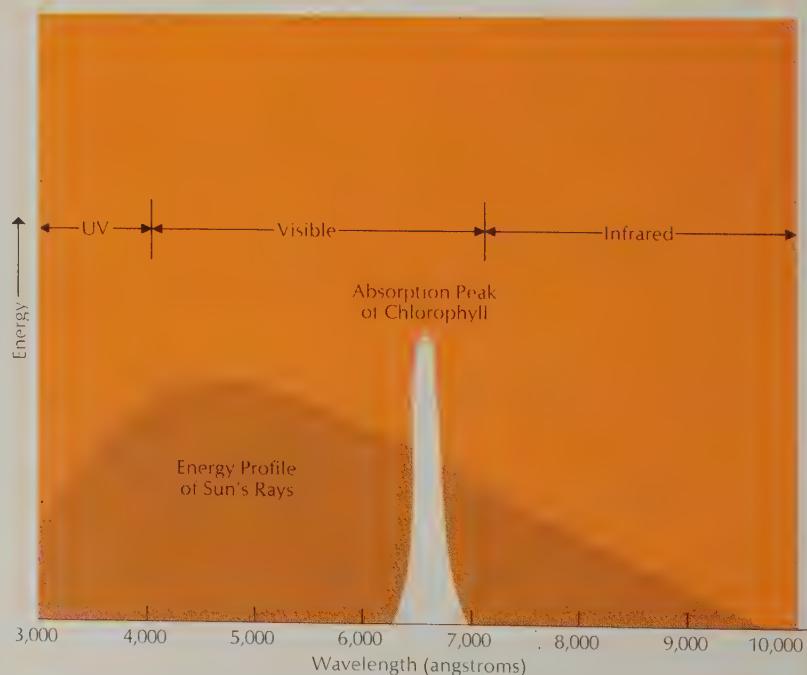
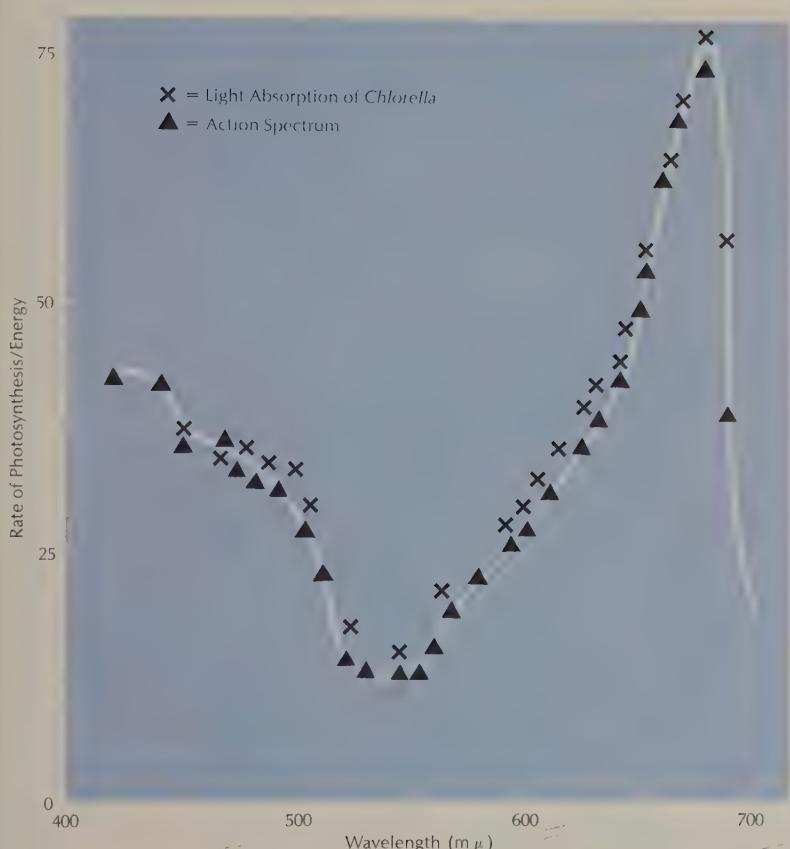


Figure 5.19 (above). Action spectrum of photosynthesis of *Chlorella*.

Figure 5.20 (below). Structural formula of a prosthetic group of a phycocyanin.



tions. Two kinds of cytochrome take part in redox reactions producing ATP in cellular respiration. At least two kinds of cytochrome, differing in their oxidation potentials, are present in photosynthetic cells. One is a form called cytochrome *b*; the other is called cytochrome *f*. It appears that cytochrome *b* may function as the oxidizing agent in PCII, accepting electrons from an as yet unknown intermediate in the $H_2O \rightarrow O_2$ sequence. Cytochrome *f* may act as an electron donor for PCI, passing electrons on to P700. Reduced cytochrome *b* and oxidized cytochrome *f* then react, probably through a sequence of intermediates, to restore each to its original

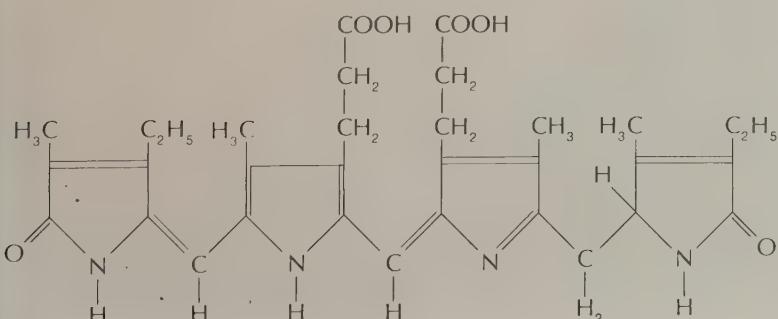


Figure 5.21. A collage of leaves, showing the abundance of pigments and forms found in nature. In addition to the green chlorophylls, there are two main accessory pigments – the carotenoids (yellows to reds) and the phycobilins (reds to blues). These pigments provide a full complement for light absorption in the visible spectrum and help to increase the efficiency of photosynthesis. Color also plays an important role in insect attraction, pollination, and seed dispersal via the fruit.



Figure 5.22. Electron micrograph of a thin section of cells of the purple sulfur bacterium *Chromatium sp.* strain D. ($\times 72,000$)

state. This reaction releases about 9 kcal/mole of energy, which is used to synthesize ATP. Pigments called plastoquinone and plastocyanin appear to act as intermediate cofactor pairs in this reaction sequence.

VARIATIONS OF THE PHOTOSYNTHETIC REACTION

Just as a chemist may learn about the structure of a molecule by taking it apart and examining the pieces, he may learn about a reaction by blocking or altering various steps in the sequence and observing the results. Progress toward an understanding of photosynthesis was impeded for a long time because this approach seemed impossible: photosynthesis appeared to be an all-or-nothing process not subject to partial alterations. It was therefore an important landmark when, in 1937, the English plant biochemist Robert Hill discovered that the oxygen-evolving photochemical processes of photosynthesis can be made to operate without the carbon-dioxide-reducing dark reactions if a substitute oxidant such as a ferric compound is supplied instead of CO_2 .

Studies of this *Hill reaction* have played an increasingly important role in recent research into the chemical mechanisms of photosynthesis. The Hill reaction indicates that PCII and the associated dark reactions that form free O_2 molecules can function by themselves without the CO_2 -transforming dark reactions. The substitute oxidant can be provided before or after PCI. In other words, the Hill reaction may involve only one photochemical step or may involve both of them. A weaker oxidant is used if PCI is to be eliminated, because only the first upward push of the hydrogen atoms is to be accomplished.

Biologists have prepared samples of material from photosynthesizing cells that are apparently enriched in one or the other of the photochemical systems. These samples show different capacities for carrying out the Hill reaction. Such studies have helped to clarify parts of the mechanism involved in the photochemical steps and the intermediate dark reactions.

Another variation of the photosynthetic process occurs in pigmented

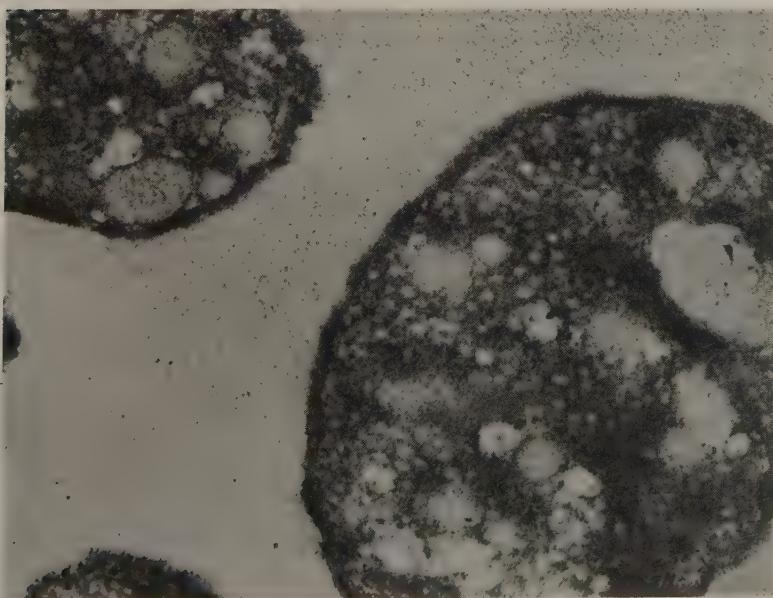


Figure 5.23. Fossil plants. These fossil ferns were found in Illinois and are approximately 300 million years old. They were a dominant plant form during the Upper Carboniferous period.



bacteria. These organisms are unable to oxidize water, but they can synthesize sugar in light if they are supplied with reductants such as hydrogen sulfide (H_2S), free hydrogen, or some organic hydrogen donors. These reductants are oxidized to produce various materials (such as sulfur in the case of H_2S), but no oxygen is liberated.

Most of the pigmented or purple bacteria contain as their main pigment bacteriochlorophyll, which is very similar to chlorophyll but absorbs light of long wavelengths at the very end of the visible spectrum and even in the near infrared. Thus, bacterial photosynthesis can proceed in infrared light. The bacteria store much less energy than do the green plants. Whether this low efficiency is due to the low-energy content of the far-red photons is not yet known. The bacterial photosynthesis also requires eight quanta for each molecule of CO_2 reduced and thus apparently also involves two photochemical steps, although the energy stored in the process is quite small.

Other bacteria utilize the process of *chemosynthesis*, in which the energy needed to reduce carbon dioxide is supplied by exothermic chemical reactions rather than by capture of photons. The hydrogen bacteria, for example, synthesize carbohydrates using energy derived from oxidizing hydrogen gas to form water.

EVOLUTION OF PHOTOSYNTHESIS

Because every living system today uses ATP as a medium of energy exchange, it seems likely that the earliest organisms also used ATP or a similar molecule. Molecules of ATP may have been present in the primeval “organic soup” and could have been used by the early organisms. However, the supply of randomly created ATP could not have supported life for long. Early in the history of life on earth, successful organisms must have been those with some mechanism that would convert chemical energy in the other organic molecules into the common coinage of ATP, which could be utilized throughout the living system.

Mechanisms such as fermentation and glycolysis, which convert sugars to waste products such as alcohols or organic acids, are used by modern bacteria that survive in reducing environments. These mechanisms are also used by other organisms for temporary energy supply when oxygen is not available. Similar though simpler mechanisms were probably developed by the early organisms.

Eventually, the supply of organic molecules suitable for these reactions must also have been depleted. The supply of new organic molecules created by random processes probably decreased over the first billion years of the earth's history, not only because early organisms consumed the molecules at an increasing rate but because the amount of ultraviolet light available at the surface decreased.

At first, most of the ultraviolet light in the solar radiation reached the earth's surface, where it could provide energy for organic syntheses. However, the light molecules of hydrogen gas must have gradually escaped from the earth's gravitational pull and been lost to space. After most of the free hydrogen gas was gone from the atmosphere, free oxygen and ozone (O_3 or $O—O=O$, an unstable and highly reactive molecule) would begin to form in the upper atmosphere as the ultraviolet light broke down molecules of water vapor. As long as free hydrogen was available, the water molecules would have been quickly re-formed by combination of hydrogen and oxygen. The layer of oxygen and ozone absorbed much of the ul-

traviolet light before it reached the surface, thus probably significantly decreasing the rate at which organic molecules were synthesized.

The diminution of ultraviolet radiation did have some advantages for living systems. Complex organic molecules decompose readily when struck by the high-energy photons of ultraviolet light. In fact, the existence of modern land organisms is made possible by the shielding effects of the oxygen and ozone in the atmosphere. As long as the unshielded ultraviolet radiation reached the surface, even the simplest organisms would have been forced to remain several meters below the water surface. Thus, the appearance of the oxygen-ozone layer in the upper atmosphere may have been the event that made possible the development of the first organisms.

As traces of free oxygen began to reach the lower atmosphere, the rate of synthesis of organic molecules must have decreased still further. With the supply of organic molecules in the environment dwindling, organisms apparently were forced to develop other sources of chemical energy and precursor molecules.

Some scientists have argued that chemosynthesis was the earliest mode developed by living systems for building organic molecules and for obtaining free energy from the environment, with bacterial photosynthesis developing later in evolution, to be followed by green-plant photosynthesis when more efficient pigments and enzymes had been developed. In this view, the production of oxygen would have been a late stage in the evolution of photosynthesis, coming long after the ability to synthesize carbohydrates was developed. Others feel that chemosynthesis and bacterial photosynthesis are special adaptations developed late in evolution to permit bacteria to survive in environments that could not be utilized by normal photosynthetic organisms.

It is becoming clear that many of the reactions that make up the sequences in the photosynthetic process are similar or identical to reactions that occur in other metabolic processes. Even the photosynthetic pigments may have existed in biological systems before the entire photosynthetic mechanism was developed. Thus, the evolution of the first photosynthetic organism may not have required the development of a complex mechanism from nothing but simply may have required the recombining of existing mechanisms in a new way.

No matter how the first photosynthetic organisms arose, there can be no doubt that they had a profound effect upon the earth. Carbon dioxide, which had been accumulating as a waste product of fermentation processes, now became a useful source of carbon for building new organic molecules. Oxygen, given off in increasing quantities as photosynthetic organisms became more common, became a major constituent of the atmosphere. The old reducing atmosphere was replaced by the present oxidizing one, and it was no longer possible for organic molecules to be synthesized spontaneously. From the time of the development of photosynthesis, all organisms were dependent upon the photosynthetic organisms for their supply of organic molecules and of chemical energy.

FURTHER READING

The most useful recent discussions of photosynthesis are those by Fogg (1968), Levine (1969), and Rabinowitch and Govindjee (1969). Of largely historical interest—as an indication of how ideas have changed in the past few decades—are books and articles by Arnon (1960), Bassham (1962), Bassham and Calvin (1957), Kamen (1963), Rabinowitch (1948), Rabinowitch and Govindjee (1965), and Wald (1959).

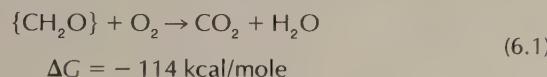
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Respiration



Through the process of photosynthesis, plants are able to convert solar energy into chemical energy and to synthesize all the organic molecules they require from such simple inorganic compounds as carbon dioxide, water, and ammonia. The plants are the major group of *autotrophic* ("self-feeding") organisms. The photosynthetic and chemosynthetic bacteria are also autotrophs. All other organisms are *heterotrophic*—they must obtain organic molecules as foodstuffs from the environment.

The plant photosynthesizes sugar, and from this substance it can derive practically everything else it needs for life: polysaccharides—such as starch and cellulose—proteins, nucleotides, nucleic acids, coenzymes, and lipids. When an animal eats a plant, all of these organic molecules are brought to the metabolic machinery of the animal. The animal organism, however, cannot simply use these molecules to construct its own tissues. The plant substance must first be demolished, the chemical energy converted to usable form, monomers salvaged, and new macromolecules characteristic of animal systems built up. In most heterotrophs, the breakdown of foodstuffs is accomplished in an oxidation process called *respiration*, whose overall chemical equation is the reverse of the overall photosynthetic process:



This chemical process is sometimes called cell respiration to avoid confusion with breathing and other physical methods by which oxygen is brought into large organisms. Plants also carry out respiration, breaking down some of the glucose they have photosynthesized.

Why convert CO_2 to carbohydrates in the first place, if they are only to be broken down again later? The carbohydrates and other macromolecules play many important roles in the structure and function of the organism. Furthermore, the carbohydrates provide a good form for long-term energy storage. The sugars are an ideal medium in which to transfer energy from one organism to another or, in polymerized forms, to store energy within the organism for long periods of time. In the process of respiration, energy is transferred from carbohydrates and other food molecules to ATP, which can be used by the cell to perform cell work. Carbon dioxide and water are liberated as waste products and are cycled back to the photosynthetic organisms to be used again as building blocks for organic macromolecules.

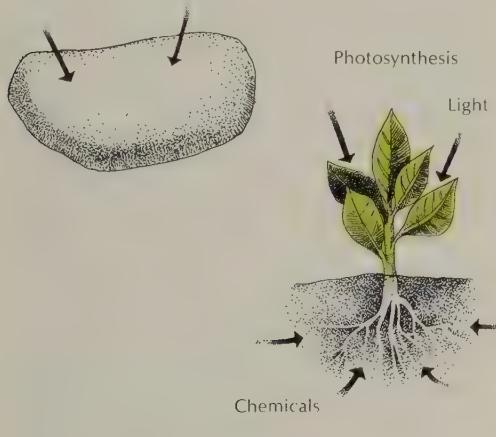
The *metabolism* of a cell or of an entire organism is the sum of all the chemical processes that the cell or organism is capable of performing. The metabolic pathway from which most plants and animals derive energy for cell work involves the breakdown of the six-carbon sugar glucose. Laboratory measurements of the energy released by the complete oxidation of one mole of glucose (180 grams) are made using a device known as a bomb calorimeter. As the name of the device implies, it can measure large amounts of energy release in a short period of time. Such a measurement shows that the complete oxidation of glucose to CO_2 and H_2O releases 686 kcal. The same amount of energy is released when a cell oxidizes one mole of glucose.

However, there is one important difference: the cell releases and captures the energy in small steps instead of one great explosive process. Energy that is released in small increments is far more useful than an explosive,

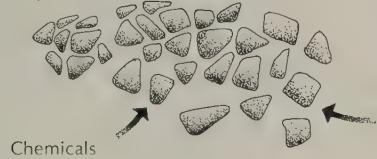
Figure 6.1 (left). Schematic diagram illustrating the principal modes of nutrition. Autotrophic organisms (above) utilize materials from the physical world by structuring them into organic molecules with either light energy via ATP (photosynthesis) or chemical bond energy (chemosynthesis). Heterotrophs (below) make use of preexisting organic molecules that are acquired through an engulfing or swallowing process (holotrophism), through absorption (saprotrophism), or through absorption from a living host (parasitism).

AUTOTROPHISM

Nutrients from Physical Environment

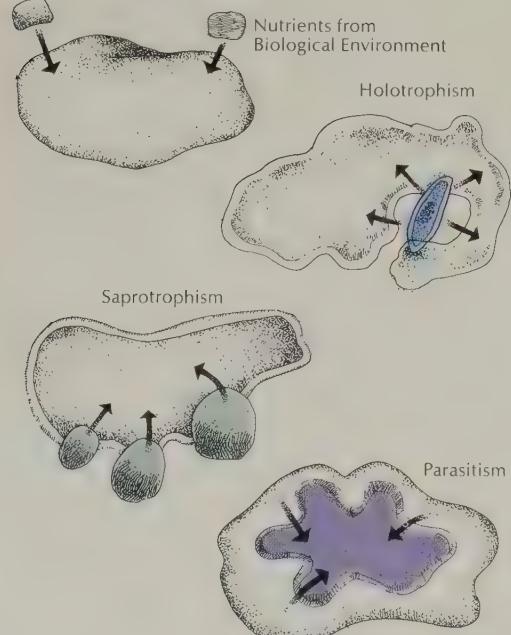


Chemosynthesis



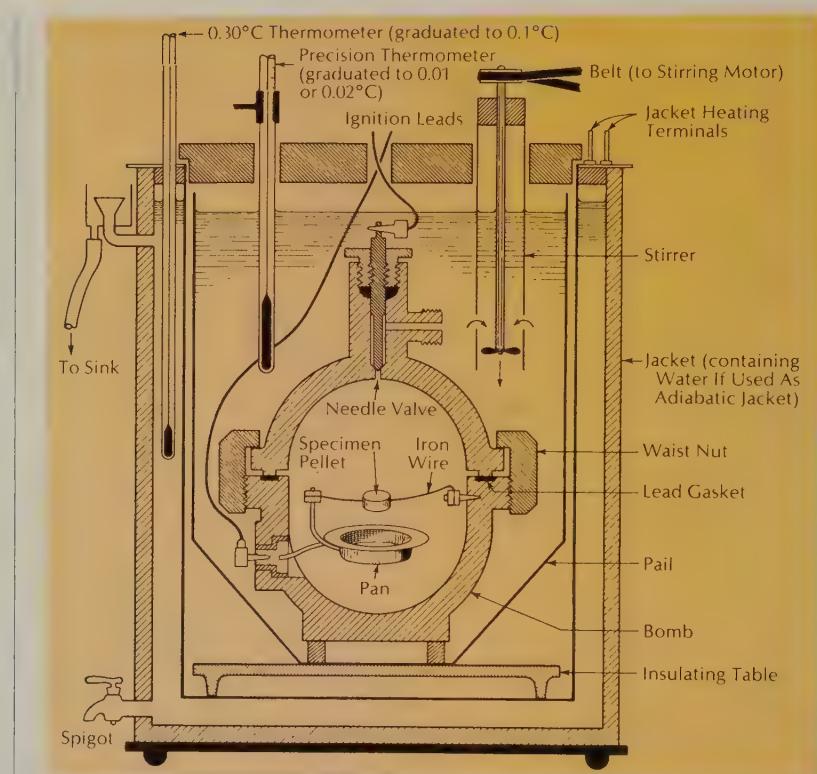
HETEROTROPHISM

Nutrients from Physical Environment



Nutrients thus acquired are chemically processed and the necessary energy extracted.

Figure 6.2 (right). A bomb calorimeter used in laboratory measurements of energy released during the oxidation of glucose.



uncontrollable release. In a gasoline engine, small amounts of fuel are burned under controlled conditions to push the pistons and to transform energy fairly efficiently into power for the automobile wheels. Far more usable energy is obtained than could be derived from the sudden and explosive burning of several gallons of gasoline.

The first phase in the breakdown of organic molecules with release of energy is accomplished in processes known as fermentation and glycolysis, processes that do not require free oxygen and stop before the organic molecules are completely broken down to CO_2 and H_2O . Heterotrophic organisms living in reducing or anaerobic environments must use these processes as their only source of chemical energy. Other organisms make use of these processes as an emergency source of energy when oxygen is temporarily unavailable. These processes are not as efficient as respiration in capturing free energy during the breakdown of organic molecules.

THE EMBDEN-MEYERHOF PATHWAY

The sequence of reactions involved in the metabolism of glucose (Figure 6.3) is called the Embden-Meyerhof pathway in honor of the two men who did the definitive research on this sequence in the 1920s and 1930s. This pathway begins with the input of a simple six-carbon sugar molecule, glucose, and ends with either the production of two molecules of a three-carbon acid, lactic acid, or with the production of ethanol and CO_2 , depending on the cell in which the process occurs. In the sequence of reactions, the stable glucose molecule is first energized and made unstable; then it is partially dismantled or degraded, liberating a small part of the chemical

energy in the glucose and partially oxidizing it. A description of the detailed steps is useful as an example of the kinds of mechanisms involved in metabolic pathways.

In the steps 1–3 of the pathway, glucose is converted to fructose diphosphate by the addition of two high-energy phosphate groups from ATP molecules. The net result of these three steps is to convert the glucose to a higher-energy, unstable intermediate, while converting two molecules of ATP to ADP. Thus, the organism has invested two ATP “coins” of energy to prepare the glucose molecule for breakdown.

In step 4, the unstable six-carbon molecule is split into two three-carbon units, phosphoglyceraldehyde and dihydroxyacetone phosphate. These two compounds exist in equilibrium with each other (step 5).

In step 6, the phosphoglyceraldehyde gains a phosphate group from inorganic phosphate (P_i) and loses two hydrogen atoms to the coenzyme nicotinamide adenine dinucleotide (NAD). As the phosphoglyceraldehyde is oxidized to diphosphoglycerate, the NAD is reduced to NADH. As in all redox reactions, energy is transferred from the reductant (phosphoglyceraldehyde) to the reduced product (NADH). As the phosphogly-

Figure 6.3. The Embden-Meyerhof pathway of glucose metabolism. Note that the first 7 steps consist primarily of endergonic reactions that require ATP. In steps 7–10, partial oxidation of the three-carbon sugar takes place with simultaneous production of ATP.

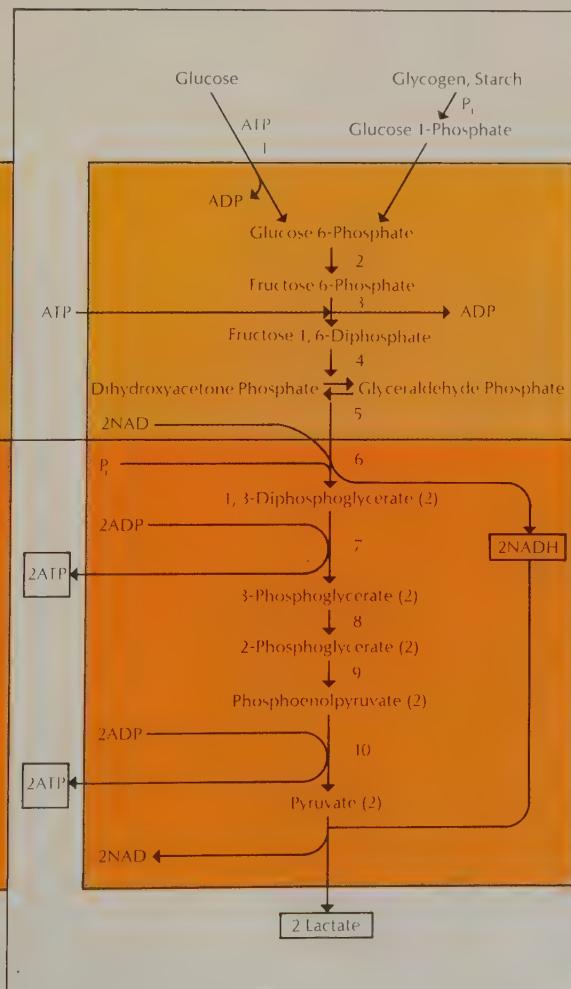


Figure 6.4. Alcoholic fermentation involves the metabolism of the six-carbon sugar glucose (top) to 2 two-carbon ethanol molecules. The six-carbon molecule is split when fructose is fragmented into 2 molecules of glyceraldehyde phosphate. Each of these molecules is further metabolized through four more intermediate compounds before ethanol (ethyl alcohol) is produced.

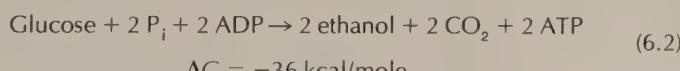


ceraldehyde is used up in step 6, the dihydroxyacetone phosphate is converted to phosphoglyceraldehyde by step 5. Thus, eventually two molecules of phosphoglyceraldehyde will enter step 6 for each molecule of glucose that entered step 1.

In step 7, a phosphate group is transferred from diphosphoglycerate to ADP, forming phosphoglycerate and ATP. In this step, some energy is transferred from the diphosphoglycerate to the ATP. Because two molecules of ATP are produced in this step for each molecule of glucose entering the pathway, this step restores the ATP that was invested in steps 1 and 3. Such a reaction, in which ADP is converted to ATP by the simple transfer of a phosphate group from an organic molecule, is called substrate-level phosphorylation.

In steps 8 and 9, the bonds of the organic molecule are rearranged with the loss of a water molecule, forming phosphoenolpyruvate. This molecule is unstable enough to carry out another phosphorylation of ADP (step 10), forming pyruvate and ATP. As one glucose molecule moves through the sequence, two molecules of ATP are used in steps 1 and 3, but four molecules of ATP are produced in steps 7 and 10. Thus, the net result for the organism is the production of two molecules of ATP that can be used to supply chemical energy elsewhere for useful work.

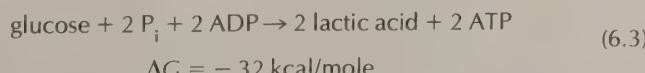
These ten steps of the Embden-Meyerhof pathway occur in both fermentation and glycolysis. In alcoholic fermentation, which is carried out by organisms such as yeast, CO_2 is released from pyruvate, forming acetaldehyde. Acetaldehyde is reduced at the expense of one NADH to form ethanol. The overall reaction for alcoholic fermentation may be written as



The breakdown of the glucose to ethanol and CO_2 releases about 56 kcal/mole; about 20 kcal/mole of this energy is stored in the ATP molecules for use elsewhere in the organism; the balance of the energy is accounted for by an increase in entropy.

Some microorganisms carry out fermentations of amino acids; others use sugars as energy sources but produce waste products other than alcohols. The types of organic molecules fermented and the waste products emitted are used as a means of classification for many bacteria and other microorganisms. In every case, the fermentation process is a relatively inefficient means of retrieving chemical energy from organic molecules.

Glycolysis is a particular extension of the Embden-Meyerhof pathway in which pyruvate is reduced to lactic acid using NADH. Some bacteria utilize this process; soured milk is caused by bacterial production of lactic acid. It also occurs in muscles forced to contract under anaerobic conditions. The process of glycolysis has been thoroughly studied because it occurs as the first step in glucose breakdown in the respiration of most animal species. The overall reaction of anaerobic glycolysis is:



The breakdown of glucose to lactic acid releases about 52 kcal/mole, of which about 20 kcal/mole is stored in the high-energy linkages of ATP.

Figure 6.5 (above). The structural formula of glycogen.

Figure 6.6 (below). During intense muscular activity, animals often supplement the energy from aerobic respiration by another pathway, anaerobic glycolysis. This process results in the accumulation of toxic lactic acid, and it builds up an oxygen debt. During the resting period, faster breathing occurs, which repays this debt and causes the complete oxidation of the lactic acid. In mammals, the lactic acid is carried to the

liver, returned to the muscle, and transformed into glycogen.

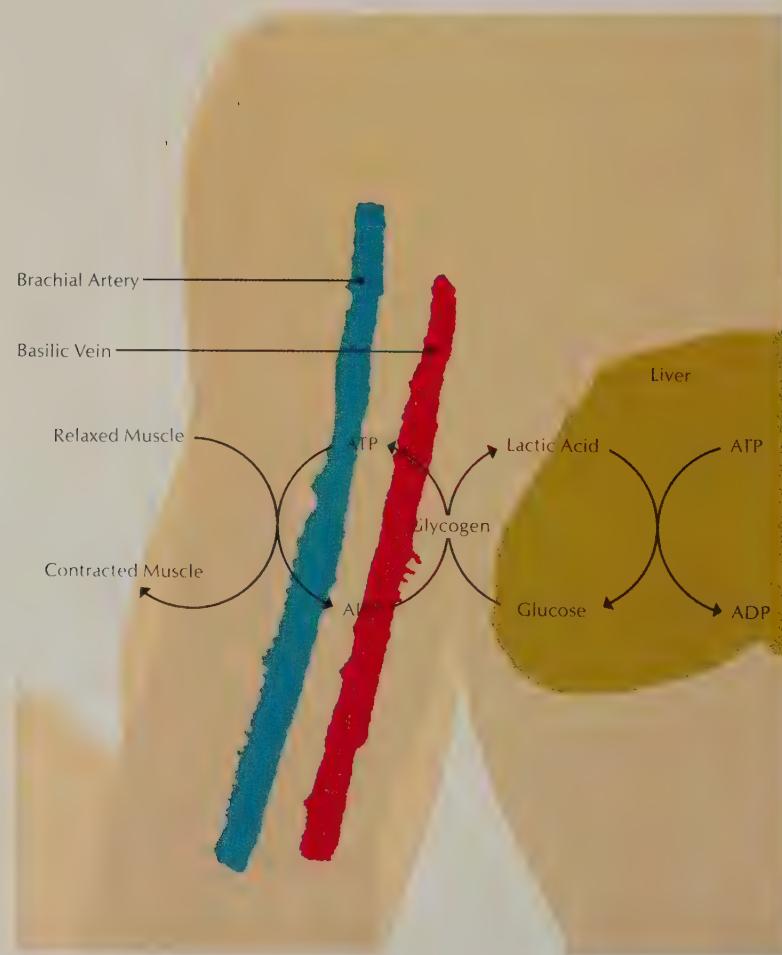
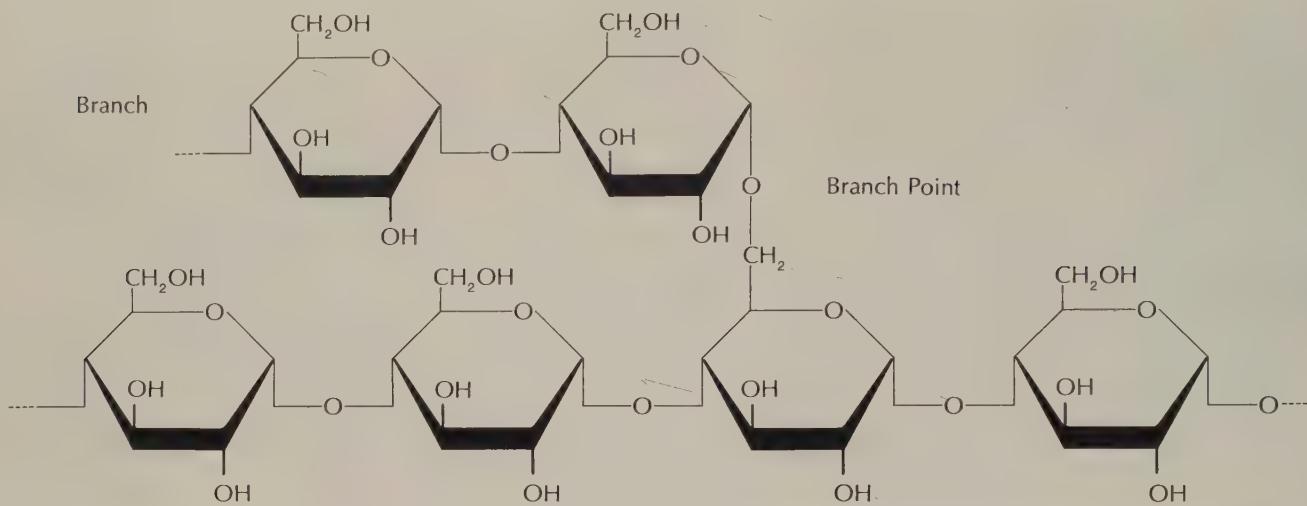


Figure 6.7 (right). Schematic diagram illustrating the sequential action of the glycolytic chain of enzymes. The product of one reaction becomes the substrate of the next enzyme. Thus, the chain of enzymes can be considered a type of disassembly line that takes in glucose at one end and turns out lactic acid at the other. (Refer to the key for specific enzymes and intermediate compounds in the series.)

Figure 6.8 (left). The redox reactions of the coenzyme nicotinamide adenine dinucleotide (NAD). NAD, the

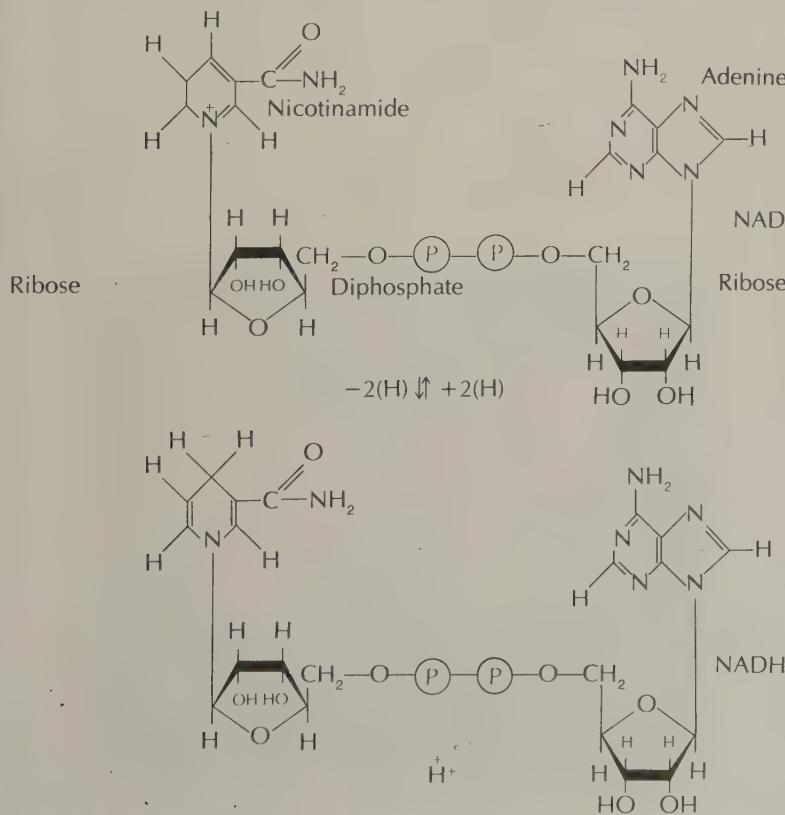
Much of the chemical energy of glucose remains stored in lactic acid.

In the muscles of animals, glucose is stored in the form of glycogen, a complex polymer of glucose (Figure 6.5). When a muscle contracts and relaxes rapidly, glycogen disappears and lactic acid accumulates. When the concentration of lactic acid becomes high enough, the muscle ceases to contract. Energy to power contraction is obtained in the form of ATP through glycolysis of the glycogen, but lactic acid accumulates as a toxic waste product. When the blood circulation returns to such a muscle, the lactic acid diffuses into the blood and is transported to the liver, where glucose is resynthesized from the lactic acid. This process involves the expenditure of energy by the liver cells.

Only a small percentage of the energy released in the reaction is captured by ATP, and a great deal of chemical energy remains stored in the organic molecules discarded as waste products. In organisms capable of respiration, fermentation is usually carried out as a first step in the breakdown of organic molecules. The pyruvate of the fermentation is then oxidized further to release CO_2 and H_2O .

Each step in the pathway is catalyzed by a specific enzyme. The reaction sequence may be regarded as a chain of enzymes, each of which converts its substrate into a form suitable to act as substrate to the next enzyme (Figure 6.7). This chain of enzymes can be regarded as a "disassembly line" that takes in glucose at one end and turns out lactic acid at the other.

The NAD^+ - NADH coenzyme pair is found in all organisms. Just as the ATP-ADP pair plays a universal role in transferring phosphate groups and



oxidized form, is a hydrogen acceptor. When combined with two hydrogens, it becomes NADH_2 , the reduced form. The release of hydrogen converts it back to NAD and decreases the free energy of the molecule; acceptance of hydrogens increases its free energy.

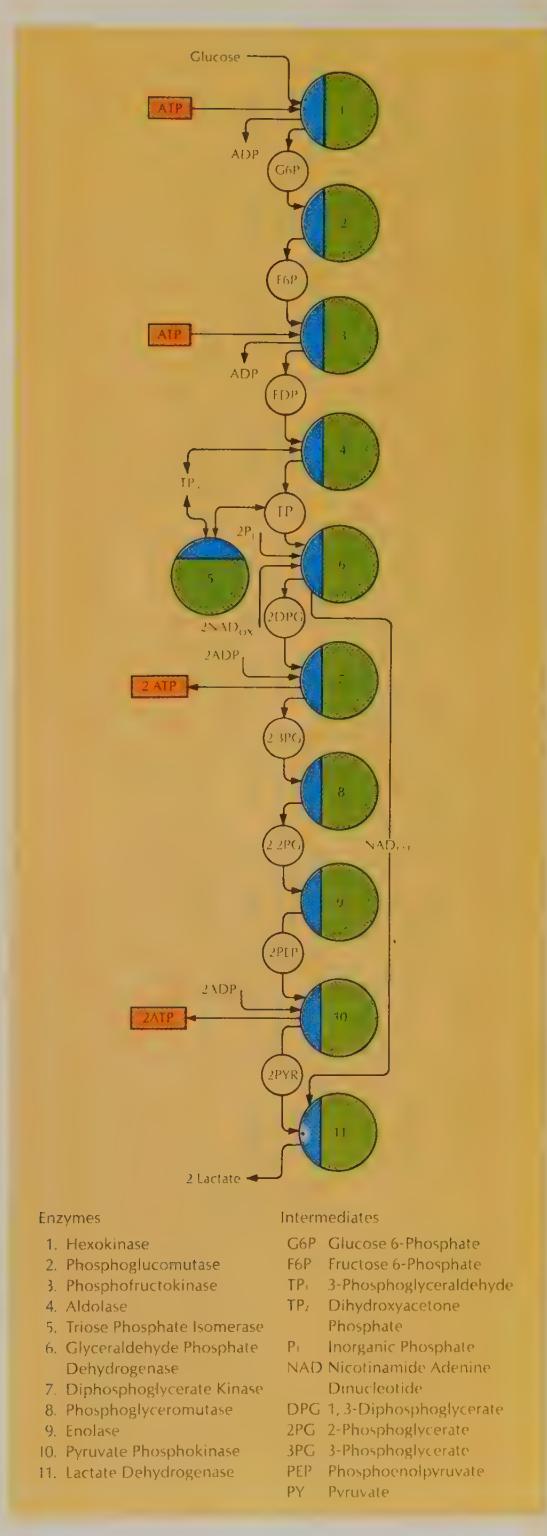


Figure 6.9. Dietary absence of nicotinamide, one of the B vitamins, can cause the disease pellagra. The secondary effects of this disorder are shown in this photograph of scaly dermatitis over the back of the hands and wrists.

chemical energy in all living systems, the NAD-NADH pair plays a universal role in transferring electrons (or hydrogen atoms). Notice that in both glycolysis and alcoholic fermentation, reduced NADH must be oxidized back to NAD, using as its final electron acceptor pyruvate or acetaldehyde. If this impotent carrier were not reoxidized, the metabolic machinery would soon be halted because the continued dissimilation of glucose requires a constant supply of NAD (step 6). Because there are only very small quantities of this coenzyme in the cell, it must constantly shuttle back and forth in a cyclic fashion between oxidized and reduced states. As soon as NADH donates its electrons to an acceptor, it is ready to accept electrons from step 6 or other oxidative reactions of cellular metabolism. NAD⁺ was formerly known as DPN (diphosphopyridine nucleotide), and this older name may be found in much of the older literature on metabolism. NAD⁺ is a complex molecule, containing a group called nicotinamide, which acts as the electron donor and receptor. Nicotinamide is one of the B vitamins, and its absence from the diets of humans and other higher animals causes the disease pellagra.

THE KREBS CITRIC ACID CYCLE

In aerobic organisms, the pyruvate produced as the end product of glycolysis is degraded to CO₂ in a reaction sequence called the Krebs citric acid cycle. During this cycle, hydrogen is removed by coenzyme teams and moved through the oxidative phosphorylation sequence, where it ultimately reduces oxygen to water.

The oxygen-requiring continuation of the Embden-Meyerhof pathway was postulated in 1937 in a simplified form by Hans Adolf Krebs and W. A. Johnson. In aerobic organisms, the pyruvate produced in glycolysis moves directly into this reaction sequence. Compounds derived from the breakdown of lipids, fatty acids, and amino acids can also enter this sequence at various points along the way.

During glycolysis, glucose is partially oxidized and split into two three-carbon molecules. In the Krebs cycle—the heart of the metabolic proc-



Important biochemical concepts often become lost in what appears to be a series of dry reactions depicted by circles, arrows, and strangely named substances. These concepts can become more meaningful by the introduction of a major approach in modern biology—that of structure and function. By looking at structural parts, biologists can better understand the functioning of systems and can then use this knowledge of components to understand how these systems interact to perform specific tasks. With the use of modern techniques, biologists have probed the subcellular world and elucidated much of the mystery of larger biosystems.

This concept is not new; it has its basis in all inquisitive thought. It has been expressed in invention, in primitive man's understanding of natural forces, and in the work of poets and architects. During the Middle Ages, man viewed the universe as an interaction between the macro and microcosm. Today, through a more sophisticated use of technology, man has further developed his understanding of larger systems (behavioral, organismic, ecological) and noted parallels in the elucidation of smaller systems (molecular pathways, organelles, cells). These systems do not mirror one another—each has its own unique aspects—but there is a continual interaction of architecture and function between the two. An understanding of this interrelationship ultimately expands the way we view our universe.

The panels that follow will graphically show the relationship between the biochemical pathways described in Chapters 5 and 6 and the structural units that carry out these processes. These structural units are two main organelles that have extremely important roles in plants and animals; they are the mitochondrion and the chloroplast.

Both mitochondria and chloroplasts are energy units. Their efficiency is increased by the inward convolutions of their membranes. This arrangement is common in living systems because it increases the surface area to volume ratio and thereby maximizes the space available for reactions to occur. Plants use sunlight, CO_2 , and water as fuels to produce the energy molecule ATP and the electrons necessary for sugar formation. Animals, on the other hand, depend on oxygen and the metabolic breakdown of food for ATP synthesis. Plants also have mitochondria, but 30 times as much ATP is produced in the chloroplasts. Much of what follows is condensed and abbreviated, but it is hoped that the combination of this brief introduction and the detailed graphics will give the reader an appreciation of the concept of structure and function.

The Mitochondrion

The use of the electron microscope has revealed much of the structural mystery of subcellular systems. Although there is a limitation in dealing with processed and fixed material, much can be learned from reading electron micrographs. They provide a tangible base from which structural details can be studied. Electron micrographs of mitochondria show a highly complex ultrastructure. A mitochondrion is basically a double-membrane system with an outer membrane that envelops a highly convoluted inner membrane. Both membranes have what is termed the unit membrane structure; that is, they are composed of a double layer of lipids with interspersed protein molecules. These membranes also provide the basic building blocks upon which more ornate structures are added for specific functions. The main regions of the mitochondrion are labeled in panel B.

Further details of structure can be seen after a treatment that causes the mitochondria to swell. New particles, previously within the membrane, now cover the cristae surface with a polygonal head attached to the membrane by a stem (Figure 3). These particles have been variously termed F_1 , or elementary, particles. They appear regularly dispersed along the cristae, with some estimates of up to 10,000 per mitochondrion. They are an important link in understanding the energy role of the organelle.

The functions of mitochondria are shown in panel E. The metabolic breakdown of food pumps intermediaries through a complex series of enzymatic reactions

Continued on H

Interleaf 6.1 STRUCTURE AND FUNCTION

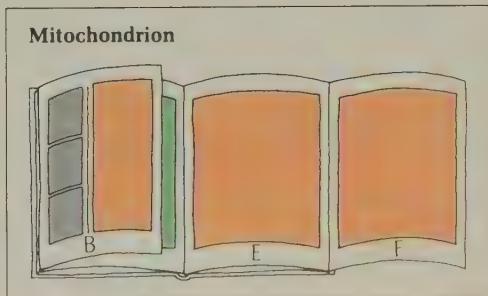




Figure 4 ($\times 10,700$)

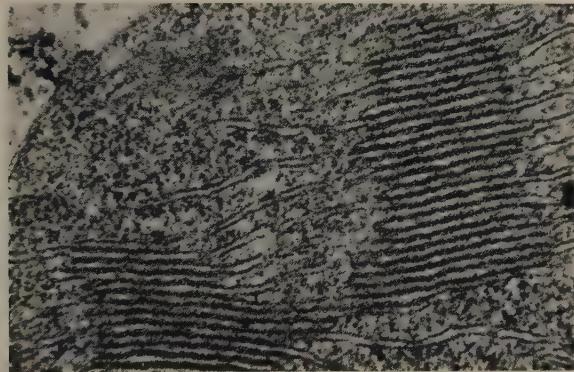


Figure 5 ($\times 58,000$)

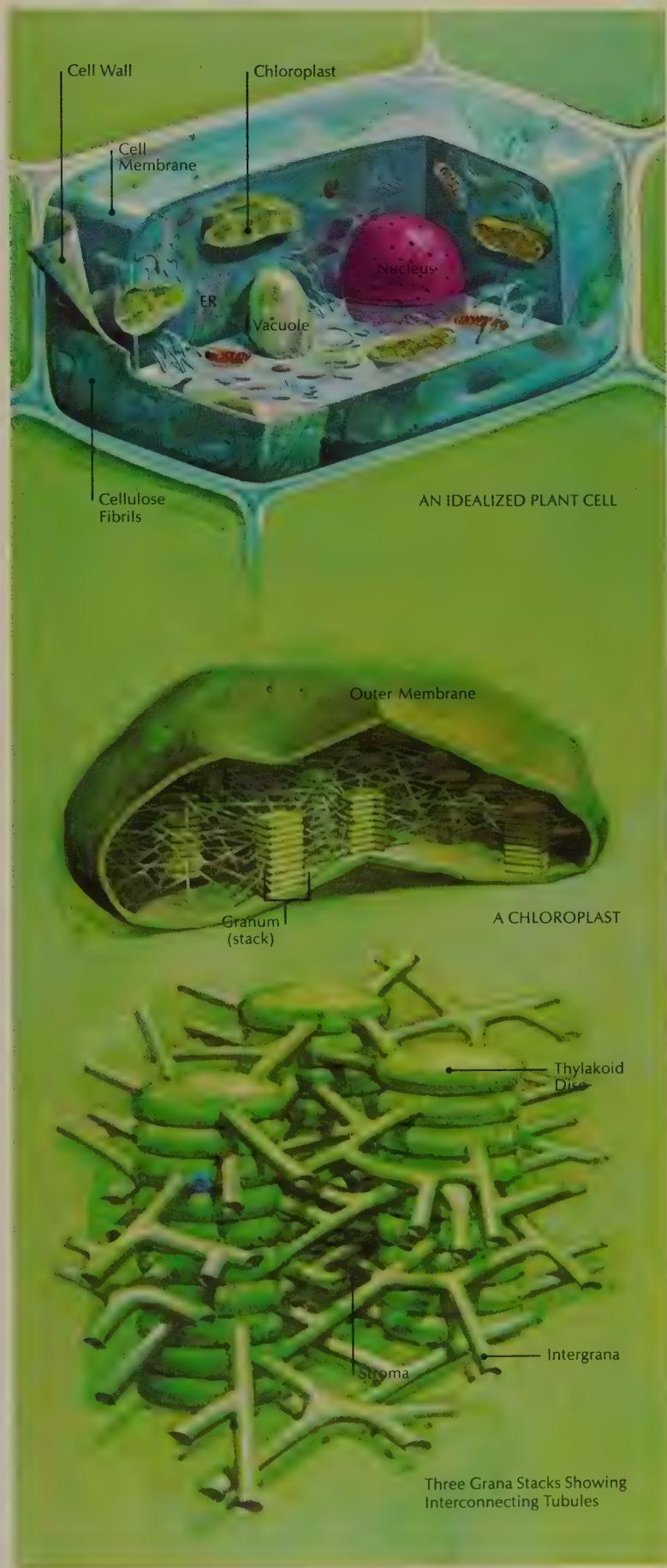


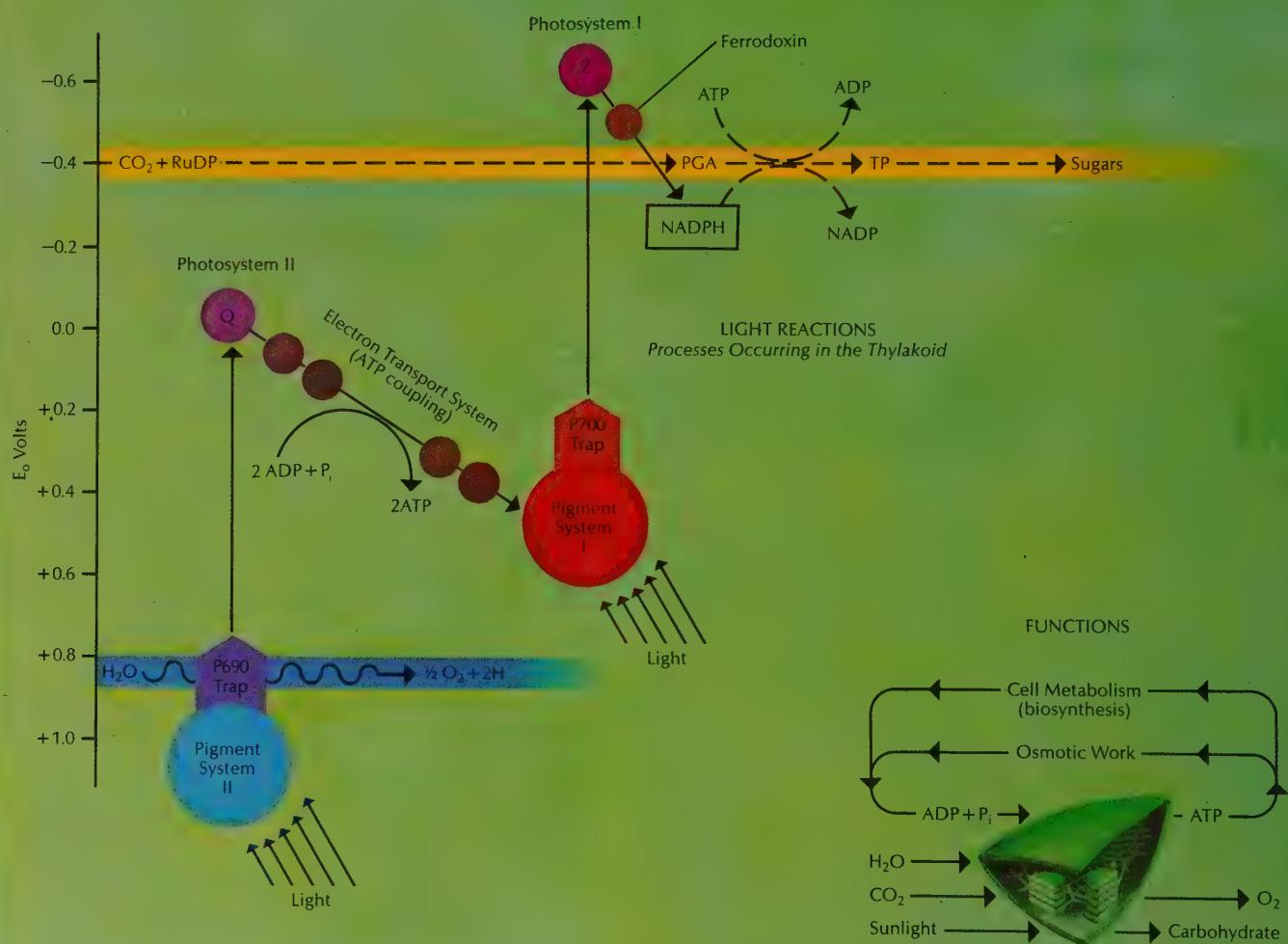
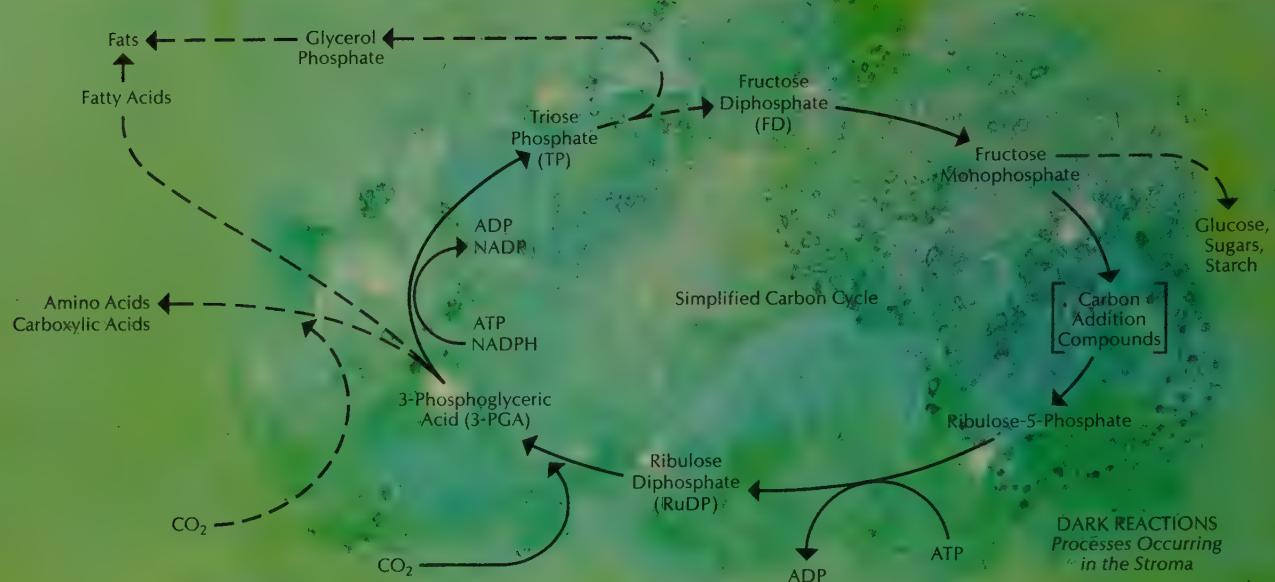
Figure 6 ($\times 310,500$)



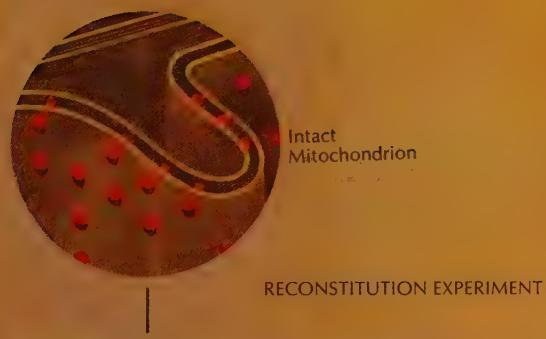
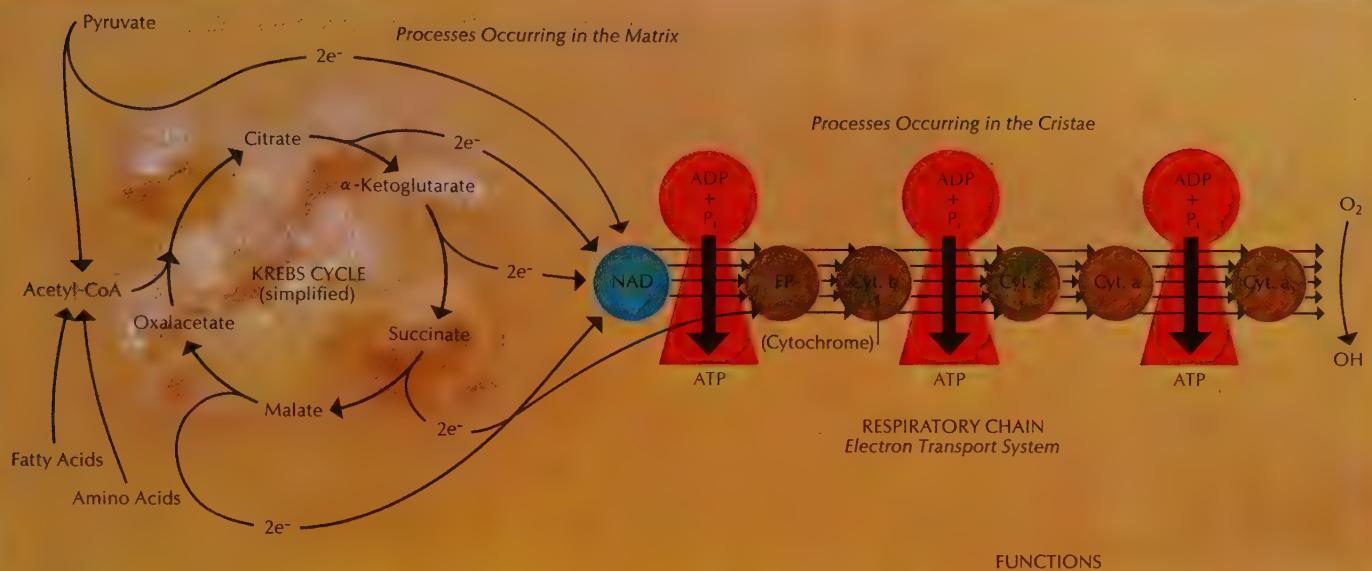
Figure 7 ($\times 38,500$)

A series of electron micrographs showing increased magnification of chloroplast structure (Figure 4). Figure 5 shows two grana stacks and a portion of the outer membrane. Figure 6 shows an enlarged region of a granum. In this figure, the staining techniques are reversed from Figure 5 so that the loculus is black (L), and the inner membrane of the thylakoid is white. Figure 7 is an electron micrograph taken by the freeze-etch technique showing the ultrastructure of thylakoids. The different subunit arrays may correspond to Photosystems I and II and the ATP coupling system.





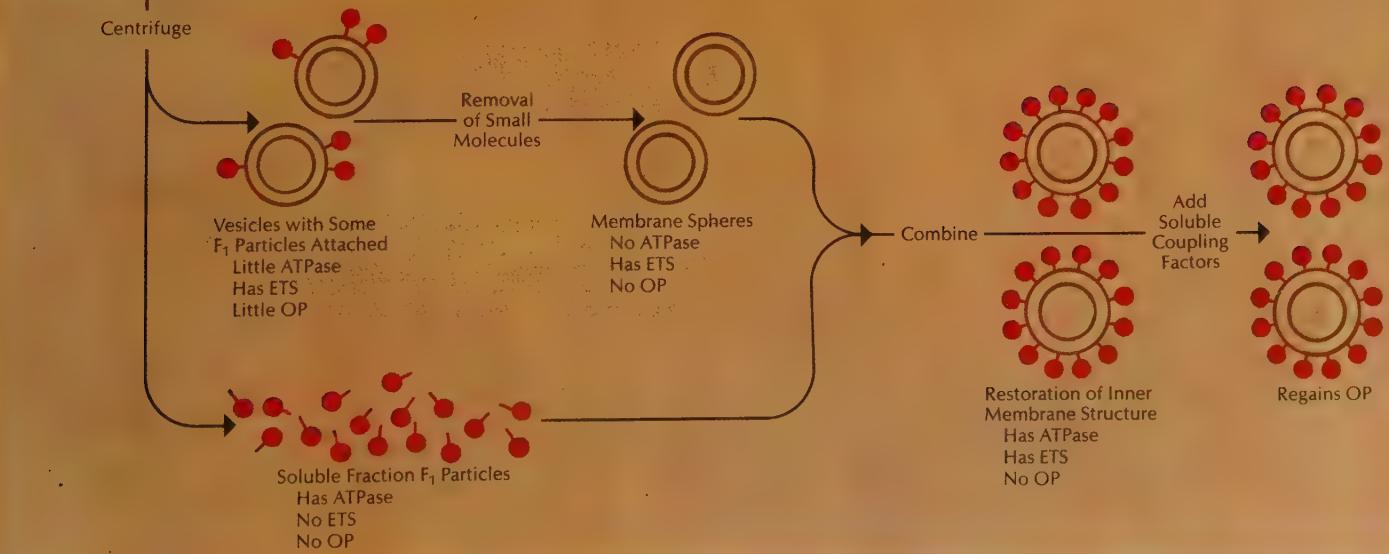
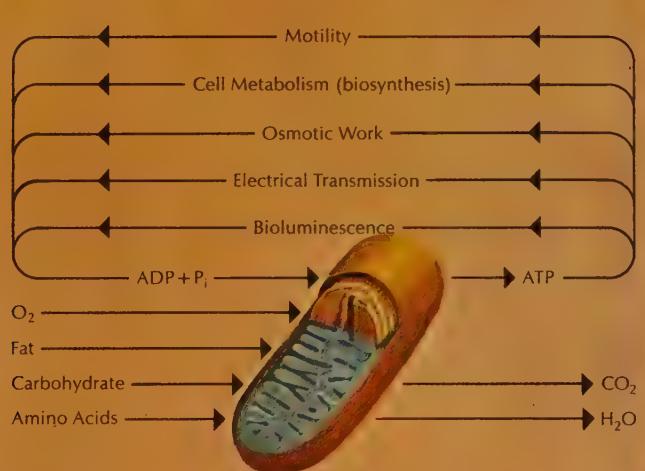




RECONSTITUTION EXPERIMENT

Sonic Disruption

Inner Membrane Spheres
ATPase Activity
Electron Transport System (ETS)
Oxidative Phosphorylation (OP)



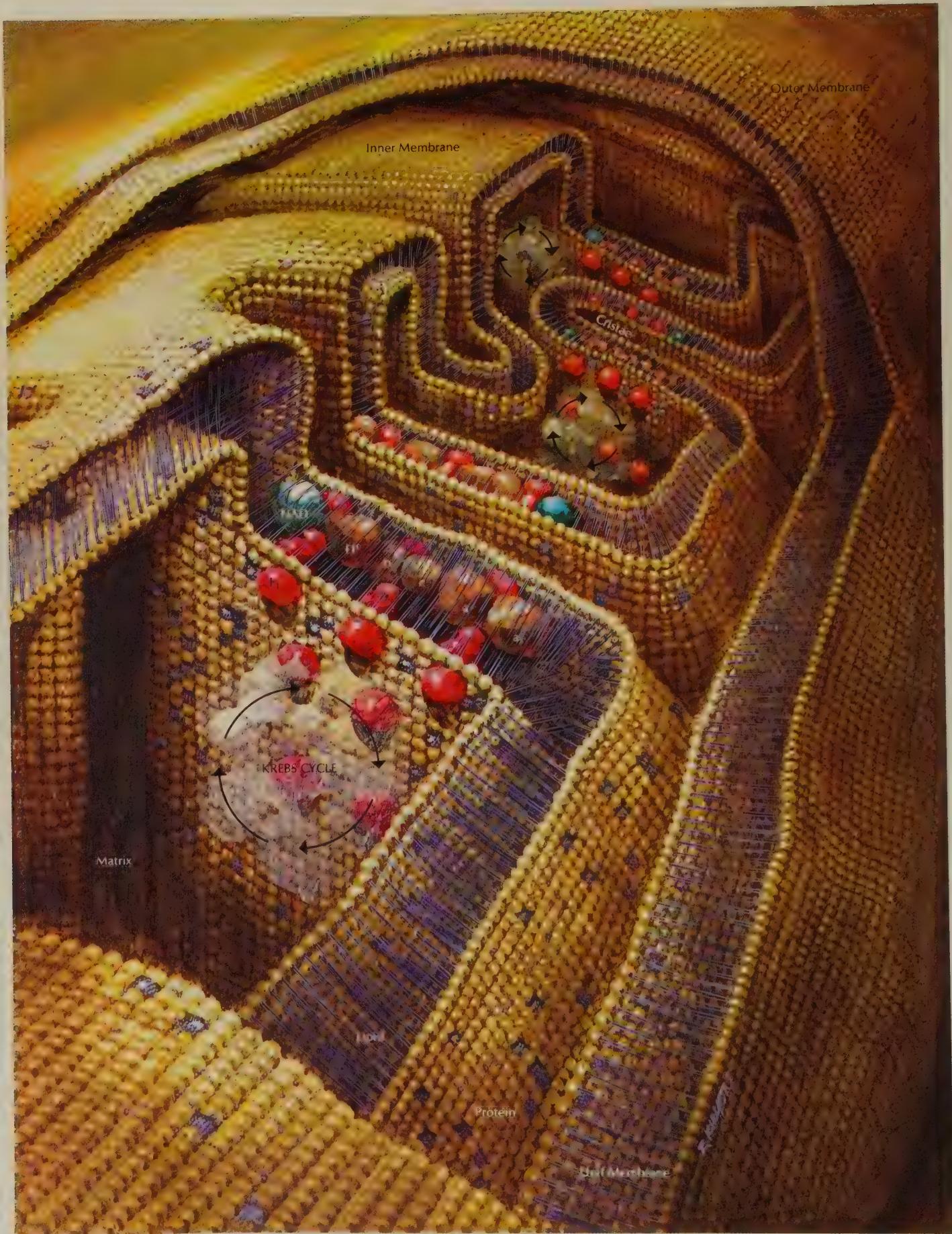
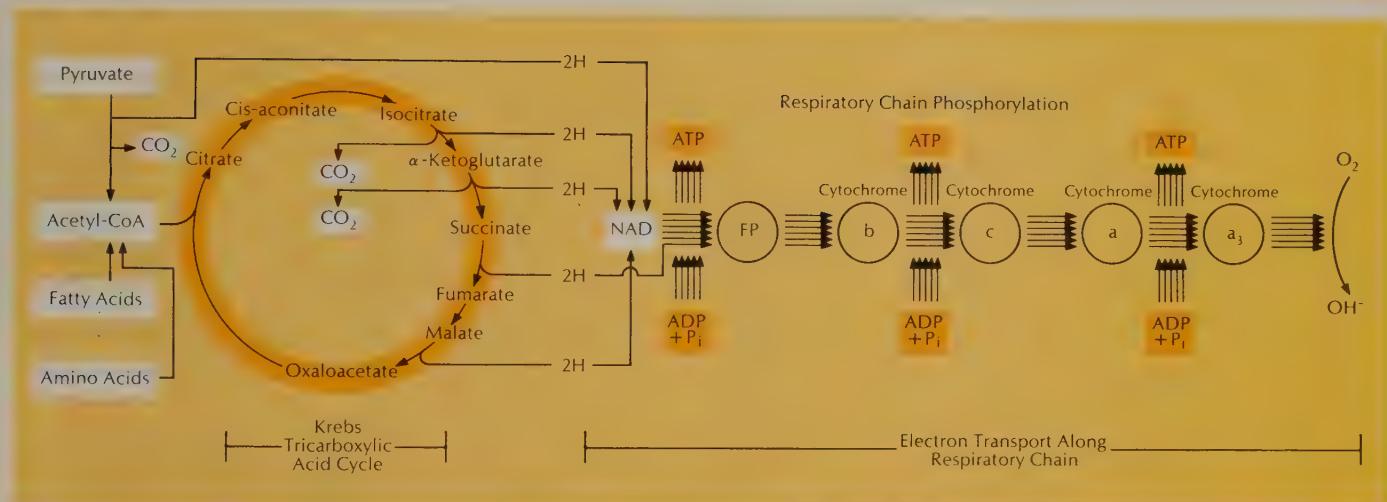
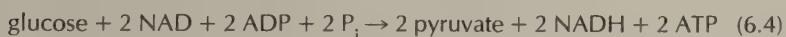


Figure 6.10. The Krebs citric acid cycle and respiratory chain phosphorylation. Major intermediate compounds, each requiring a specific enzyme to continue the reaction series, are shown in the Krebs cycle. Note the function of NAD in transporting high-energy electron (H) to the respiratory chain, where it is passed along to various cofactors and ultimately reduces oxygen and forms metabolic water.

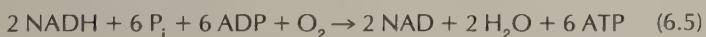


esses—the oxidation process is completed. Eventually, all of the hydrogen atoms are removed from the carbon compounds and are used to reduce cofactor molecules.

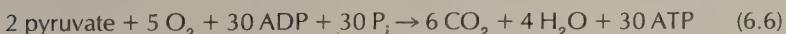
The process of aerobic glycolysis can be represented by the following overall reaction:



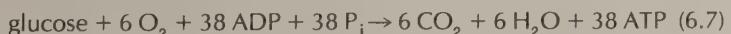
The two molecules of NADH enter the oxidative phosphorylation sequence directly



The two molecules of pyruvate pass through the Krebs cycle and oxidative phosphorylation with the following overall reaction:



The sum of reactions 6.4, 6.5, and 6.6 yields the overall equation for respiration



For every molecule of glucose that is completely oxidized in respiration, 38 molecules of ATP are produced. The free energy of oxidation of glucose is about -686 kcal/mole . Because each mole of ATP stores about 7 kcal of energy, the respiration of a mole of glucose stores about 266 kcal in the form of 38 moles of ATP. The efficiency of respiration is thus about 42 percent ($266/686$); the other 58 percent of the chemical energy in the glucose is lost.

The complete reaction sequence of the Krebs cycle is shown in Figure 6.10. Before it can enter the main cycle, the pyruvate is oxidized to a two-carbon compound, acetic acid. The acetic acid is bonded to a coenzyme molecule called coenzyme A (CoA), which acts as a carrier of acetyl

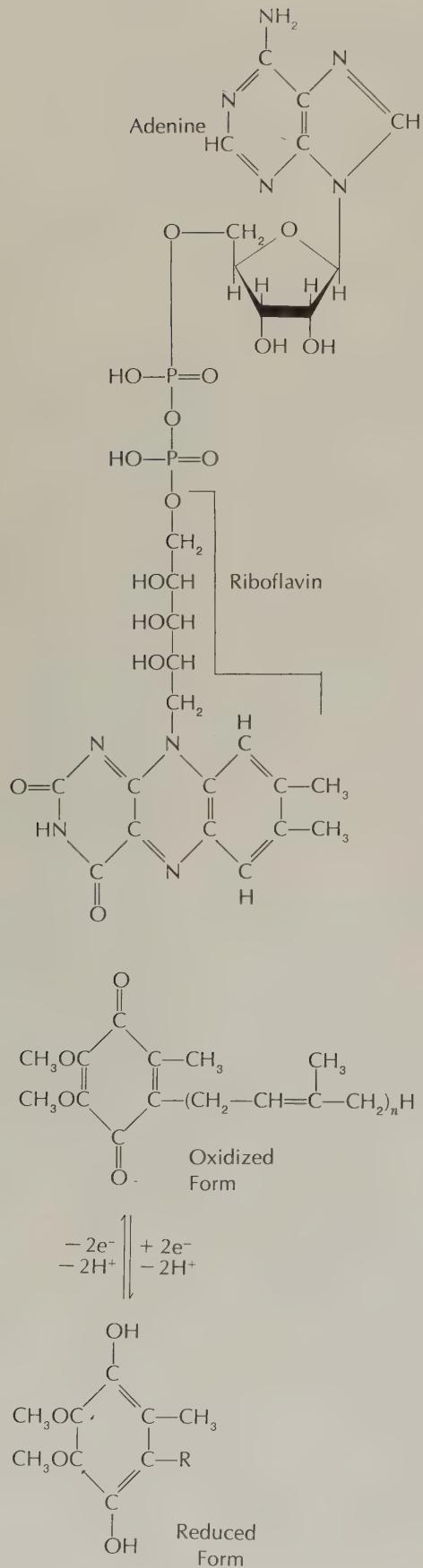


Figure 6.11. The flavin adenine dinucleotide (FAD) is shown above and coenzyme Q (CoQ) is shown below. CoQ_6 is found in a few microorganisms, and CoQ_{10} is present in numerous mammals.

groups, much as ATP acts as a carrier of phosphate groups. During this step, the third carbon atom of the pyruvate is oxidized to CO_2 . The oxidant for this reaction is NAD, which is reduced to NADH. The NADH enters the oxidative phosphorylation sequence.

The acetyl-CoA complex is also formed in the breakdown of amino acids, fatty acids, and lipids. Acetyl-CoA enters the Krebs cycle by donating its acetyl group to a four-carbon acid to form the six-carbon *citric acid*, for which the cycle is named. At three more steps in the cycle, reduction of nicotinamide cofactors (NAD or NADP) occurs. The reduced cofactors move into the oxidative phosphorylation sequence. In two other steps of the cycle, ATP is produced directly by substrate-level phosphorylation.

Many of the intermediates formed in the Krebs cycle can serve as precursors for other reaction pathways.

OXIDATIVE PHOSPHORYLATION

Two turns of the Krebs cycle completes the oxidation of glucose to CO_2 , yet no more ATP is available to do cell work. The energy released from the glucose is captured by the compounds NADH and FADH_2 . In order to convert this energy into energy stored in ATP, these reduced compounds are gradually oxidized by a series of electron transfer reactions at the same time ADP is phosphorylated to ATP. This process is known as oxidative phosphorylation.

During oxidative phosphorylation, enzymes called dehydrogenases transfer hydrogen to various cofactors such as nicotinamide nucleotides (NAD^+), flavin nucleotides (FAD), quinones (coenzyme Q), and the iron-containing pigments cytochromes. The final step of oxidative phosphorylation requires oxygen as a hydrogen acceptor, thus forming H_2O .

The engineer designing a gasoline engine will be extremely pleased with himself if he succeeds in designing an engine that turns 25 percent of the chemical energy of the gasoline into useful work. The reactions of the Krebs cycle and of oxidative phosphorylation add 34 ATP molecules to the 2 formed during glycolysis, so that a total of 36 ATP molecules are formed during the complete oxidation of glucose to CO_2 and H_2O . This formation represents 360 kcal/mole captured in ATP compared to 686 kcal/mole available in glucose for an efficiency of 54 percent.

REGULATION OF RESPIRATION

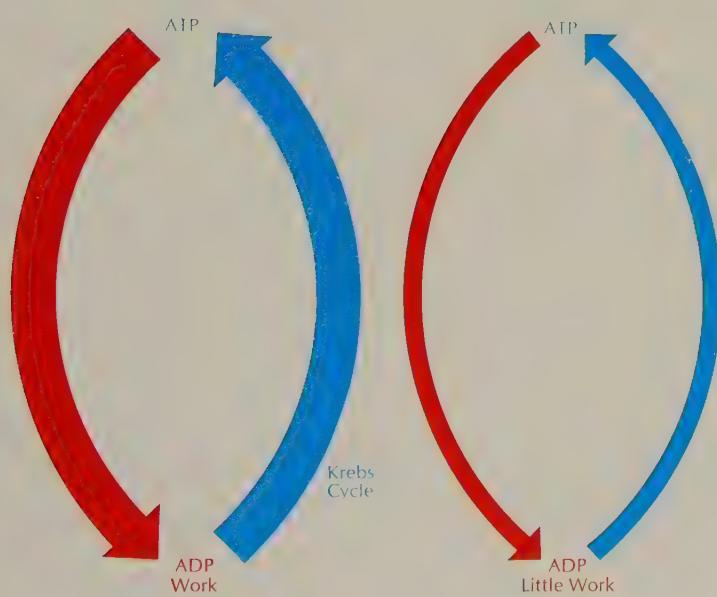
In most organisms, there is usually an abundant supply of glucose and oxygen. What, then, prevents the organism from breaking down all of its supply of glucose through respiration? This total consumption would be most undesirable, for energy stored in ATP is lost rapidly through the breakdown of ATP to ADP and P_i , unless the ATP is used rapidly in coupled reactions.

The rate at which the Krebs cycle reactions occur appears to be limited chiefly by the ratio of ATP to ADP in the organism. When a great deal of work is being done, ATP is converted to ADP and the ADP concentration rises. With an abundance of ADP available, the reactions of the Krebs cycle proceed rapidly, turning the ADP back into ATP. If little work is being done, the ATP concentration rises and the ADP concentration decreases. With little ADP available, the reactions of the Krebs cycle cannot proceed very rapidly. This feedback mechanism quite precisely regulates the rate of glucose oxidation to match the amount of work being done.

If the supply of oxygen to a microorganism or cell is limited, the final

Figure 6.12. ATP-ADP feedback system. The diagram illustrates how ATP-ADP concentrations in organisms regulate the rate of respiration. When a great deal of work is being done, ATP is converted to ADP and the ADP concentration rises. With an abundance of ADP available, the reactions of the Krebs cycle can proceed rapidly, converting ADP back into ATP. If little work is being done, the ATP concentration rises and the ADP

concentration decreases. With a diminished ADP supply, the reactions of the Krebs cycle cannot proceed very rapidly. Thus, the ATP-ADP feedback system precisely regulates the rate of glucose oxidation to match the amount of work being done.



step of oxidative phosphorylation cannot proceed very rapidly. As each enzyme in that sequence becomes so overloaded with substrate molecules that it cannot pass along to the next step, NADH begins to accumulate and the supply of NAD dwindles, slowing down the reactions of the Krebs cycle so that pyruvate begins to accumulate. This reaction supplies enough NAD to keep the glycolytic process going for some time. Thus, the organism can continue to produce small amounts of ATP without oxygen, but at the cost of accumulating the toxic waste product, lactic acid. Some microorganisms are able to eliminate this substance from the cell and thus to survive indefinitely in anaerobic conditions. In human muscle cells, on the other hand, the accumulation of lactic acid eventually interferes with the process of muscle contraction, and after a period of extreme exertion, the muscles may cramp.

INTERMEDIARY METABOLISM

Until now, cellular metabolism has been discussed from the standpoint of the breakdown of food molecules, primarily glucose, through a series of discrete steps in which glucose is completely oxidized to CO_2 and H_2O . The ultimate goal of this breakdown is the generation of ATP, which can then be used by the cell to drive unfavorable chemical reactions. The process whereby the molecules are broken down is called *catabolism*. The other side of metabolism, that of biosynthesis, is called *anabolism*. Both catabolism and anabolism occur simultaneously within the cell. Catabolic processes also generate other compounds of importance to the cell.

The ATP produced in catabolic reactions is used to drive energetically unfavorable reactions, such as the assembly of macromolecules. Their direct synthesis involves only a limited number of precursors, such as amino acids, nucleotides, and sugars. These small molecules are not the only ones present in cells; there are a wealth of small molecules involved in cellular metabolism. As in glycolysis or the Krebs cycle, all molecules

are made sequentially and specifically in a number of discrete steps, each one being catalyzed by a different enzyme. Each step produces an *intermediate*, and the ordered breakdown or synthesis is called *intermediary metabolism*.

Nucleic acids are highly ordered molecules and use directly the high-energy triphosphates of adenosine, guanosine, thymine, and cytosine for their synthesis. These precursors cannot directly arise by hydrolysis of nucleic acids, for this process yields only the monophosphates. These precursors must first be resynthesized to the triphosphate level, requiring the expenditure of 2 ATP equivalents. Alternatively, the bases and sugars may be synthesized separately and then linked together. The new synthesis of amino acids includes using portions of glycine, aspartic acid, formic acid, and nitrogen atoms donated from glutamine. Energy is also required for this process. For instance, the new construction of ATP from ribose and the components of the purine ring requires the expenditure of 8 ATPs.

The monomers of carbohydrates are simple sugars. Carbohydrates, such as glycogen, starch, and cellulose, are composed of repeating units of glucose linked together in different ways to form these macromolecules. Some carbohydrates may be used only as storage products—food reserves to be used during periods of starvation—whereas others are used in building structures such as cell walls. The surface factors of red blood cells, which determine blood type, are carbohydrates, and lipids are also known to contain carbohydrates.

The most common form of fats are the triglycerides. They are synthesized by the sequential combination of glycol, which is easily synthesized from a glycolytic intermediate, with three fatty acids. The fatty acids themselves are synthesized from the repeated addition of acetyl-CoA, a 2-carbon unit from the Krebs cycle, onto a small precursor molecule consisting of CO_2 and acetyl-CoA. The fatty acids are hooked by an ester linkage onto the glycerol.

The degradation of these compounds occurs by the hydrolysis of the fatty acids from the glycerol, followed by the oxidation of the fatty acids—2 carbons at a time—in the form of acetyl-CoA units, which may pass directly into the Krebs cycle. Glycerol may enter the glycolytic pathway.

This brief outline of cellular metabolism is not complete, but it should serve to illustrate several points. All macromolecules serving as food must first be broken down, at least to the level of monomers. These monomers may serve either as sources of energy by further oxidation or they may be constructed into new and unique macromolecules typical of that cell. All metabolic conversions occur in discrete steps, each step being catalyzed by a different enzyme. Some but not all of these steps require input of ATP. The cell is thus able to maintain a balance between catabolic and anabolic processes.

RESPIRATION AND PHOTOSYNTHESIS

The processes of respiration and photosynthesis are coupled by the flow of carbon, oxygen, and hydrogen through the biosphere. CO_2 and H_2O are consumed in photosynthesis, forming organic molecules and releasing O_2 . In respiration, the organic molecules are oxidized to CO_2 and H_2O as O_2 is consumed. Each process depends upon the other for its supply of raw materials. In plants, both processes occur in the same organism. Some of the organic molecules formed in photosynthesis are broken down by respira-

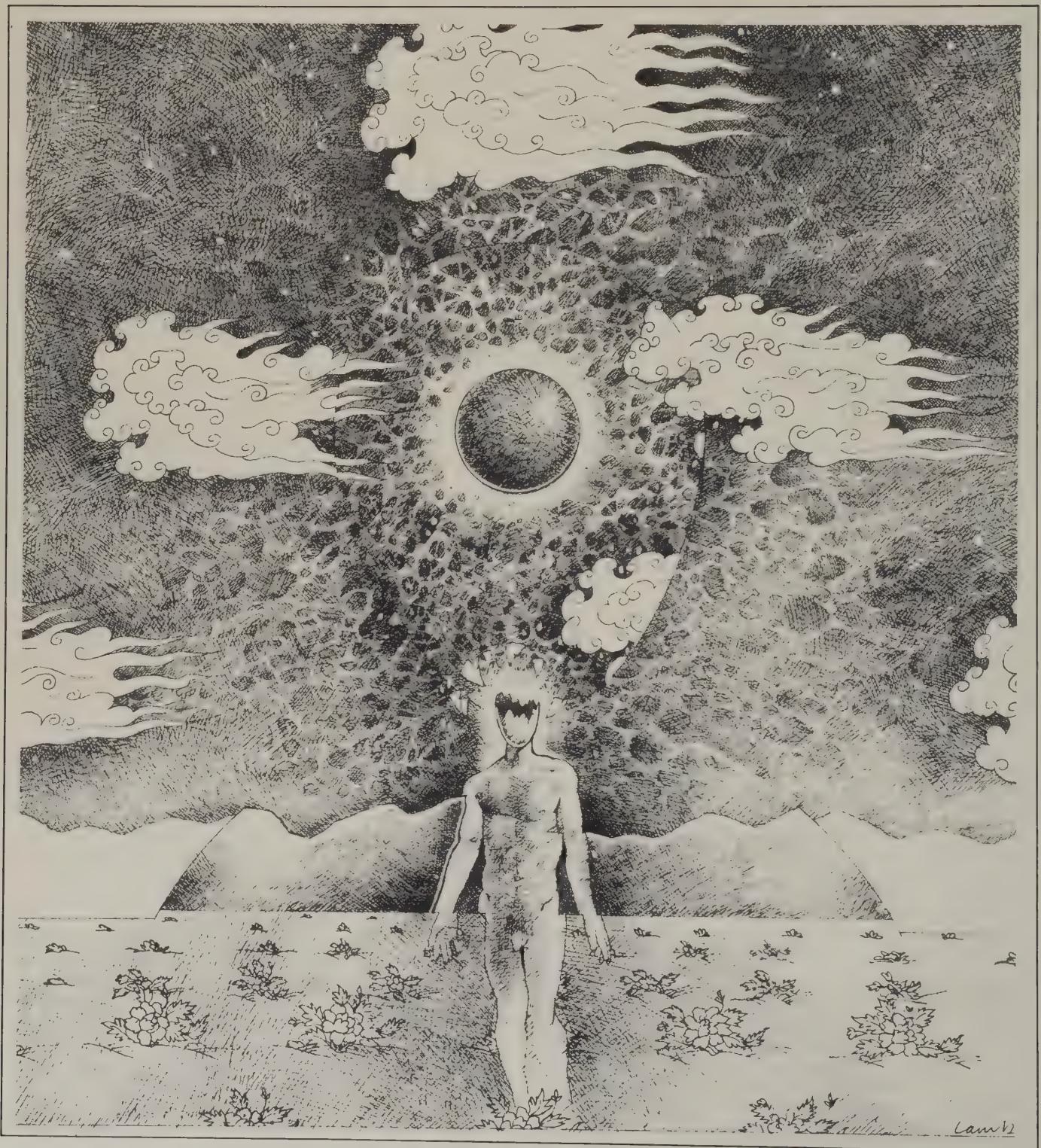
tion to provide ATP for the energy needs of the organism. Heterotrophic organisms are capable only of respiration and are dependent upon the photosynthetic organisms for a supply of organic molecules and O₂.

In the flow of energy through the biosphere, these two processes also play major roles. Radiant energy from the sun is partially converted to chemical energy through photosynthesis; the balance of the radiant energy is lost. The stable molecules formed in photosynthesis are broken down in respiration, with part of the chemical energy being stored in the short-term ATP storage and the balance being lost. The chemical energy of ATP is then used to do various kinds of useful work. However, all of the energy eventually is converted to heat as it passes through various processes. One of the most important forms of work done is the synthesis of the various special organic molecules needed for growth and reproduction of the organism.

FURTHER READING

Good general introductions to metabolic processes are given in books by Conn and Stumpf (1966) and Lehninger (1970). Lehninger (1961) briefly summarizes the energy transfer processes of photosynthesis and respiration. The initial description of the Krebs citric acid cycle was given by Krebs (1950), and more complete descriptions and discussions are given by Krebs and Kornberg (1957). Lehninger (1964) discusses the respiration processes in relation to the structure of the mitochondrion, which will be discussed in Chapter 7 of this book.

Further Readings for Chapter 5 are also relevant for this chapter.



Organization

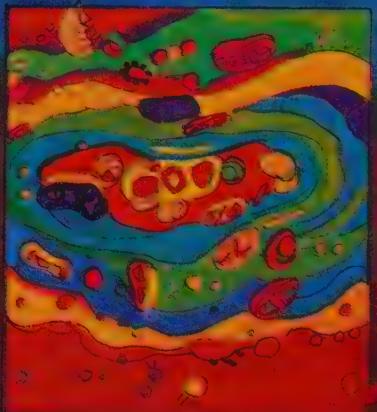
One of the insights that has been most helpful to biologists is the so-called Cell Theory. It is difficult to say precisely what that phrase encompasses. In part, this is because the cellular nature of living systems was revealed not in a dramatic single resolution but rather through a series of clarifications that were developed throughout most of the mid-nineteenth century.

The basic postulates of the Cell Theory are, first, that all organisms are composed of subunits resembling one another in the possession of a certain set of organelles and a boundary; second, that these entities arise only through the division of pre-existing cells. . . . The establishment of the Cell Theory, though attended by far less ceremony than the nearly contemporaneous Darwinian Revolution, has made it possible for biologists to deal coherently with what would otherwise be a hopeless welter of diversity. The doctrine of evolution offered explanations for the enormous breadth of the living spectrum; the cell theory offered hopeful assurances that these variations, despite their extent, had a theme, that the theme was the cellular organization of living systems, and that one might hope to comprehend some of the basic whys of life without inspecting an infinite series of special cases. That expectation has been amply fulfilled. . . .

— Donald Kennedy (1965)

7

Eucaryotic Cells



In 1969 thousands of Americans became fascinated by the microorganisms that Theodor Rosebury described in *Life on Man*. This delightful book was widely reviewed and soon after publication was prominent on the best-seller list. It sparked magazine and newspaper articles, and its author was a widely sought guest on radio and television talk shows. In contrast, the discoveries that Rosebury describes received little attention at the time they were made. Even when the cell theory—one of the great scientific steps in understanding the organization of living systems—was set forth in 1838–1839, it received no coverage in the newspapers, which were filled with articles on cross-Atlantic ship travel, international treaties, and the Opium War.

DEVELOPMENT OF THE CELL THEORY

Basically, the cell theory states that all higher organisms are composed of combinations of simpler subunits called cells. This deceptively simple idea proved to be one of the major keys to the recognition of underlying structural and functional similarities among the great variety of organisms that were being described and classified.

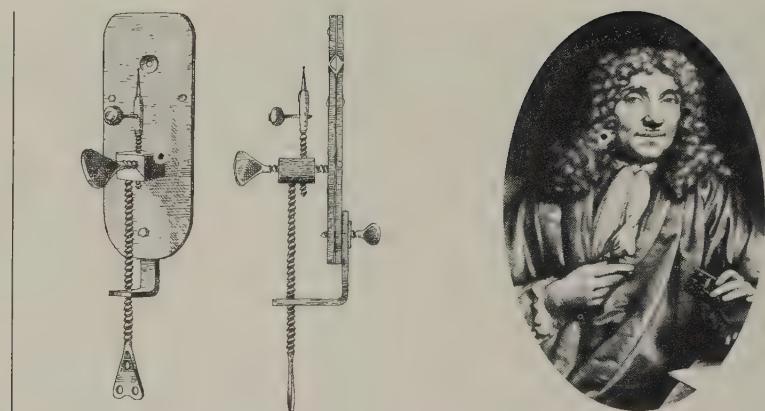
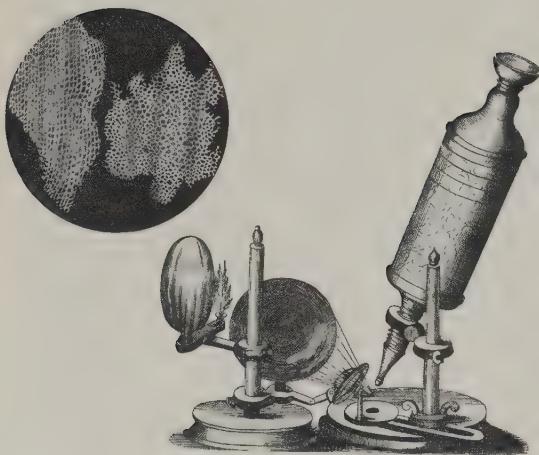
By the seventeenth century, scientists had made many observations concerning the structures and functions of living organisms, but the power of observation was limited by man's eyesight. The invention and use of the microscope gave man the power to observe smaller and smaller objects. The use of the microscope resolved many previously unanswerable questions. For example, it was known that the heart pumped the blood into the arteries and that the veins somehow collected the blood and returned it to the heart, but it was not until the microscope revealed the tiny capillaries that the connections between arteries and veins and the true nature of the circulatory system became established.

One of the earliest microscopists was Robert Hooke. In 1662 he was appointed curator of experiments for the Royal Society of London. This distinguished body of scientists met weekly, and it was Hooke's job to set up an experimental demonstration for their observation at each meeting. Many of his demonstrations were simply observations of familiar objects under the microscope. One such demonstration was made using a thin slice of cork (Figure 7.1). Hooke observed that the cork appeared to be composed of many little boxes, or cells, lined up end to end. For this observation, Hooke is credited with the discovery of the cell.

Among the great early microscopists was Anton van Leeuwenhoek, who owned a drapery shop in the Dutch town of Delft. In his spare time, Leeuwenhoek built hundreds of tiny, single-lens microscopes and used them to make remarkably accurate observations. Although Hooke and others developed more powerful compound microscopes, Leeuwenhoek managed to see more than anyone with his delicately ground lenses, many no larger than a pinhead. Many of his drawings show cellular structures in plant and animal tissues, but Leeuwenhoek limited himself to reports of what he saw and offered no interpretations. Biologists were interested in his descriptions of "animalcules" ("little animals"), which he first saw in 1675 and thereafter observed in great variety. Leeuwenhoek also described the small objects in blood later called red blood cells. Although microscopists continued to describe cellular structures throughout the eighteenth century, they failed to suspect that a better understanding of organisms could emerge from careful comparison of microscopic structures.

Figure 7.1 (below). The microscope Robert Hooke used to observe the microscopic structure of cork.

Figure 7.2 Anton van Leeuwenhoek (far right) ground hundreds of fine lenses in order to observe sperm cells, yeasts, and bacteria. One of his early microscopes is shown at right.



About the same time, microscopists were discovering that the interior of the cell is not the simple fluid originally described. Leeuwenhoek had described small dark objects within some cells, and other investigators began to report a confusing array of "inclusions" in various kinds of cells.

Among the biological writings of the early nineteenth century, there are precursors of the cell theory. The German natural philosopher Lorenz Oken elaborated a complex theory in which all of nature was regarded as reflecting the ideal characteristics of man. His extensive writings include one passage that seems to be a brilliant forecast of the cell theory. He states that "all organic beings originate from and consist of vesicles or cells."

Another early cell theory was set forth in 1824 by the French physiologist René Joachim Henri Dutrochet. He concluded from his microscopic studies that plants are composed entirely of cells and that plant growth occurs both through increase in the volume of cells and through the addition of new cells. Dutrochet then turned to the study of animal tissues and concluded that they too are composed of fluid-filled cells. He theorized that various plant and animal tissues are of different natures only because they contain different fluids in their cells.

With the benefit of hindsight, it is easy to see that Oken and Dutrochet were on the right track. However, as Canadian physician William Osler once commented, "In science, the credit goes to the man who convinces the world, not to the man to whom the idea first occurs." Neither Oken nor Dutrochet convinced the scientific world of the importance or the universality of cells.

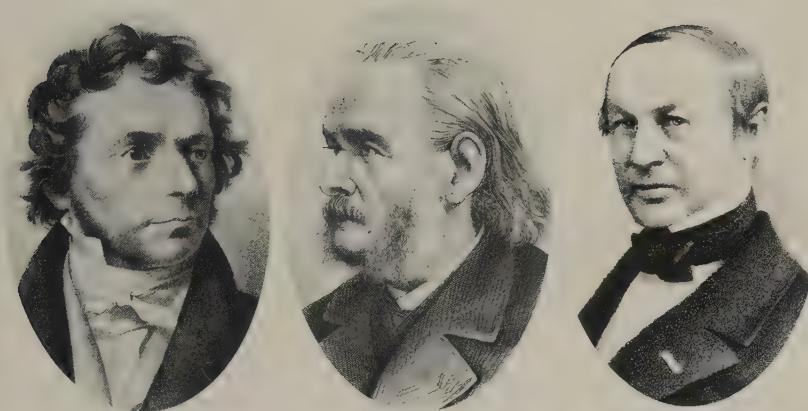
That task was successfully accomplished by two young biologists—the botanist Matthias Jakob Schleiden and the zoologist Theodor Schwann, who in 1839 joined forces to become the "public relations" men for the cell theory. Schleiden had begun his career as a lawyer but became so depressed over his lack of success in that profession that he attempted suicide. Upon his recovery, Schleiden turned to the study of plants. He denounced the systematic collections of various species made by most botanists as so much "hay" and devoted his own efforts to careful microscopic analysis of plant structures. Schleiden (1838) argued that all higher plants "are aggregates of fully individualized, independent, separate beings, namely the cells themselves."

During a visit to the University of Berlin, Schleiden enthusiastically described plant cells and their nuclei to his friend Theodor Schwann, a me-

Figure 7.3 (left). Lorenz Oken was among the first theorists to set forth an early version of the cell theory.

Figure 7.4 (middle). Matthias Jacob Schleiden, a German botanist whose ideas were instrumental in the development of the cell theory.

Figure 7.5 (right). Theodor Schwann, a German zoologist and a colleague of Schleiden's.



thodical young physiologist and researcher. On the basis of his own independent study, largely with animal tissues, Schwann (1839) greatly extended Schleiden's conclusions. Schwann pointed out that animal tissues also are universally composed of cells. He wrote a book setting forth the idea that all organisms—from oak trees and tigers to men—are composed of individual cells. The fertilized egg from which an animal grows—whether the large egg of a bird, the small egg of a frog or a fish, or the microscopic ovum of a mammal—is a single cell, with a surrounding membrane and a nucleus much like those of any cell found in animal tissues. The development of an animal occurs, said Schwann, through the creation of new cells. He concluded that animals and plants are composed entirely of cells and of substances produced by cells and that the cells, to some extent, are independent living units, although they are subordinate to the entire organism.

By the fortieth anniversary of the publication of Schwann's book, the cell theory was so well established that an international ceremony was held in Schwann's honor. Tribute was also given to Schleiden for his important contribution to the recognition of the universal importance of cells in plants. Thus, by the late nineteenth century, Schleiden and Schwann were being credited as the "fathers" of the cell theory. The publications of Schleiden and Schwann were followed by rapid progress in the understanding of plant and animal organization.

The cell is recognized today as the basic subunit of any living system. A single cell is a clearly defined unit, bounded by a membrane that separates it from other cells or from the outside environment. The definition of the cell as a biological unit, however, has more basis than merely the existence of a physical boundary. A cell contains all of the genetic information, all of the translational molecules, and all of the enzymes that are essential to the life of that cell. In short, a cell is the simplest unit that can exist as an independent living system.

THE STRUCTURE OF CELLS

To most of the early investigators—particularly those specializing in the study of plant tissues—the cell appeared to be a fluid-filled wall or bladder with a granular or dense nucleus in the fluid. In later studies, attention shifted from the cell wall to the material inside the cell.

Gradually, biologists found that most animal cells are filled largely with protoplasm, a viscous, granular, constantly moving fluid inside the cell.

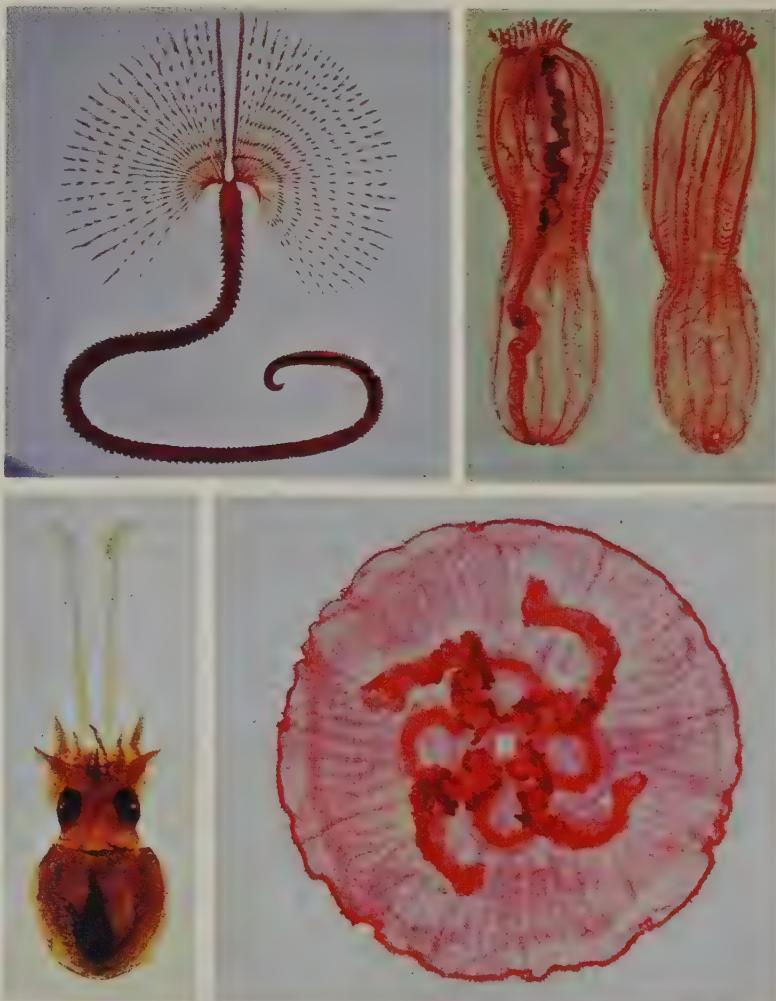
Figure 7.6. Early staining techniques were often crude and inefficient. The use of stains, however, helped in the microscopic examination of cell nuclei and other large organelles. These specimens of various marine animals were made in the middle of the nineteenth century using carmine dye, port wine, and other primitive staining media.

Furthermore, they discovered that the boundary of the living animal cell is a very thin membrane; the highly visible walls of plant cells are nonliving structures that lie outside the membrane.

With the further development of microscopes and staining techniques, more details of cellular structure were discovered. The term "protoplasm," with its implications of a homogeneous living substance, has now been almost entirely abandoned. The terms "nucleoplasm" (for the material of the nucleus) and "cytoplasm" (for the material inside the cell but outside the nucleus) are still used. Still, even these terms are decreasingly appropriate in view of the modern recognition that they refer to collections of distinct molecular structures rather than to simple and uniform substances.

The cells of plants, animals, and most microorganisms are similar in their basic structure. These *eucaryotic* ("having a true nucleus") cells are surrounded by a cell membrane, or plasma membrane, and contain a nucleus surrounded by a nuclear membrane.

Within the cytoplasm of the eucaryotic cell are a number of *organelles*, various specialized structures that perform particular functions and contain



specialized membranes. Although there are many variations on the basic theme of eucaryotic cell structure—for example, kinds of cells in which one or more parts are developed in greater numbers or complexity—the basic structural pattern of eucaryotic cells is remarkably constant throughout the realm of life.

OBSERVATION OF CELLULAR COMPONENTS

What am I, Life? A thing of watery salt
Held in cohesion by unresting cells . . .

These lines by John Masefield convey a sense of the dynamic, continuous activity in living cells. Nevertheless, much of what has been learned about cells has been gained from the study of static structure, and static representations of cell structure are mere snapshots—frozen moments picked out of a miniature, tumultuous maelstrom of unending biochemical activity. If the descriptions, photographs, and diagrams of this chapter create an image of cells as complex but motionless pieces of molecular machinery, perhaps Masefield's words will help to stress their active, ever-changing nature.

Eucaryotic cells vary greatly in size. Some single cells, such as eggs and certain protozoans, are large enough that they can be seen with the unaided eye. Most eucaryotic cells, however, are about ten times smaller than the smallest object visible to the naked eye, but they can be seen with the aid of a microscope. Some of the larger organelles within eucaryotic cells can be distinguished with a light microscope, but their structural details can be detected only with the electron microscope (Interleaf 7.1). Many cellular components are too small to be seen except by electron microscopy.

It is difficult to develop a feeling for the sizes of submicroscopic cellular components. In examining micrographs, one must keep in mind that large and striking structures, such as the nucleolus, may be only 0.00005 inch in diameter. Cellular features are so small that it is awkward to express their size in fractions of an inch. Therefore, sizes of submicroscopic features are expressed in appropriately small units, *microns* and *angstroms*. One micron (μ) is equal to 10^{-6} meter (0.000001 meter, or 0.0001 centimeter), or about 0.00004 inch. One angstrom (A) is equal to 10^{-10} meter, 10^{-4} micron, or about 0.000000004 inch. The *millimicron* ($m\mu$), equal to 10^{-3} micron or 10 angstroms, is also used occasionally. Most eucaryotic cells are approximately 10 to 30 microns in diameter.

The human eye sees most things by reflected light—the light that bounces off the objects being observed. An object under a microscope, however, is normally observed by transmitted light—the light that has passed through the object. To be seen with a microscope, the object must be optically dense—it must absorb some of the light that passes through it. If it absorbs light of some wavelengths (colors) more than others, it appears to have the color of the wavelengths that are not absorbed. To be observed in the electron microscope, objects must absorb (or, more accurately, scatter) electrons.

Until recently, observation of a cell or subcellular structure was possible only after a lengthy treatment. The cell is killed, chemically fixed so that its constituents do not decompose, and its water then replaced with some solvent as a preparation for the addition of an embedding matrix. This matrix—usually a wax or plastic—holds the cell and its components rigidly in position while the cell is sliced into minutely thin sections. The sections then are stained with colored dyes (for light microscopy) or are stained with

Figure 7.7. A size comparison chart of different types of cells. The ostrich egg and the bird eggs are reduced in size by one-half.

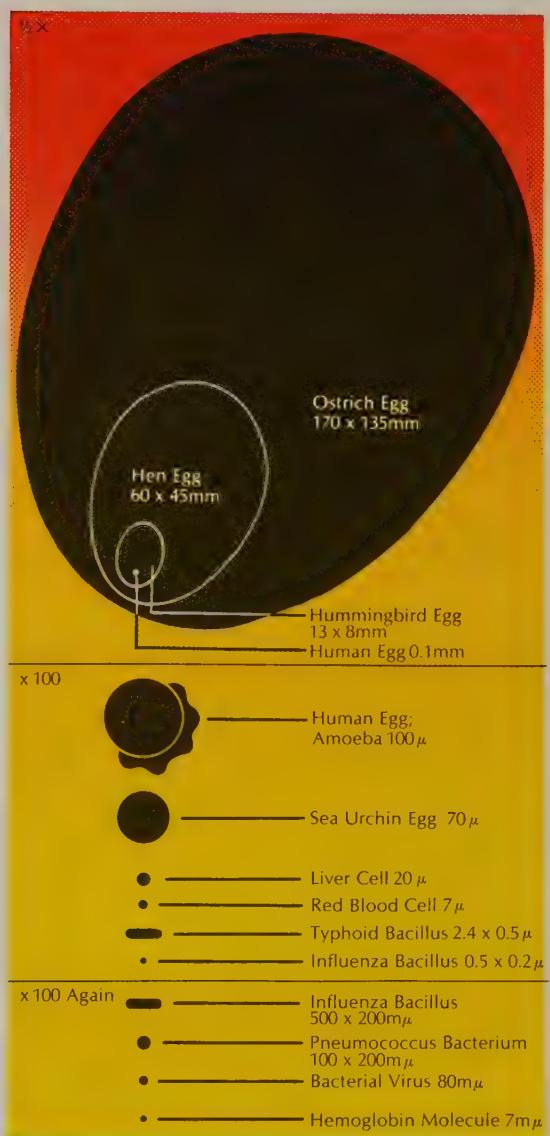
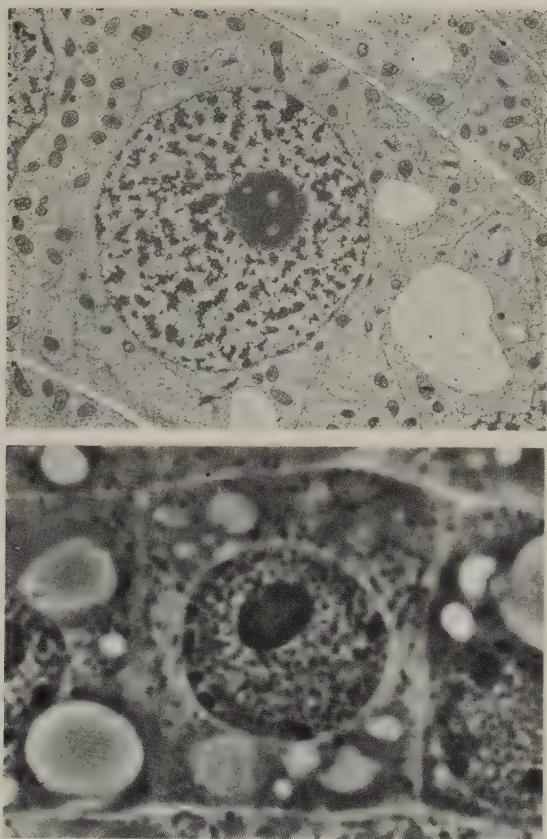


Figure 7.8. A comparison between an electron micrograph of an onion root cell (above) and a light micrograph of the same type of cell (below).

Interleaf 71
LIGHT AND ELECTRON MICROSCOPY



The ability to see details of small structures is limited by the *resolution* of the optical system being used. Resolution refers to the minimum distance between two points at which they can be distinguished; any two points separated by a distance smaller than the resolution will be seen as a single point. Figure 7.8 shows two sections through cells of an onion root. Both micrographs have a linear magnification of X 1,000 (that is, the distance between two points in the image is 1,000 times as great as the distance between the corresponding points in the actual specimen). One micrograph was taken with a light microscope, and the other was taken with an electron microscope. The image obtained with the light microscope is blurry and indistinct, whereas much finer detail can be seen in the electron micrograph. The finer resolution of the electron micrograph is of obvious value in studying cellular structure. In fact, much greater magnifications can be reached with clarity of detail.

The resolution of a light microscope is limited both by the wavelength of light being used and by certain characteristics of the microscope itself. The most significant limitation is due to the diffraction of light—the bending of light waves as they pass around the edge of an object. Diffraction causes the edge of an image to be blurred, and it is impossible to distinguish two point images very close together. In a light microscope, the diffraction becomes greater (and the resolution becomes poorer) as the wavelength of light is increased, as the optical density of the material between the object and the lens is reduced, and as the aperture (angle through which light is admitted) of the lens is reduced. The best resolution is achieved by using a dense oil between the object and the lens and by using violet light of short wavelengths. Even with an ideal microscope, the best resolution that could be achieved under such conditions is about 1,700 Å, or 0.17μ . In fact, the best light microscopes have a resolution of about 0.25μ with white light.

Other effects that once posed limitations to the resolution of light microscopes have been overcome through the design of lenses that compensate for these effects. *Chromatic aberration* results when a lens focuses light of different colors (wavelengths) at slightly different distances from the lens. This effect severely hampered the observations of early microscopists, who saw each small object surrounded by rings of various colors. Achromatic lenses, which eliminate chromatic aberration through the use of two kinds of glass that counteract each other, were introduced about 1830. *Spherical aberration* causes light passing through the center of the lens to be focused in a different plane from light passing through the edges of the lens. This effect also was soon minimized by proper lens design.

Figure 7.9 shows simplified diagrams of light and electron microscopes. In order to emphasize the similarities between the two instruments, the light microscope is inverted, and the dimensions and details of the two instruments have been somewhat distorted. In the light microscope, light from a hot filament (or from the sun) is passed through a condenser lens to produce a parallel beam of light. This beam passes through the specimen and is then focused by the objective lens. An eyepiece lens is used to magnify the image produced by the objective lens.

Light microscopes reached essentially their theoretical limits of resolution late in the nineteenth century, when instruments with oil immersion lenses and condenser lenses became generally available. Even the use of shorter wavelength ultraviolet light would only improve the resolution by a factor of about 2 to about 0.1 micron.

The electron microscope is based on the fact that a beam of electrons has wave properties with very short wavelengths. The first experimental electron microscopes were built in the early 1930s, and commercial models became available in 1939. The electrons are drawn from a hot filament by an electric field. The beam of electrons is focused by magnetic fields, which are produced by electromagnets. A visible image is produced when the electrons strike a coated screen, whose molecules emit visible light when struck by electrons. In most electron microscopes, this screen swings out of the way so that the electrons can fall directly onto photographic film and produce a micrograph. Because electrons are scattered by gas molecules, clear

Figure 7.9. A diagram comparing similar components in a light microscope (left) and an electron microscope (right). Shown below is a scanning electron micrograph of the compound eye of the fly.

images are formed only if a vacuum is maintained within the electron microscope. Because electrons are scattered so easily, the specimen must be very thin—a few hundred angstroms or less for most biological specimens.

In the light microscope, contrast between dark and light areas of the image results chiefly from absorption of light by parts of the specimen. In the electron microscope, dark areas result where parts of the specimen scatter electrons out of the beam so that they are not focused onto the image. With a specimen thicker than a few hundred angstroms, most of the electrons are scattered and a uniformly dark image results. With a very thin specimen, most of the electrons pass through the specimen except where they are scattered by the heavy atoms of a metal stain.

In the light microscope, the image is focused by moving the glass lenses. In the electron microscope, the focal length of the magnetic lenses is changed by altering the current flowing through the electromagnets. In general, however, the path of the electron beam through the electron microscope is similar to that of the light beam through the light microscope.

The wavelength of the electron beam used in the typical electron microscope is about 0.05 \AA . With an ideal instrument design, it would theoretically be possible to achieve resolutions of about this order in electron microscopy. At the present state of the art, however, the resolution of the electron microscope is limited primarily by factors other than diffraction. The equivalent of chromatic aberration exists in the electron microscope if the electron beam contains electrons of varying velocities, but this effect has largely been eliminated by use of very stable voltage supplies to draw the electrons from the filament. However, spherical aberration is a very serious limitation in the electron microscope because the magnetic field in the lenses differs greatly in strength from the edge to the center of the lens. In order to minimize spherical aberration, it is necessary to use a very narrow aperture, which permits the image to be formed only from the part of the electron beam that passes through the center of the lenses. This narrow aperture greatly increases the diffraction effects, so that the best resolution achieved by current electron microscopes is around 1 to 2 \AA . Further improvements in instrument design should make possible a tenfold increase in the resolution and make possible the visualization of molecular structures.

A major problem in electron microscopy has been the development of techniques for specimen preparation. New devices were developed to cut extremely thin sections without seriously distorting the structures. Methods were devised to support these thin slices on metal grids covered with fine films of carbon or collodion. Materials were found for embedding, fixing, and staining the specimens. Because the heavy-metal stains that must be used to make biological specimens visible under the electron microscope are largely toxic to living organisms, because living organisms are too thick to be transparent to an electron beam, and because specimens must be completely dehydrated before being placed in the vacuum of the microscope, it is impossible to observe living cells with the electron microscope. Therefore, there is no direct way to determine how much the *ultrastructure* (small details of structure visible only by electron microscopy) is altered by the techniques of preparation.

Various techniques of specimen preparation are used for electron microscopy. *Electron stains* are electron-scattering (or electron-dense) materials that combine preferentially with certain parts of the biological structure. Like the stains of light microscopy, electron stains darken certain parts of the structure in the image. *Negative staining* involves the use of a general film of electron-dense material that is pushed aside by the biological molecules, producing a negative or light image of the biological structure. *Heavy-metal shadowing* is accomplished by spraying a fine film of electron-dense metal over the specimen at an angle, so that heavy deposits are built up on one side of the structures and a transparent shadow is left on the other side. In the recently developed *scanning electron microscope*, a beam of electrons is scattered from the surface of a thicker specimen, making possible micrographs that show three-dimensional structures.

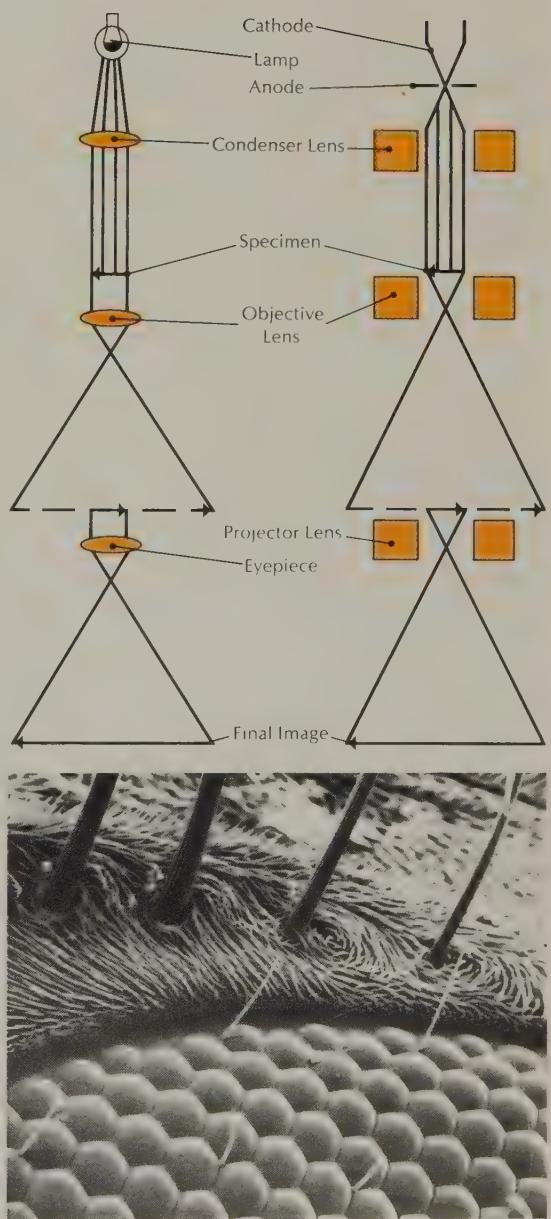


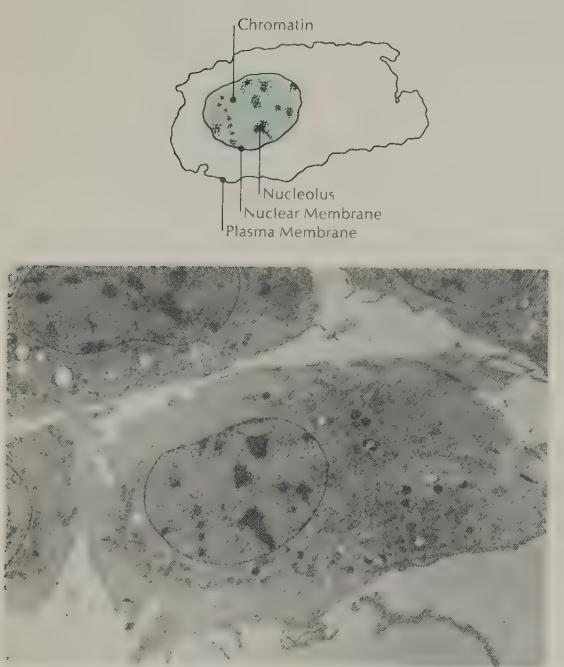


Figure 7.10. A three-dimensional drawing of a eucaryotic animal cell showing its organelles suspended in the cytoplasm (opposite page). Shown at upper left on this page is the nucleolus, which contains RNA and protein and is the site of rRNA synthesis. At the upper right is a Golgi apparatus, a stack of folded membranes that packages and transports materials to be secreted. The mitochondria (lower left) are the sites of cellular respiration. The endoplasmic reticulum (ER) at the lower right forms a complex system of cisternae, or sacs, throughout the cell. Rough ER is studded with

ribosomes and is extensive in protein-secreting cells. Smooth ER can be seen in the lower righthand corner.



Figure 7.11a. Electron micrograph and diagram of a cell nucleus, the most prominent feature of a eucaryotic cell. Several dense nucleoli and scattered chromatin material can also be seen. ($\times 4,000$)



electron-dense metals (for electron microscopy). Some dyes are general or nonspecific stains; others specifically stain certain cellular constituents.

Biology is the study of life, but the student of cell structure usually observes only dead, preserved materials. He must infer how they looked when they were alive. He does not see the actual materials of the cell but only the staining materials. Furthermore, he is forced to deduce a dynamic, continually changing picture of cellular activity from a series of instantaneous, static "snapshots" of the structure.

Special phase contrast and interference microscopes have been developed to enhance the visibility of structures within living cells. With these instruments, it is possible to observe processes in living cells, processes that formerly had been deduced only from the study of a great many sections of fixed and stained cells. However, no means of observing living cells in the electron microscope is yet available.

THE CELL NUCLEUS

Generally, the *nucleus* is the most prominent feature of a eucaryotic cell (Figure 7.11). The contents of a nucleus are separated from the surrounding cytoplasm by a nuclear membrane, which is clearly visible in electron micrographs (Figure 7.12). This membrane is double; it appears to consist of two membranes, each about 70 Å thick, separated by a distance of about 150 to 200 Å.

Closely spaced around the nuclear membrane are small, round structures called *annuli*, which are formed where the inner and outer membranes come together to form a much thinner membrane. These annuli sometimes are called nuclear pores, but this name is misleading because they are not open channels between the nucleoplasm and the cytoplasm. Annuli are thought to be selective barriers that permit the passage of macromolecules such as RNA but, at the same time, prevent the free exchange of ions between nucleoplasm and cytoplasm. The precise function of the membrane in the annuli is not known.

Early staining techniques for light microscopy revealed what appeared to be granular material scattered through the nucleus. This *chromatin* seemed to gather into threadlike bodies, or *chromosomes*, just before cell division. Biochemical analyses show that the chromatin and chromosomes consist of DNA in close association with RNA and protein. The electron microscope reveals that the granular-appearing chromatin in nondividing cells consists of the chromosomes in unwound form. During cell division, the chromosomes coil and condense to such an extent that their threadlike structure becomes visible under the light microscope. Electron micrographs of the highly compacted chromosomes of dividing cells have not revealed the detailed structure of chromosomal material. In thin sections, the chromosomes appear as densely packed aggregations of tangled fibers. Even with special techniques for isolation of individual chromosomes, the chromosome still resembles disorganized, packed masses of yarn.

Within the nucleus are one or more large bodies, the *nucleoli* (Figure 7.13). In most kinds of cells, the nucleus contains one or two nucleoli, but exceptions are numerous. The nucleolus contains large amounts of RNA and protein and is now known to be the site of the synthesis of ribosomal RNA. A small piece of chromosomal DNA, the *nucleolar organizer*, lies within the nucleolus and apparently carries information that directs the formation of the nucleolus itself and of ribosomal RNA.

Figure 7.11b (above). Electron micrograph view of the nuclear region of an onion root tip cell made by the freeze-etch preparation technique. The relatively new freeze-etching technique involves a splitting of membranes, which allows extremely detailed examination of membrane faces. ($\times 27,000$)

Figure 7.12 (middle). Electron micrograph showing the double nuclear membrane. ($\times 26,000$)

Figure 7.13 (below). A large central nucleolus is clearly visible in this electron micrograph and diagram. Also

visible are large quantities of darkly stained chromatin material. ($\times 14,000$)

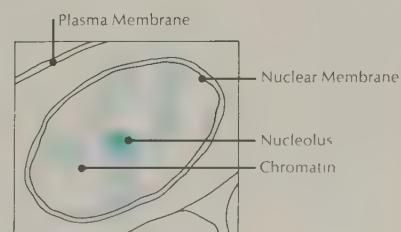
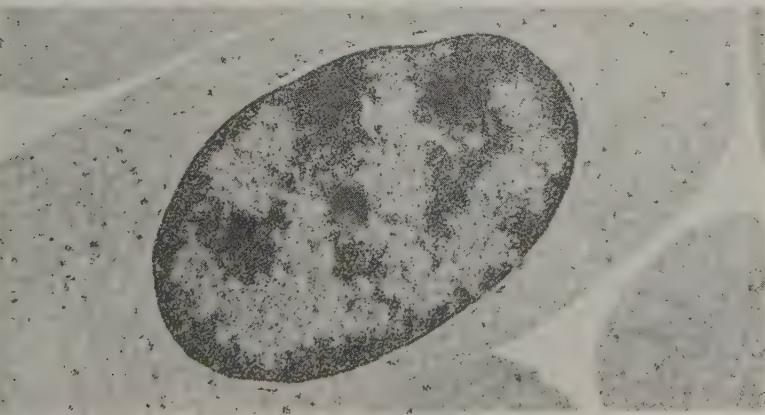


Figure 7.14. Electron micrograph of a plasma membrane. Note the two dark lines separated by a light line, together making up the unit membrane. (x 500,000)

Although nucleoli vary in size among different types of cells and among different organisms, they are usually large enough to be discerned easily with the light microscope. Nucleoli are larger and denser in cells carrying on active protein synthesis. They are smaller and less dense in metabolically inactive cells. Electron micrographs show that the nucleolus is made up of two kinds of granules. One kind is about 150 Å in diameter; the other, about 75 Å. These granules are thought to be the precursors of ribosomes. In some nucleoli, the granules are arranged into fibers called *nucleolomata*. No membrane is visible around the nucleolus, and the nucleoplasmic material appears to extend in various zones within the nucleolus.

CYTOPLASM

The cytoplasm of the eucaryotic cell consists of all the materials outside the nuclear membrane, including the outer cytoplasmic membrane of the cell. Some cells—such as amoebae and some egg cells—contain a relatively large amount of cytoplasm and relatively small nuclei. Others, such as thymus cells, are almost all nucleus with very little cytoplasm. Depending upon the functions of certain cells, the cytoplasm can contain such specialized products as hemoglobin, starch, or yolk. The cytoplasm contains the organelles and also bodies of inactive or structureless materials—such as droplets of lipid or starch—called *inclusions*. The types and numbers of organelles and inclusions in the cytoplasm vary with the activity and type of cell. Despite the variations from cell to cell, certain general features that hold true for most eucaryotic cells can be described.

Plasma Membrane

Every cell is surrounded by a plasma membrane, or cell membrane. The existence of this membrane was postulated long ago because cytoplasm flows

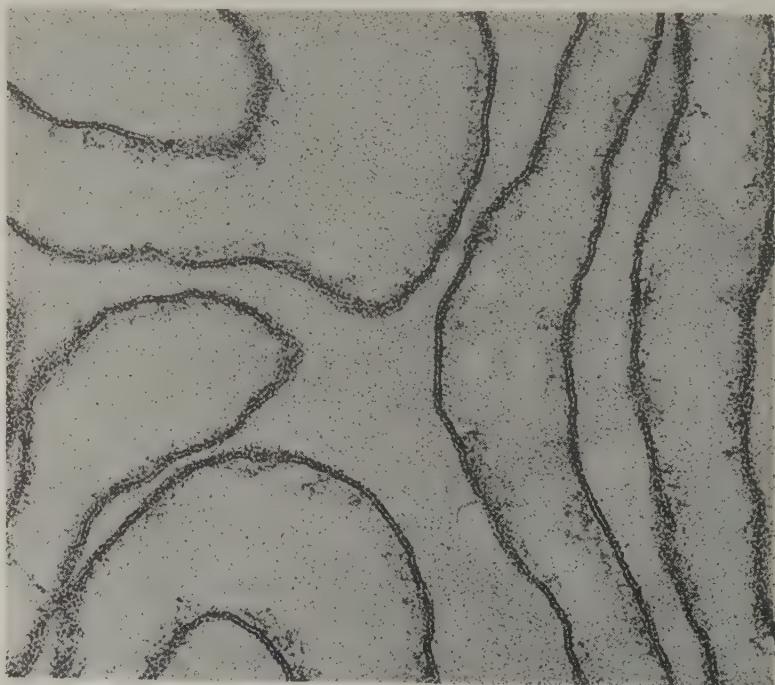


Figure 7.15. Electron micrograph of microvilli.

out of the cell if the cell surface is punctured or torn. With the light microscope, the membrane can be seen—if at all—only as a very thin edge. The electron microscope, however, shows the plasma membrane as a dense line about 90 Å thick. In high-resolution micrographs of favorably sectioned and stained material, the cell membrane appears as two 30 Å, electron-dense lines separated by an electron-transparent space about 30 Å thick. The cell membrane is composed largely of proteins and lipids, but the structural arrangement of these components within the membrane is still not known. Current theories of membrane structure and function are discussed in Chapter 10.

The plasma membrane separates the highly unstable and chemically reactive molecules of the cell's interior from the external environment. The membrane regulates the flow of materials into and out of the cell with exquisite precision and efficiency. Substances such as water freely diffuse across the cell membrane; water molecules move toward the side of the membrane where the water concentration is lowest. The movement of ions, however, depends upon their size, charge, and chemical nature.

In many kinds of cells, the membrane is folded to form tiny projections called *microvilli* (Figure 7.15). These structures are most abundant in cells that specialize in the absorption of substances from the external environment—for example, intestinal cells—and they apparently serve to increase the surface area and absorptive capability of the cell. In some specialized cells, such as nerve cells or light-receptor cells in the eye, the plasma membrane is complexly folded to produce special structures related to the specific functions of these cells. Cellular structures called *desmosomes* seem to provide a form of tight connection between adjacent cells in multicellular organisms.

The plasma membrane is far more than a simple envelope surrounding the living protoplasm. In fact, it is a complex structure that plays an active role in the life processes of the “unresting cell.”

Ribosomes

Most of the RNA found in the cytoplasm is associated with protein in distinct particles called ribosomes. The ribosomes catalyze construction of proteins and are most numerous in cells that are actively synthesizing protein. Studies indicate that a ribosome consists of two separate subunits, each containing ribosomal RNA (rRNA). These two parts can be seen in some high-resolution electron micrographs. The larger subunit provides binding sites for transfer RNA (tRNA); the smaller subunit binds to a molecule of messenger RNA (mRNA). The message that specifies the amino acid sequence is read from the mRNA, and the molecules of tRNA insert their attached amino acids into the growing polypeptide chain in accordance with that sequence.

The formation of ribosomes begins in the nucleolus, where molecules of rRNA are transcribed from part of the DNA of the nucleolar organizer. The RNA molecule is split into two unequal parts, which acquire protein to form two unequal particles (ribosomal precursors). These particles move independently into the cytoplasm, where two units (one of each kind) combine to form a ribosome. A complete ribosome is composed of about 60 percent RNA and 40 percent protein.

Ribosomes, which are about 170 Å in diameter, may be found scattered randomly through the cytoplasm, but they are often found in clusters or

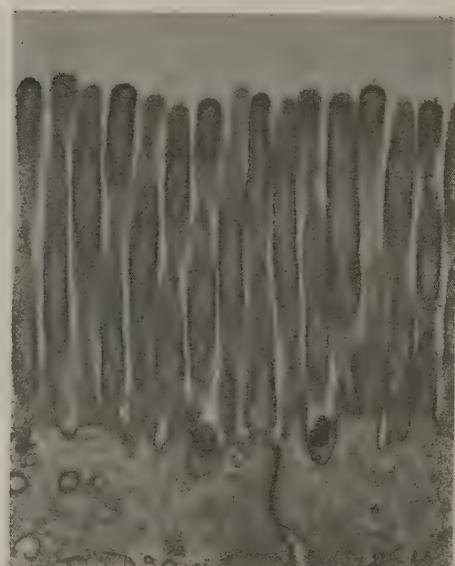


Figure 7.16. Rosettes of ribosomes of the endoplasmic reticulum. ($\times 54,000$)



rows (*polysomes*), which contain several ribosomes attached to a single mRNA molecule. In eucaryotic cells engaged in synthesis and secretion of proteins, a large proportion of the cell's ribosomes are found attached to membranes within the cytoplasm.

Endoplasmic Reticulum

Before the advent of electron microscopy, biochemists variously described the cytoplasm as a watery mixture of organic molecules and soluble salts, as a complex mixture of solutes and gels, or—in more sophisticated theories—as “a complex polyphase colloidal system.” Light microscopists, on the other hand, tended to emphasize the presence of organelles and of regions that were revealed by staining. The microscopists found considerable evidence to suggest the presence of regular submicroscopic structures within the cytoplasm. The electron microscope has confirmed that the cytoplasm of most cells contains an elaborate system of internal membranes.

The *endoplasmic reticulum* consists of membrane sheets folded through the cytoplasm, forming a complex system of tubules, vesicles, and sacs (*cisternae*). The membrane of the endoplasmic reticulum (ER) has a unit structure similar to that of the plasma membrane and nuclear membrane, and—in some places—the membranes of the ER may be continuous with the plasma and nuclear membranes.

Some parts of the ER membranes (*rough ER* or *granular ER*) are studded with ribosomes. Other parts of the membrane are smooth, with no ribosomes attached. Rough ER is particularly extensive in protein-secreting cells. Proteins synthesized on ribosomes on the ER are released into the cisternae of the ER. They then move into another part of the cell to be transported to their destination. Cells that produce and secrete nonprotein substances (for example, hormone-producing cells of the testicles and the adrenal gland) often contain large numbers of thin tubules of smooth ER. The relationship of smooth ER to hormone synthesis is not yet understood.

Golgi Apparatus

While experimenting with staining techniques on nerve cells, the Italian physician Camillo Golgi noticed the presence within the cells of a complex of vesicles (Figure 7.17). Similar structures were observed in nerve and secretory cells from many kinds of animals. For many years, the Golgi apparatus, as these vesicles came to be called, was the subject of considerable controversy. Because it cannot be seen in living cells, there was some reason to suspect that the structure might be an artifact of staining.

Electron micrographs confirmed the existence of the Golgi apparatus and provided some details of its structure. Its form in animal cells is highly variable, but in all cases it is composed of membranes similar in appearance to smooth ER. These membranes are folded into vesicles.

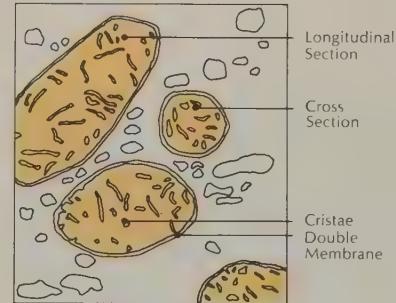
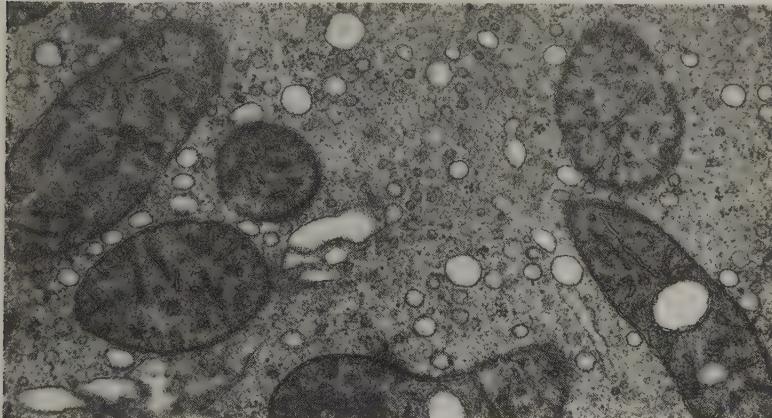
A common form of Golgi apparatus is the *dictyosome*, which consists of a stack of cisternae surrounded by a netlike halo of tubules and small spherical vesicles. Dictyosomes are found in many animal cells and are present in plant cells.

One function of the Golgi apparatus is to package and transport materials to be secreted to the exterior of the cell. Substances to be excreted accumulate in the vesicles of the Golgi apparatus. These vesicles enlarge, separate from the Golgi apparatus, and move to the plasma membrane. The membrane of the vesicle fuses with the plasma membrane, and the con-

Figure 7.17 (above). Electron micrograph showing a cross section of a Golgi apparatus, which is comprised of a stack of cisternae or vesicles. Note also the dictyosome, a stack of well-defined vesicles typically surrounded by a halo of smaller branching tubules. ($\times 50,000$)

Figure 7.18 (below). Electron micrograph and diagram illustrating the variety of shapes of mitochondria. Each mitochondrion is surrounded by a double membrane

and has the internal arrangement of folded membranes called cristae. ($\times 36,000$)



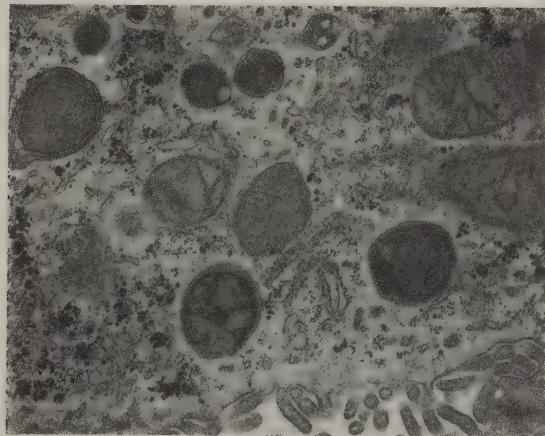
tents of the vesicle are discharged to the exterior of the cell. This process has been observed in the secretion of plant cell walls and in the secretion of enzymes and other substances by animal cells.

Mitochondria

Mitochondria are small, generally oval organelles found in nearly all eucaryotic cells, but they are absent from prokaryotic cells (Figure 7.18). These structures serve as cellular “power plants.” Cells that have high energy requirements—muscle cells, for example—contain many large mitochondria, whereas cells with low energy requirements have smaller mitochondria in sparser numbers. It is in the mitochondrion that cellular respiration takes place, with liberation of CO_2 and H_2O and phosphorylation of ADP to ATP (Chapter 6). Only a few types of highly specialized eucaryotic cells, such as mammalian red blood cells, lack mitochondria.

Mitochondria are not uniformly distributed through the cytoplasm of most cells but are often found “where the action is.” For example, there are

Figure 7.19. Electron micrograph of lysosomes.
($\times 50,000$)



large accumulations of mitochondria near the basal membrane of kidney tubule cells, where a great deal of energy is used in transporting ions across the cell membrane.

Most mitochondria are about 0.5μ in diameter and from 0.5 to 7.0μ in length. They range in number from a single mitochondrion per cell in one kind of eucaryotic alga to hundreds of thousands of mitochondria per cell in some amoebae. The mitochondrion is surrounded by a double membrane. The fluid-filled space between the membranes may be 60 to more than 200 Å wide. In most mitochondria, the inner membrane is enfolded to form *cristae*—sheets or tubules that extend across the interior of the mitochondrion. The material within the inner membrane, the *matrix*, often contains fibers, granules, or droplets.

The surfaces of the cristae in contact with the matrix are covered with small particles that are about 80 Å in diameter and are attached to the cristae by stalks. There are more than 10,000 of these particles in each mitochondrion. These stalked particles are thought to contain the enzymes that catalyze electron-transfer reactions of oxidative phosphorylation. The enzymes presumably are arranged and grouped in such a way as to lead the intermediate molecules sequentially from one reaction step to the next. The enzymes of the Krebs cycle (Chapter 6), on the other hand, are located within the fluid matrix.

The glycolytic reactions and other steps that lead to the formation of acetyl-coenzyme A occur in the cytoplasm. The acetyl-coenzyme A complex is taken into the mitochondrion, where the Krebs cycle reactions occur in the matrix. As electrons are removed in the oxidation of the carbon compound, they are passed to the enzyme sequences in the particles of the cristae membranes, where the electrons eventually reduce oxygen to form water. The ATP formed during phosphorylation is transferred back to the outside of the mitochondrion, where it may participate as an energy source in various metabolic reactions (Lehninger, 1964).

Recent experiments have shown that mitochondria contain their own DNA, which is different in size and base composition from chromosomal DNA. Mitochondria can replicate independently of the nucleus, through a process similar to binary fission. The DNA of the mitochondrion is in the form of a circular strand.

Lysosomes

Although approximately the same size as mitochondria, lysosomes are organelles of very different structure, function, and origin. They are sacs of hydrolytic enzymes enclosed within a single unit membrane that isolates the enzymes from the rest of the cytoplasm (Figure 7.19). They are believed to form by the pinching off of sacs from the Golgi apparatus (Novikoff, et al., 1964). The hydrolytic enzymes within a lysosome vary somewhat from cell to cell but typically include enzymes that hydrolyze proteins and nucleic acids.

Lysosomes, with their very simple structure, were not detected until the 1950s, and their function is still not certain. They have not been proven to exist in plant cells. They apparently act as disposal units of the cytoplasm, for within them are found the remains or fragments of mitochondria, ingested food particles and microorganisms, worn-out red blood cells, and any other debris that may have become incorporated within the cytoplasm. Lysosomes are most abundant in cells that specialize as scavengers within

the multicellular organism and in cells that participate in the breakdown of other cells (as in the absorption of the tail structure of a tadpole as it develops into a frog).

Once a lysosome has performed its function, it is expelled from the cell by the same mechanism that moves secretory materials out through the plasma membrane. In a dying cell, the membrane of the lysosome is broken down, and the hydrolytic enzymes are released into the cytoplasm—leading to irreversible changes and destruction of macromolecules, which in turn leads to the death of the cell. Malfunctions of the lysosomes are apparently involved in a number of human diseases, including cancer, and the discharge of enzymes from the lysosomes into the cytoplasm may produce cell damage or death.

Plastids

Plastids, which are present only in plant cells, are similar to mitochondria in several respects. They have a double membrane, as well as a system of internal membranes; they contain their own DNA and ribosomes and therefore may be able to reproduce independently of the nucleus; their DNA differs from that of the chromosomes in significant ways. Plastids, however, show a greater size range than do mitochondria. Whereas mitochondria are involved in making free energy available from the chemical energy of carbohydrates, most plastids are involved in the reverse process—the capture of solar energy with which carbohydrates are synthesized.

Plastids are of two general types: *chromoplasts* (which contain pigments) and *leucoplasts* (which are colorless). *Chloroplasts*, which contain the green pigment chlorophyll, are the best known of the chromoplasts (Figure 7.20). It is in the chloroplasts that photosynthesis takes place. Other

Figure 7.20. Chloroplast in a leaf cell. The stroma lamellae, or large thylakoids, can be seen extending between grana from one end of the chloroplast to the other, with small thylakoids interspersed between them. ($\times 35,000$)



Figure 7.21 (above). Electron micrograph of a leucoplast, a structure specialized for storing polysaccharides. ($\times 86,000$)

Figure 7.22. Electron micrograph of a chloroplast showing grana in portion of a developing leaf cell (middle). The stacks of membranes, or grana, in chloroplasts contain chlorophyll. ($\times 70,000$). Shown below is a diagram of the structure of the grana.

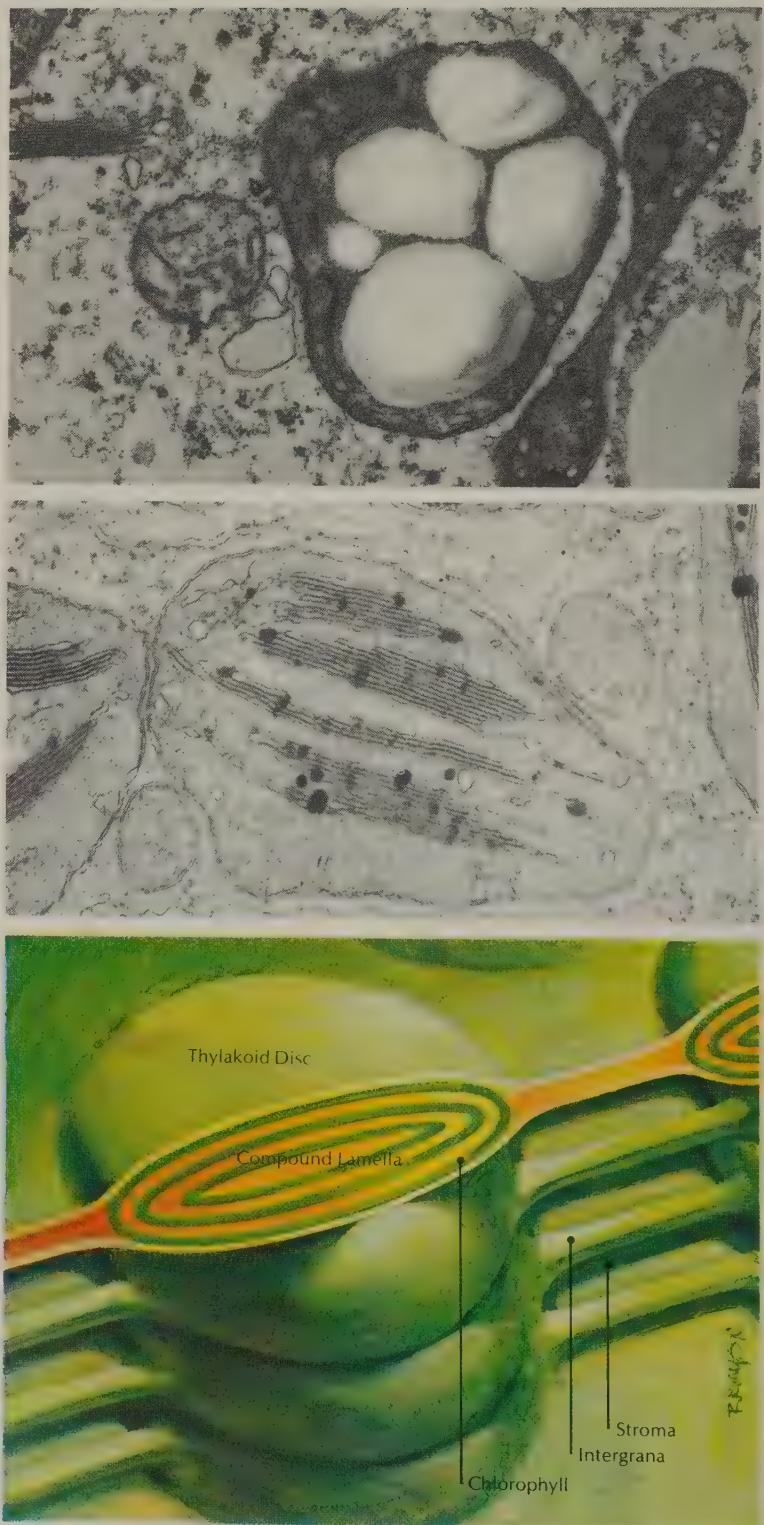


Figure 7.23. Electron micrograph and diagram of the structure of centrioles. The centriole consists of nine parallel triplets of microtubules. A similar pattern of microtubule arrangement is found in the structure of cilia and flagella. ($\times 130,000$)

chromoplasts contain other kinds of pigments and often are brilliantly colored. The yellow color of carrots, the red color of tomatoes, and—in some cases—the various colors of flowers are due to such chromoplasts.

Leucoplasts are the sites for conversion of glucose to starch and to lipids or proteins; these products then are stored in the leucoplasts (Figure 7.21). The most conspicuous group of leucoplasts is the starch-storing amyloplasts, which are found in many fruits and vegetables. The whiteness of potatoes is due to the presence of abundant amyloplasts.

Because of the great importance of photosynthesis in the carbon cycle and energy flow of the biosphere, chloroplasts have been thoroughly studied. In higher plants, mosses, ferns, and some algae, chloroplasts are disc-shape bodies about 2 to 4μ in diameter and 1μ or less in thickness. In many algae, however, chloroplasts assume more elaborate shapes: stars, spirals, perforated sheets, and so on. One of the algae most frequently used in photosynthesis research is *Chlorella*, which has a single, cup-shape chloroplast occupying the bulk of the cell.

The outer membrane of the chloroplast is similar in structure to the plasma membrane of the cell, whereas the inner membrane is a complex system made up of flattened sacs called *thylakoids*. In higher plants, small thylakoids are stacked one upon the other to form a unit called a *grana*; these grana are interspersed with larger thylakoids. The material surrounding grana and thylakoids is the *stroma*, which contains dissolved salts, enzymes, more widely spaced membranes, ribosomes involved in chloroplast protein synthesis, and the DNA of the chloroplast. In red algae, the thylakoids are tightly spaced and scattered through the stroma; in brown algae, they are grouped into small numbers of layers. In some cases, the thylakoids between grana form tubular structures. Within the thylakoids and grana, the pigment molecules are stacked in regularly arranged units that facilitate the photosynthetic reactions.

Chloroplasts generally are not present in the cells of plants grown in the dark. Present instead are much smaller bodies called *proplastids*, which develop into chloroplasts if the plant is placed in the light. Many proplastids contain an elaborate structure called a *prolamellar body*, which develops into grana in the presence of light. Chlorophyll is synthesized from a precursor called protochlorophyll as proplastids develop into chloroplasts.

The term "proplastid" also is used for small plastids that lack any elaborate internal structure. Such simple proplastids are thought to be the parent structure from which all different plastid types develop. These simple proplastids reproduce by binary fission, as do the mature chloroplasts of simple plants. Until recently, it was thought that mature chloroplasts of higher plants are incapable of division, but mounting evidence from electron microscopy indicates that chloroplast division is probably a common process in higher plants as well.

Centrioles

Eucaryotic animal cells contain two inconspicuous pairs of bodies called centrioles. These organelles are also present in the sperm cells of algae, mosses, fungi, ferns, and some gymnosperms, but they are missing from other plant cells. Each centriole is a cylinder about 0.2μ in diameter, composed of nine parallel triplets of hollow, cylindrical *microtubules*. The two members of a centriole pair normally differ in length and are at right angles to each other. One is about 0.5μ in length, the other usually about 0.2μ long.

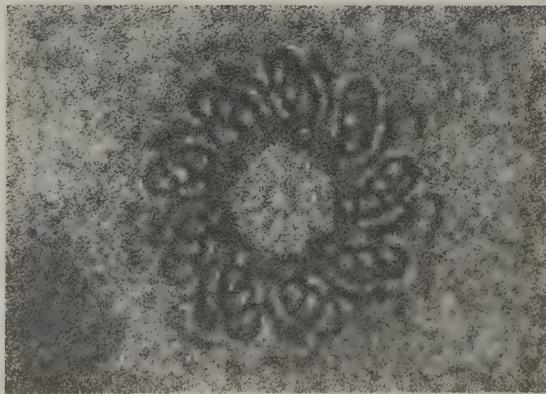
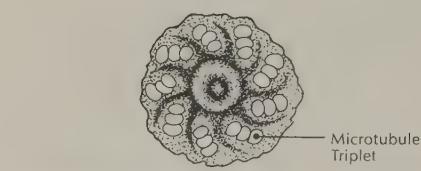


Figure 7.24. Electron micrograph of microtubules in cross section. The dark area to the right is chromatin. ($\times 35,000$)



The size and structure of centriole pairs are remarkably constant throughout all the various eucaryotic cells that contain them.

Microtubules are common in the cytoplasm of most eucaryotic cells. In some animal cells, they are oriented in regular patterns in the cytoplasm and appear to channel the flow of substances through the cytoplasm. In the cells of higher plants, microtubules are particularly abundant in the cytoplasm near the plasma membrane and are also found extending along streams of cytoplasm that move through the cell interior. In some cells, microtubules apparently serve as a "cytoskeleton" that gives structural integrity to the cell. Microtubules are a prominent feature of nerve cells and fibers, but their function is unknown. Recent findings suggest that microtubules are involved in phenomena of cell movement, including the so-called amoeboid motion characteristic of amoebae, slime molds, and certain blood and tissue cells. It has been suggested that the regular shape of annuli, or nuclear pores, in the nuclear membrane may indicate that the pore is formed by a circular array of microtubules.

Each microtubule in turn is made up of 13 filaments arranged in a circular pattern (Ledbetter and Porter, 1964). The filaments are cylinders about 40 to 60 Å in diameter. Other sorts of filaments are found free in the cytoplasm of many kinds of cells, where they act as structural supports and may serve other functions. The filaments are composed of proteins.

Cilia and Flagella

Cilia and flagella are hairlike appendages of cells. They extend from the plasma membrane to the exterior and are bounded by an outfolding of the membrane. "Cilium" and "flagellum" are relative terms. "Flagellum" generally is used for longer structures and "cilium" for shorter ones, but the two kinds of appendages have identical microstructures. Cilia are com-

Figure 7.25 (above). Electron micrograph of a sample of butterfly sperm with cross-sectional views of their flagella. Note the arrangement of microtubules within one flagellum—nine parallel ducts of microtubules with two single central fibrils. ($\times 65,000$)

Figure 7.26 (below). Electron micrograph showing large vacuoles. Starch storage plastids can be seen surrounding the nucleus, with dictyosomes, mitochondria, and ER in the cytoplasm. Chloroplasts, not seen in this cell, are also common features of plant cells. ($\times 6,000$)

monly about 10 or 20μ long, and flagella can be as much as thousands of microns long (in the sperm of some insects, for example). However, all cilia and flagella are about 0.2μ in diameter.

Most cilia and flagella are capable of motion. Because of their length, flagella usually move in an undulating fashion, whereas cilia move with simple, oarlike strokes. Their activity propels the cell to which they are attached or moves things past a stationary cell. Some kinds of cells have hundreds of cilia, some have only a few, and many have none at all. Most flagellated cells have only a single flagellum, but in algae and fungi the flagella usually occur in pairs. Many unicellular organisms move by means of cilia or flagella. A sperm cell is propelled by a single, long flagellum. The meeting of sperm and egg is further facilitated in many organisms by the motion of cilia on cells that line the female reproductive tract. In lungs, cilia move foreign particles such as dust and soot out of the respiratory tract.

A flagellum is an extension of one of the two centrioles of the flagellated cell. In a ciliated cell, many extra centrioles may be formed and may serve as *basal bodies* from which cilia develop. Two of the microtubules of each centriolar triplet extend the full length of the flagellum or cilium. One of each pair of microtubules possesses enzyme molecules that are essential to the motility of the appendage. An additional pair of microtubules extend up the center of the flagellum or cilium. If this central pair of microtubules is missing, the appendage is nonmotile. The arrangement of microtubules in the flagellum or cilium invariably forms the characteristic array called the $9 + 2$ pattern.

Vacuoles

Vacuoles are membrane-surrounded spaces found within all kinds of cells (Figure 7.26). They vary in size more than any other organelle. In fact, vacuoles might be considered to lie in a vaguely defined position between true organelles and simple inclusions. Some vacuoles play an active role in cell processes, whereas others serve merely as storage depots.

The largest vacuoles appear in plant cells, where they may make up most of the cell's volume. Vacuoles in plant cells are filled with fluid or "cell sap" under a pressure that helps to maintain the shape of the cell and to give the plant a rigid structure. A plant wilts because the amount of water available is insufficient to maintain the fluid pressure of the vacuoles. This loss of pressure leads to cell collapse.

In mature plant cells, a single vacuole may occupy 90 percent or more of the volume of the cell. The cytoplasm, nucleus, and plastids of such cells are pressed against the cell wall by the large central vacuole. Although the cells at the tips of roots and shoots as well as certain specialized cells in the plant body lack large central vacuoles, almost all of these cells have collections of small vacuoles.

Water is the major component of the fluid in large vacuoles of plant cells. Dissolved in this water are salts, sugars, pigments, and other substances. The red color of many flowers is due to pigments concentrated in the vacuoles of the flower petal cells. In citrus fruits, the contents of the vacuoles are quite acidic, giving these fruits their characteristic sour taste. In some cases, the fluid in the vacuole is so acidic that the cytoplasm would be severely damaged if it were exposed to the vacuolar contents.

Recent evidence suggests that the vacuolar membrane in many plant cells is closely associated with the ER. Some electron micrographs show the

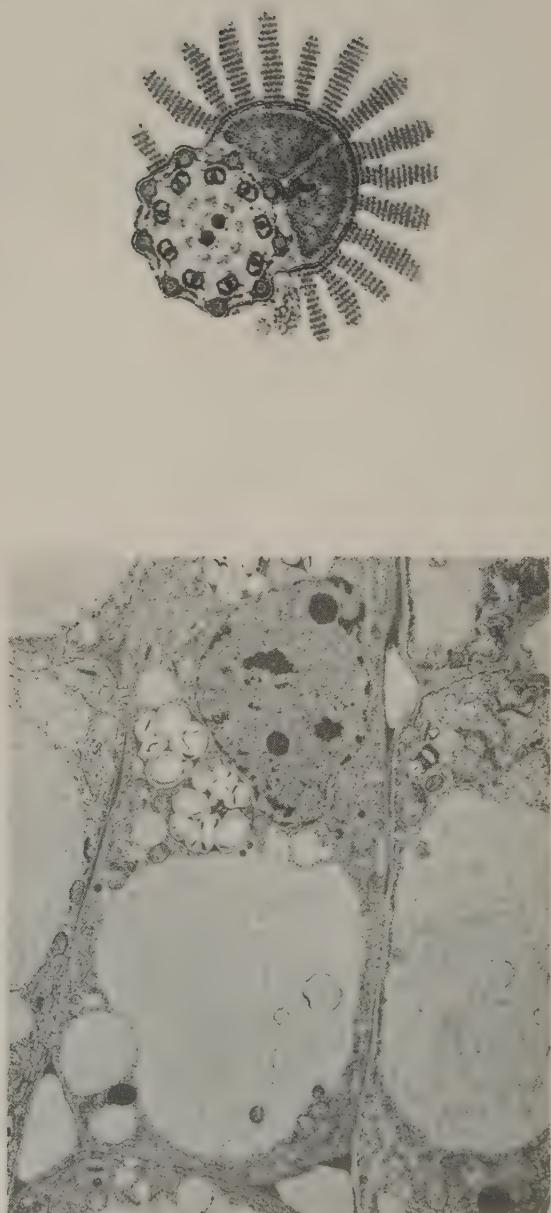


Figure 7.27. Scanning electron micrograph of chitin, an extracellular polysaccharide.

ER attached to the vacuolar membrane and continuous with it. However, ribosomes have never been observed attached to the vacuolar membrane.

The origin, development, and function of vacuoles in young plant cells are not clearly understood. At first, vacuoles are inconspicuous and, in many cases, are invisible in the light microscope. As the cells mature, many small vacuoles appear, forming larger vacuoles by joining together.

Vacuoles in microorganisms and animal cells exhibit great variability in function and in size. Fresh-water protozoans such as amoebae and paramecia possess *contractile vacuoles* which may be quite intricate and constantly changing in shape. Because the water outside the cell is at a higher concentration than the water within the cytoplasm, these small, fresh-water organisms experience a constant diffusion of water through the plasma membrane into the cytoplasm. Contractile vacuoles accumulate the excess water and periodically pump it out through a pore that they form in the cell membrane. The membrane is immediately repaired after the vacuole completes its contraction. Without contractile vacuoles, these fresh-water organisms would burst from the accumulated influx of water from the exterior. Other kinds of vacuoles in animal cells play a relatively passive role and might better be considered to be inclusions.

CYTOPLASMIC INCLUSIONS

In the rather arbitrary category of cytoplasmic inclusions are grouped all particles, droplets, storage granules, and other substances that are relatively inert with respect to the metabolic activities of the cell. They vary in size from glycogen granules (about 150 to 300 Å in diameter) to crystals of various sorts that are visible at low powers of the light microscope. In the intermediate size range are lipid droplets, yolk granules, pigment granules, virus inclusions (often in crystalline form), and other crystals. Crystals of the organic base guanine are found in the surface cells of fishes, amphibians, and lizards and in the light-reflecting cells of the eyes of many nocturnal animals. Cells that contain guanine crystals impart a silvery luster to the tissues they form. Calcium oxalate crystals are common in plant cells. They may be so numerous that sucking on the stalk of the plant (for example, "dumb cane," or *Dieffenbachia*) makes one unable to talk. The crystals become lodged in the mouth and throat, causing these tissues to swell.

EXTRACELLULAR STRUCTURES

Many types of cells are embedded in a matrix of material produced by the cells themselves. Bone cells are interspersed within a matrix formed chiefly of crystals of an inorganic compound, hydroxyapatite. Ligaments and tendons (the "gristle" of meat) derive their toughness from the substance collagen. The hard external skeleton of insects and other arthropods is composed chiefly of chitin, a polysaccharide that surrounds the cells that synthesize it. Although produced by the cells, these materials are deposited outside the plasma membrane.

Cell walls outside the cell membrane are an important part of the structure of most plant cells. Many aspects of a plant, including its general form as well as its mode of cell division and growth, are determined by the nature of the cell walls.

A young plant cell undergoing division and elongation is surrounded by a single, thin, elastic *primary wall*, about 1 to 3μ thick (Figure 7.28). This primary wall increases greatly in area as the cell grows. In many cells,

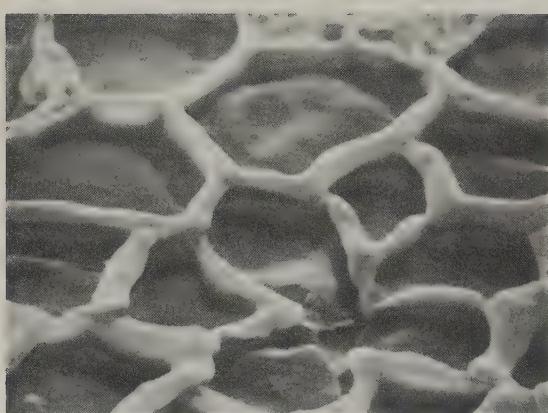




Figure 7.28 (left). Electron micrograph showing a fairly thin cell wall of a plant cell. ($\times 6,000$)



Figure 7.29 (right). Microfibrils in the primary cell wall. ($\times 2,800$)

when growth ceases, a rigid secondary wall about 5 to 10μ thick forms between the cell membrane and the primary wall. Between the primary walls of adjacent cells is a layer called the *middle lamella*, which serves as a matrix to hold the cells together. Through the walls of adjacent cells, there may be passages called *plasmodesmata*, which connect the cells' cytoplasms.

Electron micrographs reveal both primary and secondary walls as a series of layers, with each layer made up of *microfibrils* embedded within a matrix (Figure 7.29). Microfibrils are long chains of cellulose, which is a polymer of glucose. The amount of cellulose varies greatly in different kinds of plant cell walls. Some cells, such as the hair cells of the cotton seed (from which commercial cotton is obtained), have walls of almost pure cellulose. Others, such as the cells at the growing tip of a root, have little cellulose.

Microfibrils can be made up of polymers formed from units other than glucose. Such noncellulose microfibrils are less common than cellulose microfibrils in the walls of most land plant cells, but in some algae they make up the entire cell wall. Many fungi have cell walls made up of chitinous microfibrils.

The matrix between the microfibrils consists of nonfibrous macromolecules that provide flexibility while holding the microfibrils together. A substance called lignin binds the microfibrils together in many types of plant cell walls. In balsa wood, where lignin is absent, the plant tissues usually are brittle. Whereas lignin is present only in some types of plant cell walls, materials called hemicelluloses and pectic substances are present in the matrix of all plant cell walls. Both of these substances are polymers of sugar units; the exact molecular nature of the two substances is unclear. The pectic substances are soluble in hot water and appear to be the chief components of the middle lamellae. Vegetables are easier to chew after cooking because hot water dissolves the pectin of the middle lamellae, allowing the cells to separate. Changes in the cell walls of fruits during ripening also are related to modification of the chemical nature of the middle lamellae.

Pectin forms a thick solution in water and, in the presence of acids and sugars, "sets" to form a gel. Fruits that have a high pectin content can easily

be prepared into jams or jellies. Ripe fruit or fruit poor in pectin (such as strawberries) can be made into jellies by the addition of commercial pectin preparations.

The middle lamellae and primary walls of young plant cells are composed primarily of cellulose, hemicellulose, and pectic substances. All these compounds are macromolecular carbohydrates. As the cells mature, lignin may be added to the matrix of the walls. Lignin is not a carbohydrate, but its detailed molecular structure is not definitely known. Lignin never is found alone in cell walls but is always associated with cellulose. Lignin is most abundant in the secondary wall, particularly in woody plants, but is also found in the primary wall of mature plant cells.

Among other components found in plant cell walls are waxes that provide an impermeable surface for leaves, stems, and fruits. Cell walls of many grasses and plants such as the horsetails contain silica—the major component of sand and glass. Various proteins, which probably play a role in the synthesis and growth of the cell wall, have also been detected.

Current investigations of the process of cell-wall formation have revealed that the cellulose microfibrils form near to, but outside of, the cell membrane in a pattern that is determined by the cytoplasm of the cell. Microtubules just inside the cell membrane apparently play a role in the orientation of microfibrils but are not directly involved in this synthesis.

There is evidence that the Golgi apparatus is involved in the synthesis of the matrix materials of the walls and the middle lamellae. Vesicles pinched off from the Golgi apparatus apparently move to the cell membrane, fuse with the membrane, and deposit their contents in the cell wall. The ER plays a role in cell-wall synthesis in some cells, but its role is far less understood.

In many mature plant cells, particularly in the xylem—or wood—of trees, strong and rigid cell walls of dead cells provide great strength and rigidity. However, not all plant cells have rigid walls. Most plant cells are surrounded only by primary walls or by thin secondary walls, which are not strong enough to retain their shape without the additional rigidity provided by pressure of the cytoplasm and the large central vacuole. The firmness of most nonwoody plant tissues depends upon the balance between the external wall pressure and the countering internal pressure.

In organs and tissues of multicellular animals, cells are held together by various intercellular substances. Hyaluronic acid (a polymer made up of sugar units combined with proteins) is a jellylike material that binds together many animal cells. Some bacteria secrete an enzyme (hyaluronidase) that dissolves this substance and assists the bacteria in penetrating animal tissues. A similar enzyme is secreted by sperm cells, permitting them to penetrate the coat of jellylike substances that surrounds an egg cell.

Skin cells are mounted on a basal lamina made up of layers of fibers of collagen embedded in a matrix. Collagen fibers provide rigidity and strength. Fibers of an elastic protein called elastin are abundant in flexible tissues such as skin and the walls of large arteries.

The process of aging in multicellular animals is intimately involved with changes in intercellular materials. As aging proceeds, more and more collagen fibers are formed between cells, cross-linkages appear between individual fibrils, and the elastin fibers become thicker and less flexible. Thus, the skin becomes less pliant, the joints stiffen, and the muscle tissues become tougher and stringier. In effect, the processes that bind cells together merely continue to form ever more rigid connections between cells until

the rigidity of structure impedes the functioning of the organism. Similar processes occur in plants but are of less hindrance to the organism because the plant needs little mobility.

STRUCTURE AND FUNCTION

As new techniques permit increasingly close investigation of cell structure, more and more parts of the cell are found to be precisely and intricately organized at the molecular level. Specific cellular ultrastructures (structures so small that they are revealed only by electron microscopy) now are known to guide the myriad chemical reactions that must occur in coordinated fashion to maintain cells as living systems.

One noted modern cell biologist recalls that he was told in his introductory biology course that although much remained to be learned about the cell, it was certain that all important biochemical reactions occur in water solutions within the cytoplasm. The use of electron microscopy has drastically changed this view. "Now," he says, "with only slight exaggeration, I can state that it is certain that most important biochemical reactions occur on the surfaces of membranes or within other specialized structures."

Some reactions are known to occur in solution within the cytoplasm, but many reactions do appear to be carried out on membrane surfaces. The study of such reactions poses a great challenge for biochemists.

Although the emphasis of this chapter has been upon cell structure, the topics of cell structure and cell function cannot be separated. The boundaries between the study of cell structure or physiology and the study of cell function or biochemistry are largely disappearing in modern cell biology, or cytology. Biologists studying cells are beginning to approach the goal of explaining living organisms in terms of the chemical processes and physical structures within the cell. The classification of various cellular structures into neat, static categories—as has been done in this chapter—is useful for analysis. However, the living cell—an unresting cell—is constantly changing and carrying out biochemical reactions. Not all cells or cellular structures can readily fit into such neat categories, because these categories fail to represent the dynamic and continuous nature of the cell interior. A listing of cell parts no more completely describes the living cell than does a listing of organs describe a living human. For a more complete picture of the unresting cell, the structural picture of this chapter must be combined with the information about processes summarized in Unit Two and in following chapters of this book.

FURTHER READING

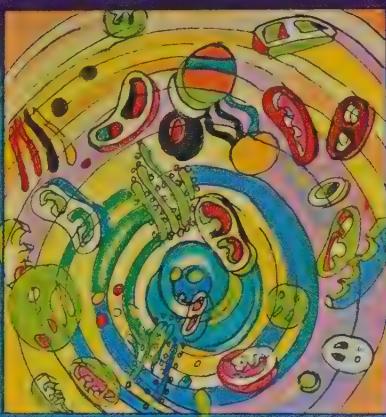
For further details of the history of the cell theory, see books by Hughes (1959), Nordenskiöld (1960), Singer (1959), and Taylor (1963).

There are a great many books available on the structure and function of eucaryotic cells. One of the most complete sources of information (although now somewhat out-of-date) is the six-volume collection edited by Brachet and Mirsky (1959–1964). Among the other useful introductions to cell biology are books by DuPraw (1968), Fawcett (1966), Gerard (1961), Kennedy (1965), Mercer (1962), Stern and Nanney (1965), and Swanson (1969). Buvant (1969), Frey-Wyssling and Mühllethaler (1965), and Jensen (1964) offer more detailed treatment of plant cells.

Further information about electron microscopy and excellent collections of micrographs of cellular structures may be found in books by Jensen and Park (1967) and Ledbetter and Porter (1970).

8

Prokaryotic Cells



Such seemingly diverse phenomena as the multicolored formations in springs and ponds in Yellowstone National Park, colored mainly by blue-green algae, and an epidemic of typhoid fever, brought on by disease-causing bacteria, actually have much in common. The blue-green algae bacteria and mycoplasmas are classified as prokaryotic cells—a fact that distinguishes these organisms from all other living things.

PROKARYOTES AND EUKARYOTES

Although prokaryotes are capable of independent existence, they lack many of the features characteristic of eukaryotic cells. Prokaryotes lack nuclear and intracellular membranes, and the hereditary material (DNA) is not segregated from the rest of the cellular contents by a nuclear membrane, although it may tend to gather in certain areas.

The prokaryotic cells of bacteria, blue-green algae, and mycoplasmas are the smallest and simplest organisms, but each prokaryotic cell is equipped with the necessary biochemical machinery to maintain itself and to divide. Although some prokaryotes attach themselves into groups of cells, they do not form cooperative units in which one cell depends upon others for its survival.

The nucleated eukaryotic cells—which include most of the cells observed by the microscopists who developed the cell theory—are larger and more complex than prokaryotic cells. A single eukaryotic cell—like a prokaryotic cell—may exist independently of other cells as a complete organism. Other kinds of eukaryotes exist in cooperative units of a few dozen cells, forming a colonial organism or a simple animal or plant. The higher animals and plants are made up of many trillions of interdependent eukaryotic cells.

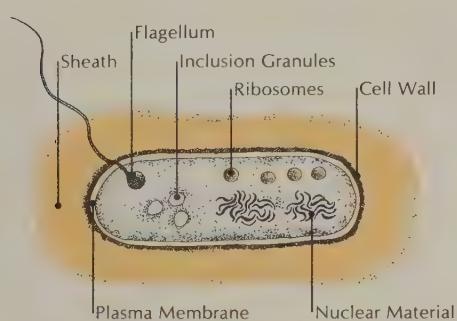
The structural differences between the most complex prokaryotic cell and the simplest eukaryotic cell are far more significant than the differences between cells from an oak tree and from a human. Plants and animals are diverging lines of organisms descended from some common eukaryotic ancestor, and their cells are constructed along the same general eukaryotic patterns. In many external features, a green algal cell and a blue-green algal cell appear to be similar, and, until recently, these groups were lumped together in most classification schemes. In cellular structure, however, it is now clear that the green algal cell is more similar to a human cell than to the prokaryotic blue-green algal cell.

Prokaryotic and eukaryotic cells do have common features. Both are limited at the outer boundary by cell membranes of similar thickness and gross structure, although the chemistry of the membrane differs for the two groups. Both use DNA as the macromolecule that carries hereditary information. Both use RNA as the macromolecule that carries information from the DNA to the ribosomes, which are the sites of protein synthesis. And there is much evidence that both prokaryotic and eukaryotic cells use essentially the same genetic code.

There are other similarities between the two kinds of cells. Most of what scientists have deduced about genetic control mechanisms comes from studies of prokaryotic cells and viruses. The fact that these findings also seem applicable to eukaryotic cells attests to the unity of life in a most compelling way.

Eukaryotic cells are generally regarded as more complex descendants of ancestral prokaryotes (Margulis, 1970). It is unlikely that the eukaryotes

Figure 8.1. Electron micrograph ($\times 40,000$) and diagram of a "typical" prokaryotic cell.



have descended from modern types of prokaryotes, but it does seem that both have descended from a common ancestor.

BACTERIA

The bacteria are a group of prokaryotic microorganisms that are so minute they can barely be seen with the light microscope. Although bacterial cells may be found in attached groups, they are never organized into cooperative, multicellular organisms in which one cell is dependent upon another for its survival. Most bacterial cells are from 2 to 5μ in length, although a few kinds are as long as 100μ and some other kinds as short as 0.2μ .

Very little of bacterial structure is revealed by the light microscope. Based upon their overall shapes, bacteria have been divided into three general groups: the rodlike *bacilli* (singular, *bacillus*), the spherical *cocci* (singular, *coccus*), and the corkscrew-shape *spirilla* (singular, *spirillum*). Other classifications of bacteria are based upon the ways that they are stained and the changes that they produce in the environment. Some types of bacteria possess long whiplike extensions called *flagella*. The rapid lashing of the flagellum propels the organism through the medium.

The light microscope did reveal some details of the processes by which bacteria reproduce. All bacteria reproduce through a process of division called *binary fission*, a continuous action typical of prokaryotic cells (Figure 8.3). A bacterium simply grows larger for a time, then divides across its middle to form two equal daughter cells. The daughter cells then repeat this process of growth and division. The time between bacterial cell divisions can be as short as 20 minutes.

Certain stains demonstrate the presence of nucleic acids and protein in the larger bacteria. Staining reveals that the nucleic acids contained in the dividing bacterial cell are apportioned about equally between the two

Figure 8.2 (above). The three major groups of bacteria based upon their overall shapes. At the upper left are both rodlike *bacilli* and spherical *cocci* bacteria. At the upper middle are predominately *bacilli*, and at the lower middle are the corkscrew-shape *spirilla*. The diagrams of bacteria denote both shape and growth pattern—that is, chains (*streptococcus*), small groupings, or irregular clusters (*staphylococcus*).

Figure 8.3. (lower left). Electron micrograph of a dividing bacterium, *Bacillus subtilis*. Note the two nuclear areas in the two daughters and the cell wall

that is forming between the two. In the diagram of binary fission at lower right, note the lack of an elaborate mitotic apparatus, as is observed in eucaryotic cells. (x 23,000)

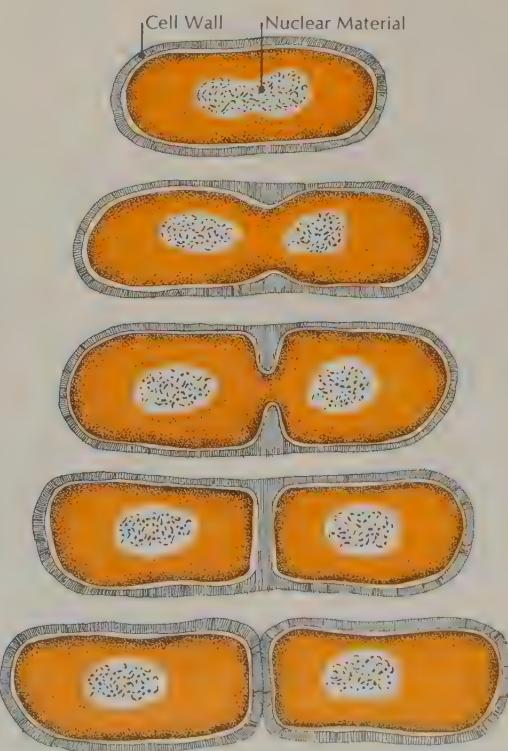
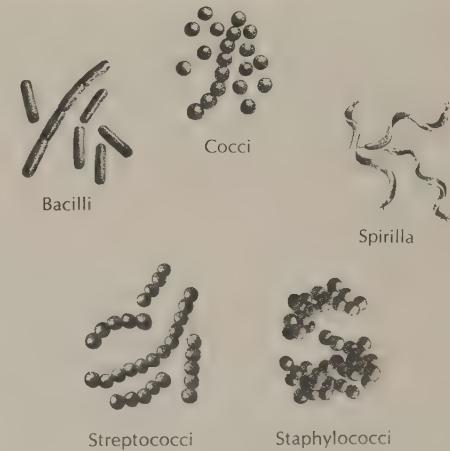
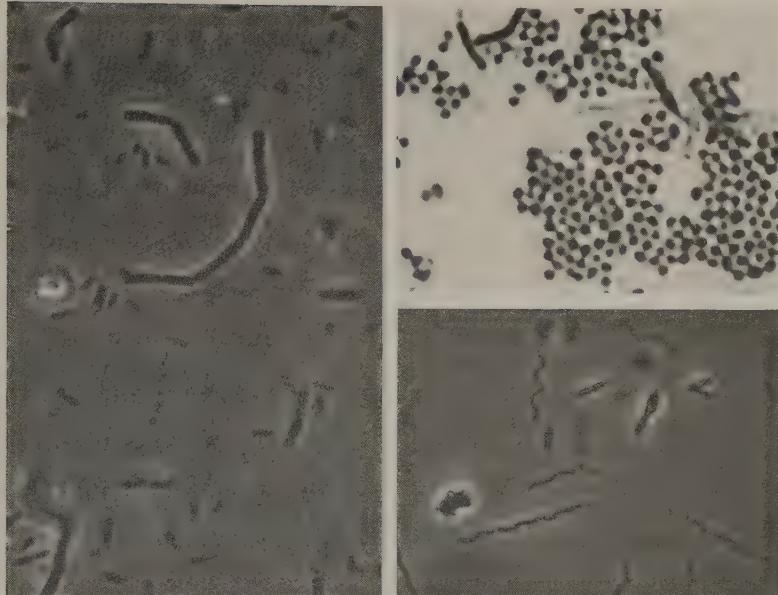


Figure 8.4. An artist's interpretation of a swarm of rapidly dividing bacteria.



Figure 8.5. Electron micrograph of a sporulating bacillus (left). The spore is the large, dark oval at one end of the cell and is surrounded by a clearly visible spore coat. The opaque areas at the other end of the cell are not vacuoles but are areas containing fatty material. In the diagram of spore formation at right, the spore coat is forming around the essential nucleic acids of the bacterium. The remaining portion of the cell disintegrates. Thus, the spore is a "resting stage" in which the vital genetic information of the cell is stored

until favorable conditions for normal function are restored.

daughter cells. However, neither chromosomes nor the other regular structures and processes associated with eucaryotic cell division are visible with the light microscope in a dividing bacterial cell. Bacteria divide very rapidly. If nutrients and space were available for continued division at the 20-minute intervals observed in laboratory cultures, a bacterium could produce offspring of mass greater than the mass of the earth in less than two days.

Some bacteria also reproduce by *budding*, a process in which a much smaller daughter cell is divided from the parent bacterium. Although the daughter cell that buds off receives a very small portion of the cytoplasm of the parent cell, the DNA is divided approximately equally between the parent and the daughter cells.

Spore formation, on the other hand, is not a reproductive process, and it is characteristic of only certain species of bacteria (Figure 8.5). When environmental conditions are unfavorable for survival, the bacterial cell of these species forms a virtually impermeable membrane—a *spore coat*—within its cytoplasm. This coat surrounds the nucleic acids and a small part of the cytoplasm. After the spore coat is formed, the remaining parts of the cell that surround the spore disintegrate. Thus, a spore represents a "resting stage" in which the vital parts of the cell are preserved in a quiescent state until favorable conditions for normal metabolism are restored.

Spores can withstand a temperature of 100° C for several hours in a slightly alkaline solution, whereas bacterial cells are killed almost instantly at this temperature. The existence of these resistant spores played an important role in the controversy over spontaneous generation. Those investigators who happened to use slightly acidic boiling water to sterilize their equipment and nutrients found no bacteria arising afterward. Others who happened to use slightly alkaline solutions did not completely destroy the spores and consequently observed bacteria that seemed to be generated spontaneously.

Exposure to a higher temperature—about 120° C for a few minutes—destroys most bacterial spores. Water cannot be heated to such a temperature

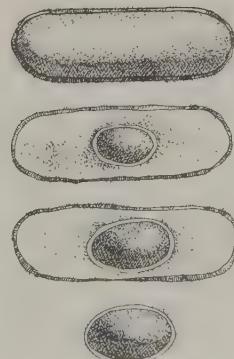
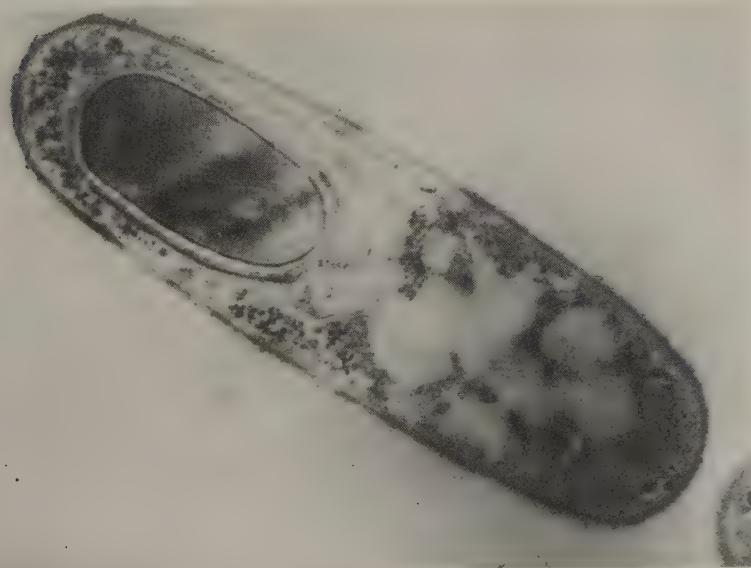


Figure 8.6. Electron micrograph of conjugation in the colon bacteria *Escherichia coli*. In this primitive type of sexual reproduction, genetic material is transferred from a "donor cell" to "a recipient cell," which will later divide by fission. Its daughter cells will possess genetic information from two different parent cells, thus increasing the genetic variation within the population. ($\times 49,000$)



at normal atmospheric pressure, but medical and laboratory equipment can be sterilized effectively in a pressurized device called an autoclave, in which water boils at about 120° C.

The electron microscope reveals that the genetic material—fine filaments of DNA—is clustered in a nuclear area of the bacterial cell but is not surrounded by a nuclear membrane. The DNA, however, does appear to be intimately associated with the membrane surrounding the bacterium. Bacterial cytoplasm contains various organelles, but some kinds of organelles found in eucaryotic cells are missing in bacterial cells. The bacterium is enclosed in a cell membrane that is surrounded by a cell wall. In some species, the cell wall is enveloped by a protective sheath, or capsule. The bacterial cytoplasm contains many ribosomes, which appear in the electron microscope as small dots. The ribosomes are composed of proteins and the nucleic acid RNA. They are often found in clusters called polyribosomes, which are the sites where bacterial proteins are synthesized.

In 1947 E. L. Tatum and his student Joshua Lederberg discovered that some species of bacteria can engage in a form of sexual reproduction called conjugation (Figure 8.6). A fine bridge is formed between the mating cells, and some DNA from the donor cell moves through this bridge into the recipient cell. The recipient cell then reproduces by the usual binary fission. Some of its daughter cells have parts of the DNA from the donor cell

Figure 8.7. Robert Koch (above), a German country doctor, developed many of the bacteriological techniques still in use today; early micrograph (middle) of anthrax bacillus; and stained anthrax bacteria (below).

in place of parts of the original DNA of the recipient cell. Thus, the daughter cells possess genetic information that is a combination of the information from two different parent cells. Such *genetic recombination* within a population creates much greater variation than could arise through random mutations.

Bacteria obtain energy through the catabolism of organic molecules in the environment. Bacteria are responsible for much of the decay of the remains of dead plants and animals, ultimately breaking down the complex organic molecules of the dead organisms into carbon dioxide, ammonia, and water that can be recycled through the biosphere. *Aerobic bacteria* use oxygen and carry out respiratory processes similar to those of eucaryotic organisms. *Anaerobic bacteria* do not require oxygen but make use of processes such as fermentation and glycolysis to obtain their energy.

More than 15,000 species of bacteria are known, many of direct importance to human life. Of particular interest in relation to theories about early life on earth are the *autotrophic bacteria*, which manufacture their own carbon-containing compounds from CO_2 . The *photosynthetic bacteria* use bacteriochlorophyll to capture solar energy; the *chemosynthetic bacteria* derive energy from the oxidation of compounds such as ammonia (NH_3), nitrites (compounds containing the ion NO_2^-), or hydrogen sulfide (H_2S). Far more common are the *heterotrophic bacteria*, which derive their energy from the oxidation of organic molecules and are thus dependent on other organisms for their food supply.

Bacteria as Disease-Causing Organisms

- Early interest in bacteria was centered around the harmful disease-causing organisms. In the late nineteenth century, Louis Pasteur, who is often considered the father of bacteriology, developed the idea that bacteria can cause disease—an idea now known as the germ theory of disease.

The work of Casimir Davaine of France in the 1860s showed that the blood of cattle dying from a disease called anthrax contained large numbers of microscopic, rodlike bodies, which he called bacteridia (the Greek *bakteria* means rod or staff). When Davaine injected a healthy animal with the smallest amount of blood he could prepare from a diseased animal, the animal that received the injection soon developed anthrax. In Germany a few years later, C. J. Eberth showed that bacteria can be filtered from the blood and that anthrax will not develop in a healthy animal injected with filtered, bacteria-free blood from a diseased animal.

In an attempt to halt an outbreak of anthrax among the cattle of his district, a German country doctor named Robert Koch developed methods for the study of bacteria (Figure 8.7). Koch found that anthrax bacteria can be grown in laboratory containers if the bacteria are supplied with the proper nutrients. He found a number of culture media in which the bacteria will thrive. Blood serum (the clear, yellowish fluid that remains after blood clots) proved to be a particularly suitable medium for anthrax bacteria. Koch also devised an incubator to maintain his bacterial cultures at temperatures similar to those of the fluids inside the body.

Koch showed that bacteria growing in the centers of his cultures were separated from one another and grew slowly. Near the surface of the culture, however, where oxygen from the air was abundant, he observed that the bacteria grew longer and joined end-to-end in long threads. Wherever these bacterial threads contacted the air, they were transformed into

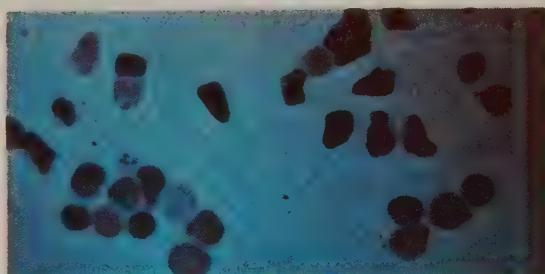
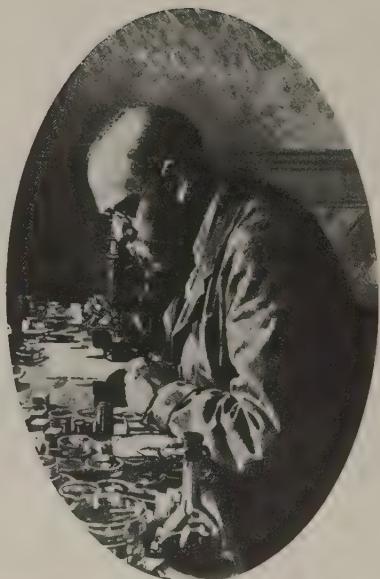


Figure 8.8. Photograph of a bacteria culture of *Clostridium botulinus* grown on an egg yolk medium. Botulism toxin in exceedingly small amounts can produce the deadly food poisoning called botulism.

minute, nongrowing, round bodies, which Koch called spores. The bacterial spores changed back again into bacterial cells when they were transferred to a fresh culture medium. Koch showed that bacterial spores are extremely resistant to damage by drying, heating, or chemical treatment.

In the 1870s, when Koch was beginning his work on bacteria, the newly established German synthetic dye industry was turning out many new dyes. Koch found several dyes that can be used to stain bacteria, increasing their visibility under the light microscope. He also developed a method for the preparation of pure cultures of a single type of bacteria. The fluid that contains the bacteria is repeatedly diluted until each drop of the final dilution contains only a few microorganisms. With a small dropper or hypodermic needle, minute amounts of this fluid are spotted onto the surface of a transparent, jellylike nutrient medium. At each spot where a single bacterium has been deposited and has reproduced, a colony of that particular type of bacteria is formed. This procedure is known as *cloning*. The techniques of bacterial staining and solid-medium culturing developed by Koch have not been greatly changed over the years, and similar techniques are still used by bacteriologists.

The bacteria most familiar to the layman are those that cause diseases in man, in his domestic animals, and in his cultivated plants. Among the diseases caused by bacterial invasion of the human body are typhoid fever, cholera, plague, dysentery, scarlet fever, diphtheria, tuberculosis, and wound infections such as gangrene.

Bacterial invasion of the human body produces the disease state in a number of ways. In some cases, the invading bacteria become so numerous that they successfully compete with the host cells for nutrients and oxygen. In other cases, the bacteria produce a poison, or *toxin*, that disrupts the normal processes of the host. The deadly food poisoning called botulism is caused by an extremely lethal toxin. It has been estimated that as little as 3.5×10^{-7} grams of botulinus toxin is sufficient to kill a human. From this information, one can calculate that a little more than one kilogram of the pure toxin would be sufficient to eradicate the population of the earth.

But, even from the prejudiced human viewpoint, bacteria do far more good than harm. Bacteria play key roles in the production of buttermilk,



Figure 8.9a (above) Photograph of the blue-green alga *Nostoc*. (Courtesy Carolina Biological Supply Company)

Figure 8.9b (below). Filamentous forms of blue-green algae. The upper filament represents the species *Spirulina versicolor*, and the lower two filaments belong to the genus *Arthospira*. In flamingos, *Arthospira* directly contributes to the birds' pink color; the pigment comes from carotenes in the blue-green algae that make up part of the birds' diet.

cheese, vinegar, and other foodstuffs. They also play key roles in modern sewage disposal plants. Bacteria are used in various industrial procedures, such as removing hairs in the preparation of leather. In promoting the decay of dead organisms, bacteria form a vital link in the carbon and nitrogen cycles of the biosphere. The bacteria help to break down the complex organic molecules and to restore carbon and nitrogen to the ecosystem in the form of simple inorganic molecules that can be used by plants. The human intestinal tract contains a bacterial population essential for normal health. Among other things, these intestinal bacteria synthesize vitamin K, which is required by the human organism for normal blood clotting.

Several methods are used to keep harmful or decay-causing bacteria out of human foodstuffs. Because the metabolic activity of bacterial cells is enzymatically regulated, it is extremely slow or completely stopped at low temperatures. Refrigeration, therefore, is an effective method of slowing food decay. Drying is an effective means of food preservation because many bacterial cells are destroyed by extreme dehydration and because their enzymes are inactive in the absence of water. Salting is effective in food preservation for the same reason.

Various organic chemicals that kill or inhibit bacteria are useful in food preservation. Many of these antibacterial chemicals are produced as waste products of fermentation by various microorganisms. Thus, wine is preserved by alcohol, sauerkraut by lactic acid, and cheese by lactic and propionic acids. In each case, the inhibitory chemicals are formed during the fermentation process that produces the particular foodstuff. Other antibacterial chemicals are artificially added to most modern packaged foods.

Sterilization by heating in a pressure cooker or autoclave destroys bacterial cells and spores and their enzymes. Home-canned foods can be dangerous because simple boiling at atmospheric pressure does not destroy spores, particularly in alkaline foods.

BLUE-GREEN ALGAE

Blue-green algae represent the other prominent group of prokaryotic organisms. As in the bacterial cell, the DNA of a blue-green algal cell is localized in a nuclear area or "nucleoid," but no nuclear membrane surrounds it. Reproduction occurs only through binary fission. All of the 1,500 or so known species of this group are photosynthetic. All species of blue-green algae contain the photosynthetic pigments chlorophyll a and phycocyanin (a blue pigment). Many species do contain additional accessory pigments of various colors, but none contains other forms of chlorophyll. The pigments are arranged on infoldings of the cytoplasm, making the algal cell interior appear more highly structured than the bacterial cell interior.

The blue-green algal cell is surrounded by a cell wall of cellulose. In many species, the outer portion of the cell wall becomes covered with a slimy substance, which sometimes is present as a very thick layer. This slime layer apparently protects the cell from dehydration and facilitates intercellular interaction and filament formation.

Some species form filaments in which a number of cells are joined together. In a few species, the filaments show slow, twisting movements, but the mechanism by which this motion is accomplished is unknown. Many species can live in moist earth, surviving long dry spells by forming resistant resting spores. Some species survive at temperatures as high as 70° C and are found growing in hot springs. The multicolored formations in springs

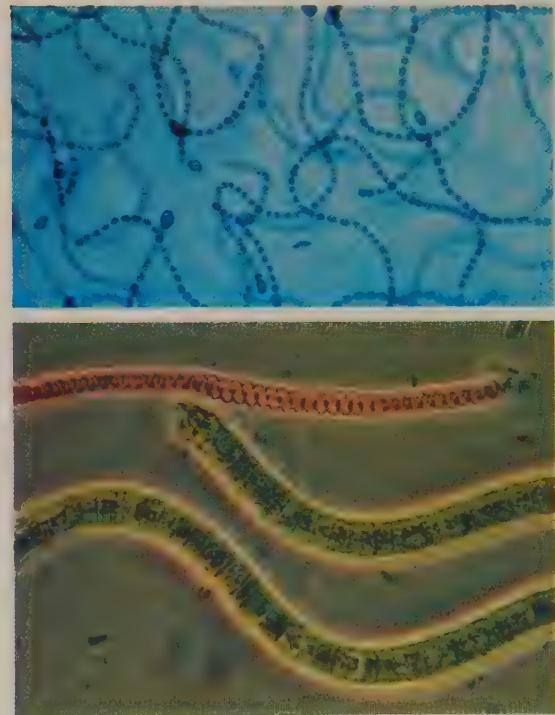
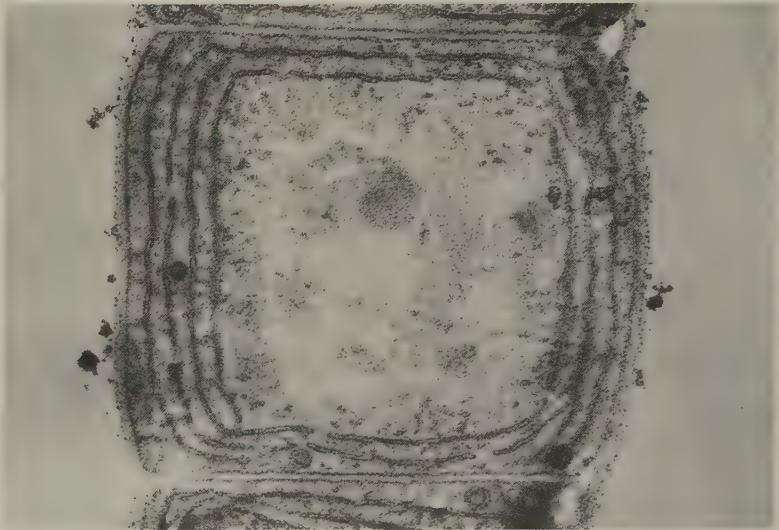
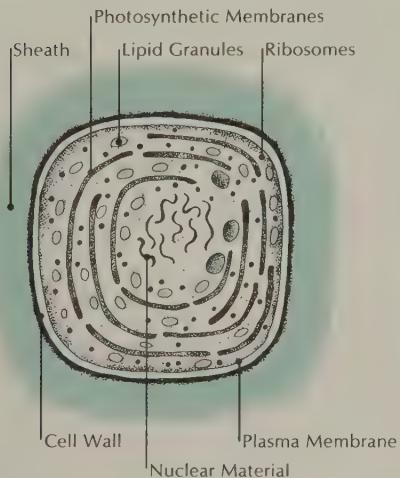


Figure 8.10 (above left). Diagram of the general structure of a blue-green alga. Note that the nuclear material, composed of DNA fibrils, is localized in a nuclear region but remains unbounded by a nuclear membrane. Shown at the upper right is an electron micrograph of the blue-green alga *Anabaena*. Clearly visible are the cell wall and the layers of photosynthetic membranes. ($\times 6,800$)

Figure 8.11 (below). Life cycle of the pleuropneumonialike organism (PPLO) *Mycoplasma*

laidlarvii. Note the double-track system of reproduction. Large cells may reproduce with or without the formation of elementary bodies. (From "The Smallest Living Cells" by H. J. Morowitz and M. E. Tourtellotte. © 1962 by Scientific American, Inc.)



and ponds in Yellowstone National Park are colored mainly by various species of blue-green algae containing a wide variety of accessory pigments.

The first photosynthetic organisms may have been similar to modern photosynthetic bacteria or blue-green algae. Some of these early organisms, incapable of adapting to the increasing concentration of free oxygen produced in the atmosphere by photosynthetic activities, may have developed the ability to invade other cells and to derive nourishment from the material of the host cell's cytoplasm. Eventually, according to this hypothesis, these invaders lost most of their cellular components and the ability to survive independently. At the same time, the host cells evolved a dependency on the symbionts as a source of carbohydrates formed through photosynthesis. A true mutualistic relation, or *symbiosis*, was the result—the ancestor of the modern photosynthetic eucaryotic cell whose photosynthetic pigments are grouped in chloroplasts.

MYCOPLASMAS

The smallest known cells belong to a group called the pleuropneumonialike organisms (PPLO), or mycoplasmas. The existence of extraordinarily minute organisms responsible for pleuropneumonia, a highly contagious disease of cattle, was suggested by Louis Pasteur. However, early investigators found that these organisms could not be trapped in porcelain filters that remove bacterial cells from blood. In 1898 E. I. E. Nocard and P. P. E. Roux succeeded in growing pleuropneumonia organisms in a complex medium, demonstrating that they are not dependent on larger cells for their survival. It was not until 1931 that W. J. Elford developed a set of special filters that revealed the pleuropneumonia organisms to be about 0.13 to 0.15μ in diameter, about a tenth the size of a typical bacterium.

About 30 different species of PPLO have been identified, ranging in diameter from 0.1 to 0.25μ (Figure 8.11). One species causes a form of human pneumonia. Harold Morowitz and Mark Tourtellotte (1962) studied one of the smallest of the PPLO, an organism that normally lives free rather than invading larger organisms. This species forms elementary bodies that are spherical and about 0.1μ in diameter. During its life cycle of a few days,

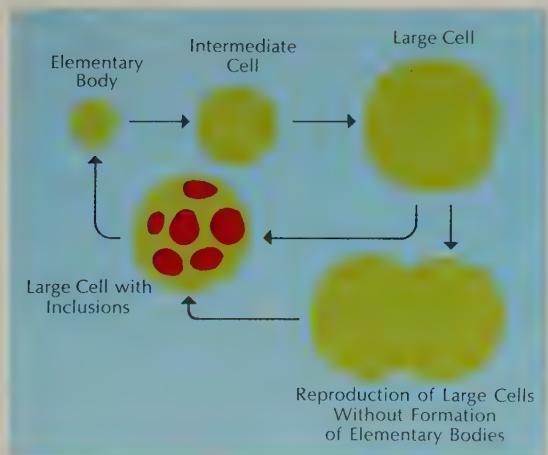


Figure 8.12. A model diagram illustrating the organization of *Mycoplasma gallisepticum*. Chemical analysis has revealed the presence of DNA in the form of a double helix and RNA in ribosomal form. In addition, the cell contains lipids similar to those found in animal cells and has a flexible membrane similar to the plasma membrane of animal cells.

the organism becomes larger and forms a cell about 1μ in diameter. The large cells may divide to form other large cells, or within themselves they may develop small bodies, which are released as elementary bodies.

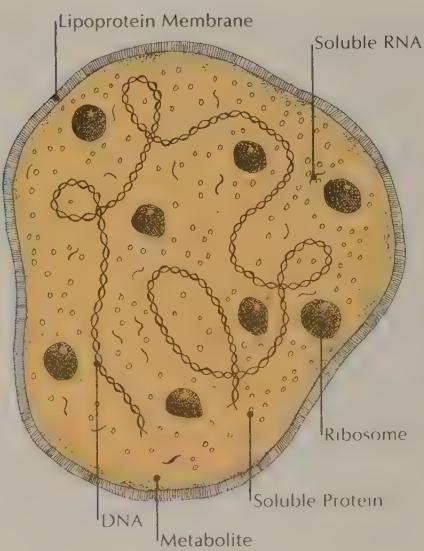
Morowitz and Tourtellotte also studied another PPLO that causes a respiratory disease in poultry. This organism is found as a cell about 0.25μ in diameter and does not appear to pass through a life cycle involving changes in size. Chemical analysis shows that the cell contains both DNA and RNA. The DNA is in the form of double helices, and the RNA is present chiefly as ribosomes. Various proteins are found in the cytoplasm of this PPLO cell. The cell contains lipids similar to those found in animal cells and has a flexible membrane about 100 Å in thickness, also similar to the plasma membrane of animal cells. Although the electron microscope reveals little of the internal structure of the cell, Morowitz and Tourtellotte were able to propose a model based upon their chemical studies (Figure 8.12).

Mycoplasmas, as these minute organisms are now called, may not be the smallest cells. If smaller organisms exist, they may have escaped detection by methods now in use. Yet no cell could be very much smaller than the mycoplasma. Because cell membranes are about 100 Å (0.01μ) in thickness, it does not seem possible for a cell to be less than about 0.025μ in diameter, or about one-tenth the size of the mycoplasma. In order to contain the protein molecules necessary for metabolic reactions, Morowitz and Tourtellotte estimated that a cell would need a diameter of at least 0.04μ , or little less than half the size of the smallest mycoplasmas.

The prokaryotic cells represent the simplest and smallest organisms yet known to be capable of independent survival in suitable environments. Even the smallest myoplasmic cell is far more complex than the very simple DNA-enzyme system postulated to have been the first step in the evolution of living systems. The prokaryotes may be descendants of organisms developed relatively early in evolution (but long after the first living systems). Or they may be descendants of organisms developed much later in evolution through simplification of more complex organisms. In any case, they represent the smallest and simplest living systems known to exist in the modern world. Mycoplasmas cannot be much larger than the smallest possible cell. If smaller and simpler living systems do exist, they cannot be based upon the common cellular structure that pervades all of life.

FURTHER READING

More information about bacteria and blue-green algae will be found in books by Gunsalus and Stanier (1960–1964), Jacob and Wollman (1961), Simon (1963), Sistrom (1969), Stanier, Doudoroff, and Adelberg (1970), and Thimann (1963); also articles by Braude (1964), Cairns (1966), Delbrück and Delbrück (1948), Echlin (1966), Hotchkiss and Weiss (1956), Wollman and Jacob (1956), and Wood (1951).



9 Viruses



Viruses have three important characteristics. First, they are very small. As many as 20,000 viruses could be fitted comfortably inside a small bacterial cell. Some large protein molecules are larger than some of the smaller virus particles. Second, viruses are structurally simple. In fact, there is little room for complexity. Simple viruses are nothing more than DNA or RNA packed inside a protective protein coating. Many viruses in purified form can crystallize just as do pure proteins. Third, viruses cannot reproduce outside of cells. When free, a virus is inert. It has none of the characteristics of a living system. It is no more alive than is the material inside a bottle of organic chemicals on a laboratory shelf. When the virus invades a cell, however, it is able to take over the metabolic machinery and to direct the cell's machinery to produce new virus particles instead of carrying out the normal cellular processes. In most cases, this takeover results in the death of the cell, which is ruptured as the newly made virus particles escape into the environment.

The debate about whether viruses are living organisms did not end with the discovery of their chemical nature and structure. In light of the characteristics described above, the question remains: Are viruses alive or not? Attempts to answer this question have not proven very fruitful, and biologists now accept the viruses as unique kinds of biological systems—neither living nor nonliving but as a sort of bridge between the two categories.

Every cell—even the simplest mycoplasma—has both DNA and RNA, ribosomes, thousands of enzymes, and an outer cell membrane that regulates the entry and exit of water, salts, foods, gases, and other components involved in metabolism. Such a cell is truly alive and independently self-sufficient. In contrast, a virus is an incomplete biological entity—a stripped-down structure usually containing only one kind of nucleic acid—either DNA or RNA, either single- or double-stranded—and a protein coat. Viruses are tiny packages of genetic information—or “genetic vectors.”

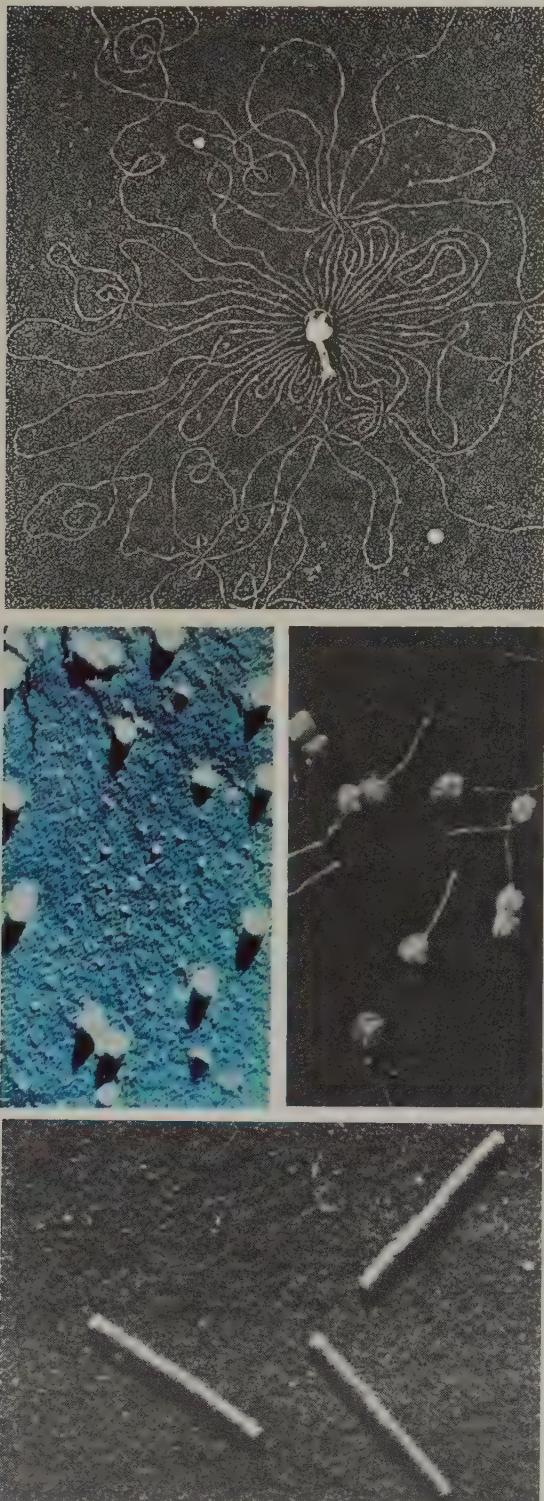
The nucleic acid of the virus carries genetic information that directs the takeover of a host cell and the production of new viruses. Like the genetic information of a cell, the viral nucleic acid is subject to mutational changes. The protein coat of the virus serves two major functions. First, it protects the viral nucleic acid from enzymes such as DNase and RNase, which are abundant in organisms and would destroy bare nucleic acid molecules. Second, by showing a specific affinity for the surfaces of certain kinds of host cells, the protein coat permits the virus to attach itself only to appropriate host cells and not to other kinds of cells within which it could not reproduce. In other words, the protein coat determines the *host range* of the virus.

The simplicity and specificity of a virus make it a remarkable research tool for molecular biologists. It can be regarded as a naturally occurring hypodermic that can inject nucleic acid into a particular kind of cell. Used in this way, viruses have revealed a great deal of information about the genetic mechanisms of cells.

Viruses exist in a wide range of sizes, structures, and types. They vary in size from poliovirus (about 100 Å in diameter) to rickettsia and other large disease-causing viruses (about 0.5 μ in diameter). Most viruses are spherical, but some are bricklike, rodlike, cubic, or irregular in shape (Figure 9.2). Some contain DNA, whereas others contain RNA; a few contain both kinds of nucleic acids. Some viruses have a coat made of a single protein, whereas others have coats made of many proteins. Some viruses contain lipid and

Figure 9.1 (above). A T-2 *E. coli* bacteriophage osmotically shocked. The long strands are DNA. ($\times 50,000$)

Figure 9.2. Examples of the shapes and sizes of viruses. Influenza virus (middle left); T-5 (middle right); and tobacco mosaic virus particles (below).



carbohydrate components in addition to their nucleic acid and protein. Cells of nearly every type of organism—from bacteria to plants, insects, and animals—are susceptible to attack by some type of virus.

TMV

The tobacco mosaic virus (TMV) is typical of the rod-shape viruses that attack plant cells (Figure 9.3). It is composed of a coiled molecule of RNA, surrounded by a protein coat, or *capsid*, made up of many identical protein subunits, or *capsomeres*. The entire particle, containing nucleic acid and capsid, is known as a *virion*.

In the mid-1950s, Heinz Fraenkel-Conrat and his colleagues separated TMV into its RNA core and protein overcoat components. These separated parts were then recombined to form infectious viral particles. This study showed that the virion is formed through a process of self-assembly. Under proper conditions of acidity, temperature, and salt concentration, capsomeres spontaneously gather around the viral RNA core to form a rod-shape virion. In another part of this study, it was demonstrated that both the RNA core and the protein coat must be present in order to produce fully infective virus particles (Fraenkel-Conrat and Williams, 1955).

In 1957 groups in both Berkeley and Germany independently reported that the RNA core of TMV, stripped of its protein coat, is still slightly infectious if rubbed into tobacco plant leaves. Typical lesions (wounds) develop on the leaves, and the infected cells produce normal virions containing both RNA and protein (Figure 9.4). The viral RNA is less than 1 percent as infectious as the normal TMV virion, but these experiments show that the RNA molecule alone contains all the genetic information necessary to infect a cell and to cause production of complete new virions. This study corroborated earlier virus and cell experiments that had demonstrated that genetic information is carried by nucleic acids alone.

The single-stranded RNA molecule of TMV contains about 7,000 nucleotides—enough to code about five to ten viral proteins. Obviously, one of these proteins must be the capsid protein of the virion. There are several different strains of TMV, each of which makes a slightly different capsid protein. After cell infection, the new virions that are formed always have capsid proteins similar to those of the parental virus, regardless of the genetic make-up of the host cell. Therefore, the information that specifies the amino acid sequence of the capsid protein must be stored in the viral RNA, not in the genetic molecules of the host cell.

The nucleic acid core of the TMV particle consists of a single strand of RNA. In order to reproduce itself, this RNA strand must serve as a template for the transcription of a complementary RNA strand. Such a process of RNA replication does not normally occur in cells and is apparently accomplished by an RNA replicase enzyme that must be one of the other proteins coded by the viral RNA. Thus, the first step after the viral RNA enters the cell must be production of the RNA replicase through the use of the cell's ribosomes to translate the viral RNA. After the replicase is formed, the viral RNA can be duplicated, and production of capsid and other proteins that assist in the takeover of the cellular machinery proceeds more rapidly.

As the capsid protein molecules are formed, they spontaneously fold into the uniquely shaped capsomeres. These capsomeres tend to aggregate into disc-shape units, which are aggregated to form the rodlike capsid around an RNA molecule. In the absence of viral RNA, capsomeres can be

Figure 9.3. Sequence of steps in the assembly of TMV from its RNA and protein subunits. A typical TMV has a rod shape and is composed of a coiled molecule of RNA (yellow) surrounded by a protein coat, or capsid, made up of many identical protein subunits, or capsomeres (blue). The entire particle is known as a virion.



Figure 9.4 (above). A bottle containing TMV crystals frozen by W. M. Stanley in 1935. When rubbed into a tobacco plant, the crystals still cause infection.

Figure 9.5 (below). Diagram and electron micrograph of a T-even bacteriophage. ($\times 420,000$)



induced to aggregate into three different kinds of rodlike structures. Around the viral RNA, however, the capsomeres spontaneously form only the structure unique to the TMV virion (Durham, 1971).

A final confirmation of the role of viral RNA in capsid protein synthesis came from studies in which mutations were induced chemically. A change of a single base among the 7,000 bases in the viral RNA molecule may cause a corresponding change of a single amino acid in the capsid protein. Many of these mutations profoundly affect the biological behavior and infectivity of the mutant viruses, suggesting that the folding of the capsid protein can be altered by the change of a single amino acid in its sequence.

BACTERIOPHAGE

In 1915 an English bacteriologist, Frederick William Twort, was dismayed to find that a number of his bacterial colonies were dying. The cells of the bacteria ruptured, leaving nothing but a foggy liquid. He discovered that this liquid infected other bacterial cells, even after filtration. Thus, Twort announced the discovery of viruses that infect bacteria. This discovery was

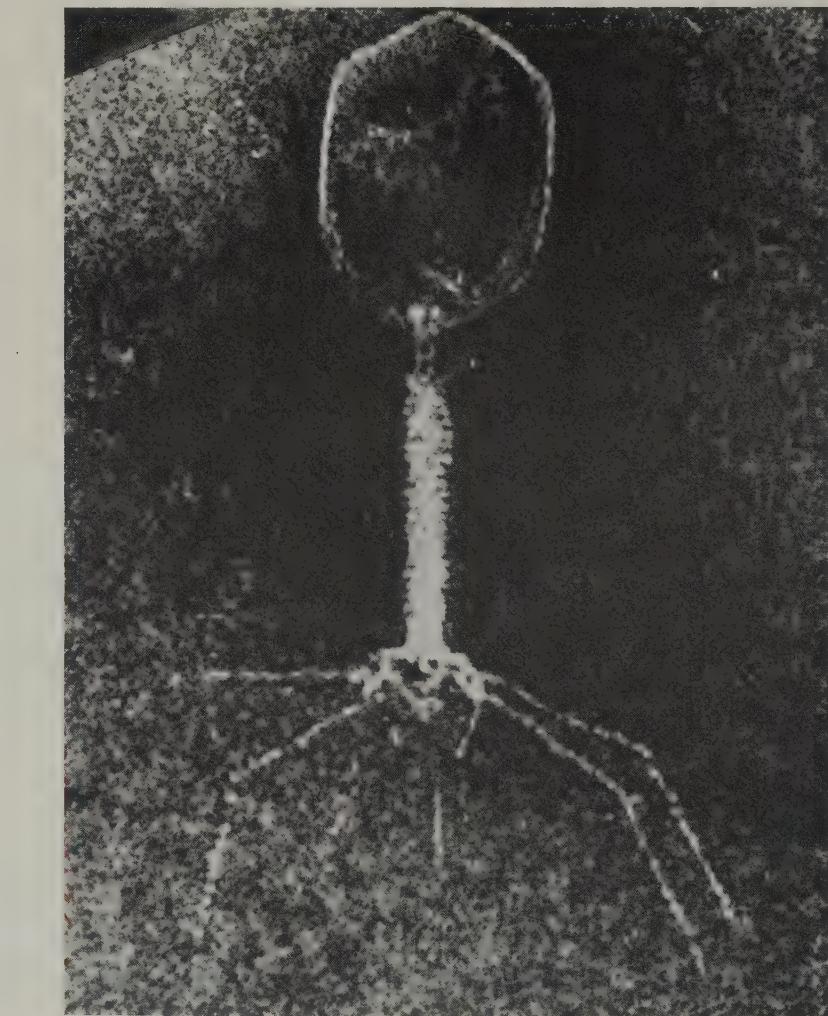


Figure 9.6 (above). T-4 bacteriophages adsorbed on an *E. coli* B cell wall. Phages are connected to the bacterial cell surfaces by short tail fibers, which will eventually penetrate the cell wall.

Figure 9.7 (below). Electron micrograph of a T-4 infected *E. coli*. The bacteriophage protein remains as an empty "ghost" attached to the outer surface of the cell wall. ($\times 55,000$)

independently repeated two years later by the Canadian bacteriologist Félix Hubert d'Hérelle, who named the viruses *bacteriophage* (bacteria eaters). Although Twort emerged the victor in the subsequent dispute about who should get credit for first discovering bacterial viruses, d'Hérelle's name for the viruses is still used.

The DNA-containing bacteriophage—often called phage by biologists who work with them—have been of enormous help in understanding gene action. One family of phage that infect the bacterium *Escherichia coli*—a common subject for laboratory studies—is generally designated by strain as T-1, T-2, T-3, and so on. The T-even strains (T-2, T-4, T-6, and so on) are *virulent* particles that kill the host bacterial cell. Expression of the DNA in T-even bacteriophage now is better understood than the gene action of any other virus or of any organism.

A typical particle of a T-even phage has a large hexagonal head, which contains its genetic material—double-stranded DNA (Figure 9.5). The DNA must be tightly coiled within the head, for it is a long molecule with a molecular weight of about 120 million—long enough to carry information for the production of more than 100 average-size proteins. The protein coat of the head is composed of subunits, each with a molecular weight of about 80,000. Associated with the DNA inside the head is an internal protein, which probably helps to hold the DNA in a tightly coiled position. At the base of the head is a protein collar with a tail assembly attached. The intricate phage tail consists of a sheath of 144 contractile protein subunits wrapped around a hollow protein core. The top of the core-sheath complex is attached to the collar region of the head. The bottom of this complex is attached to a flat, hexagonal base plate, which has six spikes protruding from it. Extending from these spikes are long, kinked tail fibers made up of several different proteins.

This elaborate structure acts as a microsyringe to inject its viral DNA through the tough polysaccharide cell wall of the bacterial host cell. To infect a bacterial cell, a T-even phage must make contact with it (Figure 9.6). The phage, with no mechanism for moving itself, is carried along in the random thermal motion of molecules and other submicroscopic particles. Eventually, the phage happens to bump into a host cell that possesses appropriate protein receptor sites on its surface. The tail fibers of the phage "recognize" the receptor sites and attach themselves by weak, noncovalent bonds. Inappropriate species of bacteria do not possess suitable receptor sites, and the phage does not attach itself to such a cell.

Although the attachment process is reversible, what immediately follows is not. An enzyme produced by the attached phage digests a small hole in the tough bacterial cell wall. The contractile sheath of the phage tail then contracts (possibly using energy provided by hydrolysis of bound ATP), the head of the phage collapses, and the DNA contents of the head are extruded through the tail into the cytoplasm of the host cell. Only the viral DNA and a small amount of its internal protein enter the bacterial cell. Most of the phage protein remains as an empty "ghost" on the outer surface of the cell wall (Figure 9.7).

Before injection of the viral DNA, the biochemical activities of the *E. coli* cell are governed by its own chromosome, a single, long, circular molecule of DNA. After injection, the bacterial cell contains an additional chromosome—the viral DNA. The consequences of this extra chromosome are disastrous for the bacterial cell. In less than 30 minutes, the injected

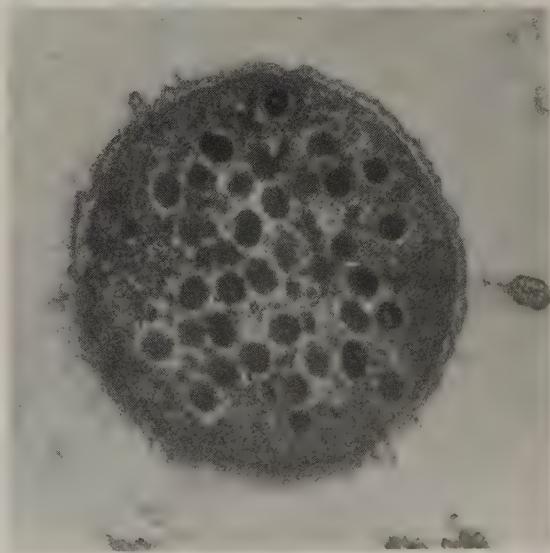
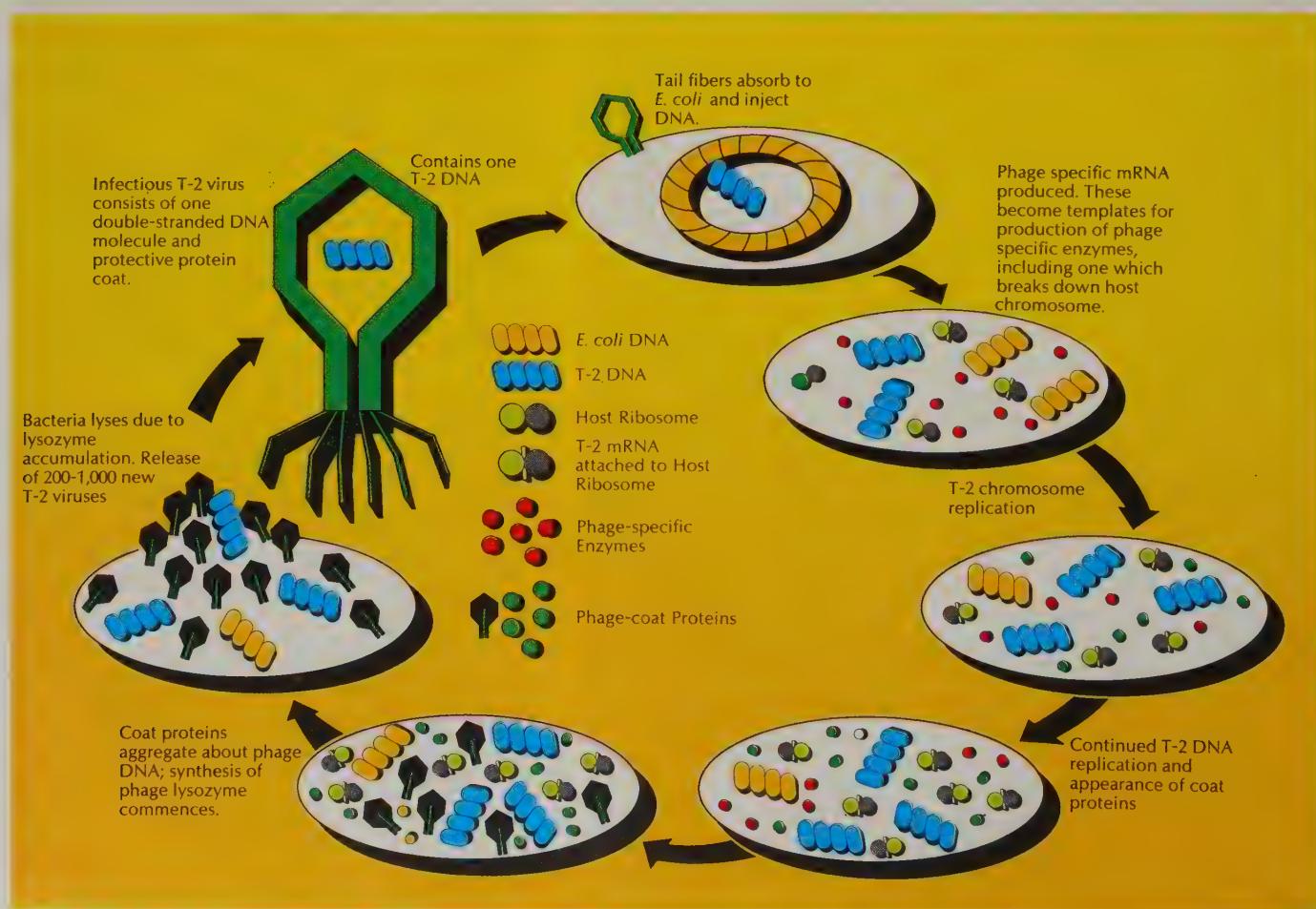


Figure 9.8. Life cycle of the double-stranded T-2 virus.
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of the Gene, © 1965, J. D. Watson

viral DNA takes over the direction of the cellular mechanism and directs the production of 100 or more new viruses. The release of the newly produced viruses is brought about by the disruption and death of the host cell (Figure 9.8).

The sequence of events during production of new phage particles is quite well known. Immediately after the viral DNA enters the cell, it interacts with the host cell's RNA polymerase and ribonucleotide precursors to make early viral mRNA. From this mRNA, the ribosomes and enzymes of the host cell produce early viral enzymes and other proteins that are necessary for replication of the viral DNA.

The viral DNA differs from the bacterial DNA in that it has a base called 5-hydroxy-methylcytosine in place of normal cytosine. Glucose molecules are attached to this unusual base (Figure 9.9). Among the early viral enzymes produced are DNases that attack the bacterial DNA but do not degrade the viral DNA. Apparently, the modified cytosine of the viral DNA protects it from the action of these enzymes. Other early viral enzymes include RNases that help break down the RNA of the host cell, and enzymes that convert cytosine to 5-hydroxy-methylcytosine. The latter process prevents the replication of host cell DNA and promotes the replication of viral



DNA. The DNA of the host cell is broken down by the DNase into its nucleotide subunits, which then are assembled by another early enzyme (a specific DNA polymerase) and produce about 100 copies of the viral DNA.

Although the viral DNA contains specifications for about 100 viral proteins, only a few of these are produced in the early stage of viral infection. The various parts of the viral DNA are activated to produce mRNA in a regular time sequence. Thus, various viral proteins are produced as needed in an assembly-line sequence, culminating in the production and release of complete phage particles.

Many control processes are needed to achieve this time sequence. For example, the host RNA polymerase has a control protein (sigma factor) that causes the polymerase to attach to certain sites on the host DNA. One of the proteins produced by the viral DNA inactivates the sigma factor of host polymerase molecules. Apparently, these sigma factors are replaced by new control proteins that are synthesized according to information in the viral DNA. The viral sigma factors then combine with and modify the host RNA polymerase so that the polymerase will activate synthesis of mRNA from parts of the viral DNA that become active late in the viral replication sequence.

Other viral enzymes guide the production of new tRNA molecules at appropriate times. It even appears that some viral proteins modify host ribosomes to make them suitable for viral protein synthesis.

Finally, as the synthesis of copies of viral DNA is completed, the late viral proteins are produced. These include internal protein and the proteins needed to build the head and tail assemblies of the 100 or more new viruses. The process of phage assembly was studied by R. S. Edgar and his colleagues, who demonstrated that this complex structure is produced through a process of self-assembly (Wood and Edgar, 1967).

The studies were carried out by using many phage mutants, each strain being defective in one essential viral protein. Each of these mutant strains is unable to complete the production of phage particles. Because a specific protein that is necessary for the next step is missing, the assembly of phage particles comes to a halt at some intermediate stage.

Suppose, for example, that two defective mutant phage strains are unable to complete the production of the head unit of the phage particle. Cultures of strain A produce incomplete head structures (H_A), whereas cultures of strain B produce different incomplete head structures (H_B). When extracts of the two strains are mixed together, however, complete phage head units are formed.

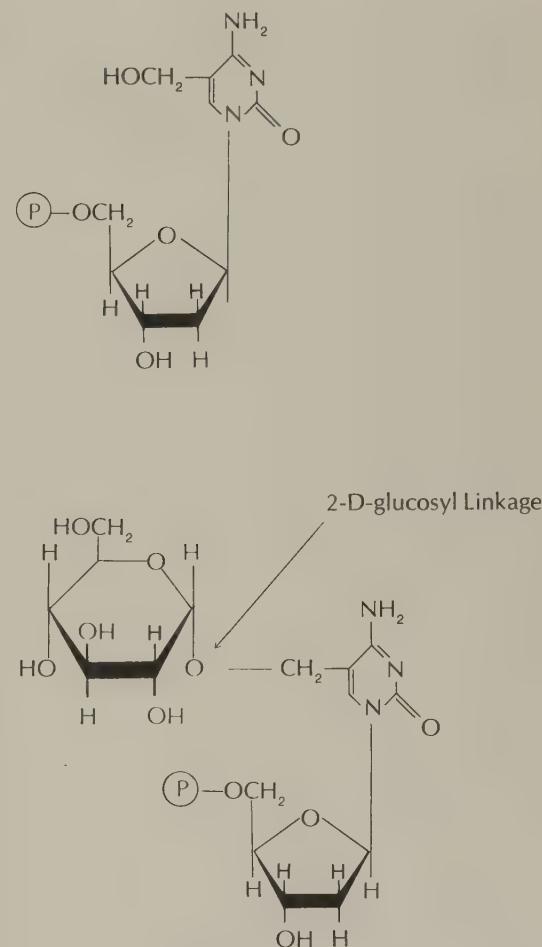
Apparently, H_A and H_B are precursors of the normal head structure:



One mutant strain lacks protein I and therefore halts assembly with precursor I. The other strain lacks protein II and halts production with precursor II. When extracts of the two strains are mixed, both proteins are present and normal head assembly can be completed.

Is H_A precursor I or precursor II? Does strain A lack protein I or protein II? In some cases, these questions can be answered by examination with the electron microscope—one precursor may be more similar to a completed head structure than the other. If the phage assembly sequence cannot be

Figure 9.9. Structural formulae showing the base 5-hydroxy-methylcytosine (above) and the same base with a glucose molecule attached (below). Viral DNA contains this base instead of the normal cytosine.



determined by electron microscopy, it can be determined by experimental tests. H_A can be isolated from strain A and added to extracts of strain B. If H_A is precursor I, strain B will possess protein I and lack protein II. Therefore, the H_A will be converted to H_B , but assembly will stop there. On the other hand, if H_A is precursor II, strain B will lack protein I and possess protein II. Therefore, the H_A will be converted into complete head structures.

After many such experiments, Edgar and his colleagues determined the sequence in which the phage particle is assembled. They discovered not a single linear assembly line but several subassembly lines converging to the final phage particle. Different proteins, produced by different parts of the viral DNA, control each assembly step.

Most of the information of the viral DNA codes for structural proteins, but apparently some of the information leads to production of proteins that act as catalysts (enzymes) for the assembly process. There is a head subassembly line, a tail subassembly line, and still another subassembly line for tail fibers. When completed heads encounter tails that are complete except for tail fibers, the two join together spontaneously. Note that this joining occurs only when each of the separate parts is completed. Finally, when the head-tail assemblies encounter completed tail fibers, the fibers attach to the base-plate spikes to complete the formation of fully infectious phage particles.

When the new phage particles are completed, the last step of viral infection, *cell lysis*, takes place. A very late portion of the viral DNA codes for the production of the enzyme lysozyme, which breaks down the rigid cell wall of the bacterium. With the bursting of the bacterial wall, hundreds of new viruses are released into the medium, where they eventually collide with new host cells to trigger a new cycle of infection and viral replication.

ANIMAL VIRUSES

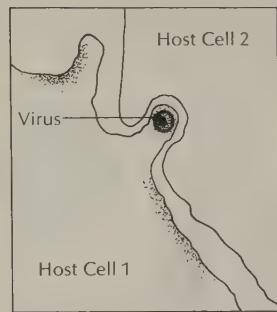
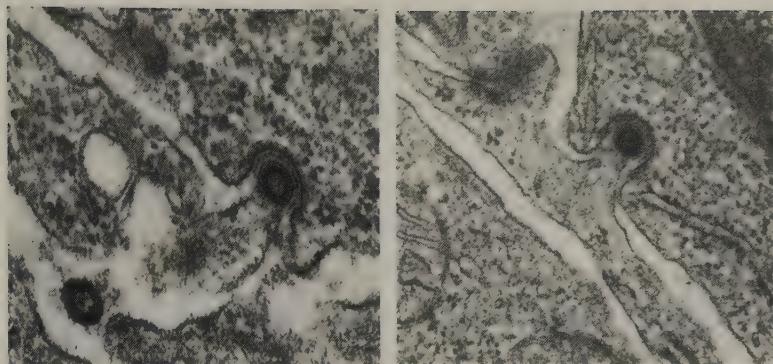
Most viruses that attack human and other animal cells reproduce in a fashion similar to the phages, producing virus particles with an exposed protein coat surrounding a DNA or RNA core. With some viruses, however, the protein coat is enclosed by a membrane derived from the host cell. The cell surface buds off to form a lipid-protein coating over the nucleic acid–protein virus particle. Among the viruses with such membrane coatings are the DNA-containing herpes virus and the RNA-containing influenza virus.

Some of the more complex viruses contain their own enzymes, such as RNA polymerase, nucleotide hydrolases, and so forth. These enzymes assist the viral nucleic acid in the rapid takeover of the host cell. Some viruses, such as the influenza virus, contain the enzyme neuraminidase in their membranous envelopes. Apparently, this enzyme plays a role in the budding of the viruses from the surface of an infected cell.

Animal viruses do not possess an injection apparatus like that of the T-even phages, but their capsid proteins do recognize specific receptor sites on the host cell membrane. It appears that the virus particle (or a portion of it) passes directly through the cell membrane into the cytoplasm (Figure 9.10). Encapsulated viruses apparently fuse their viral membrane with the host cell membrane, thus delivering the internal viral nucleic acid and internal proteins into the cytoplasm of the host cell.

After entry into the cytoplasm, the viral particle is soon stripped of capsid proteins by cell enzymes, resulting in the release of the viral nucleic acid. In the case of DNA viruses, the viral DNA acts directly as a template

Figure 9.10. Electron micrographs and a diagram showing the direct cell to cell transfer of a Rittner virus.



(in most cases with host RNA polymerase) for the transcription of viral mRNA (which guides the formation of viral proteins) and as a template for the replication of viral DNA:

RNA viruses replicate in much the same way, but in most cases the viral RNA acts as a messenger RNA that codes directly for viral proteins by association with host ribosomes. As with TMV, one of the proteins produced by viral RNA is an RNA replicase, which then catalyzes the replication of the viral RNA. The RNA replicase makes complementary strands of the viral RNA and then makes duplicates of the original strands on the complementary strands.

Recent experiments with certain RNA cancer viruses indicate that these viruses produce a DNA polymerase that brings about formation of viral DNA by using the viral RNA as a template. The viral DNA formed inside the host cell then serves as the template for the transcription of new viral RNA. The discovery of DNA synthesis guided by an RNA template forced the modification of the so-called central dogma of molecular biology, which asserted that genetic information flows only from DNA to RNA to protein. (F. H. C. Crick, however, has pointed out that he was careful not to exclude the possibility of RNA-directed DNA synthesis from his original statement of the central dogma.) Now that biologists have begun to look for RNA-directed DNA synthesis and RNA replication in normal cells, they are finding that even in cells not infected by viruses the picture is not as simple as indicated by the simplified form of the central dogma. Enzymes capable of guiding DNA synthesis from RNA templates apparently do exist in normal mammalian cells, where they may play a role in processes such as differentiation or memory (Scolnick, et al., 1971). The existence of these enzymes in normal cells is not yet fully confirmed, and the significance of RNA-directed DNA synthesis will probably not become clear until its role and extent have been more fully studied.

Both RNA and DNA viruses act as packets of genetic information. The details of the information vary in different viruses, but the overall message they bring into infected cells is similar. In effect, the viral nucleic acid tells the cell: "Make the proteins that I specify. Use some of these proteins to make many copies of my nucleic acid. Wrap these new viral genes in new viral capsid protein to assemble complete new viral particles."

If this cycle of virus reproduction and cell destruction were to continue indefinitely, no animal could survive a viral infection because all its cells would soon be destroyed. In fact, the organism possesses various defense

mechanisms that destroy many of the virus particles before they can infect other cells. In addition, each virus attacks only certain kinds of cells. For example, common-cold viruses grow mainly in the cells lining the respiratory passages, viruses causing intestinal disorders grow mainly in gut cells, and hepatitis virus invades chiefly liver cells. This specificity is partly due to the fact that the virus particle attaches only to cells possessing appropriate receptor sites on their membranes. Thus, most viral diseases attack only certain tissues of the organism.

The destruction of cells that are susceptible to viral infection may be prevented by the immune responses of the animal body. The immune system, stimulated by the presence of the foreign virus particles, produces molecules called antibodies, which combine with the viral coat protein. The antibodies adhere firmly to the viral particle and prevent it from attaching to or penetrating cells. Antibodies that neutralize a particular kind of foreign particle are produced in great quantities after that kind of particle enters the body. The immune system can produce antibodies much more quickly in response to any future invasion of the same kind of particles (Chapter 22). Thus, one attack by a particular virus (or microorganism) confers greater or lesser immunity to ill effects from future attacks by the same type of particle.

Immunity to viruses can last for varying periods of time. Most childhood virus diseases (measles, mumps, chickenpox) usually induce lifelong immunity. These diseases are childhood diseases because most people suffer an attack during childhood and thereafter are immune.

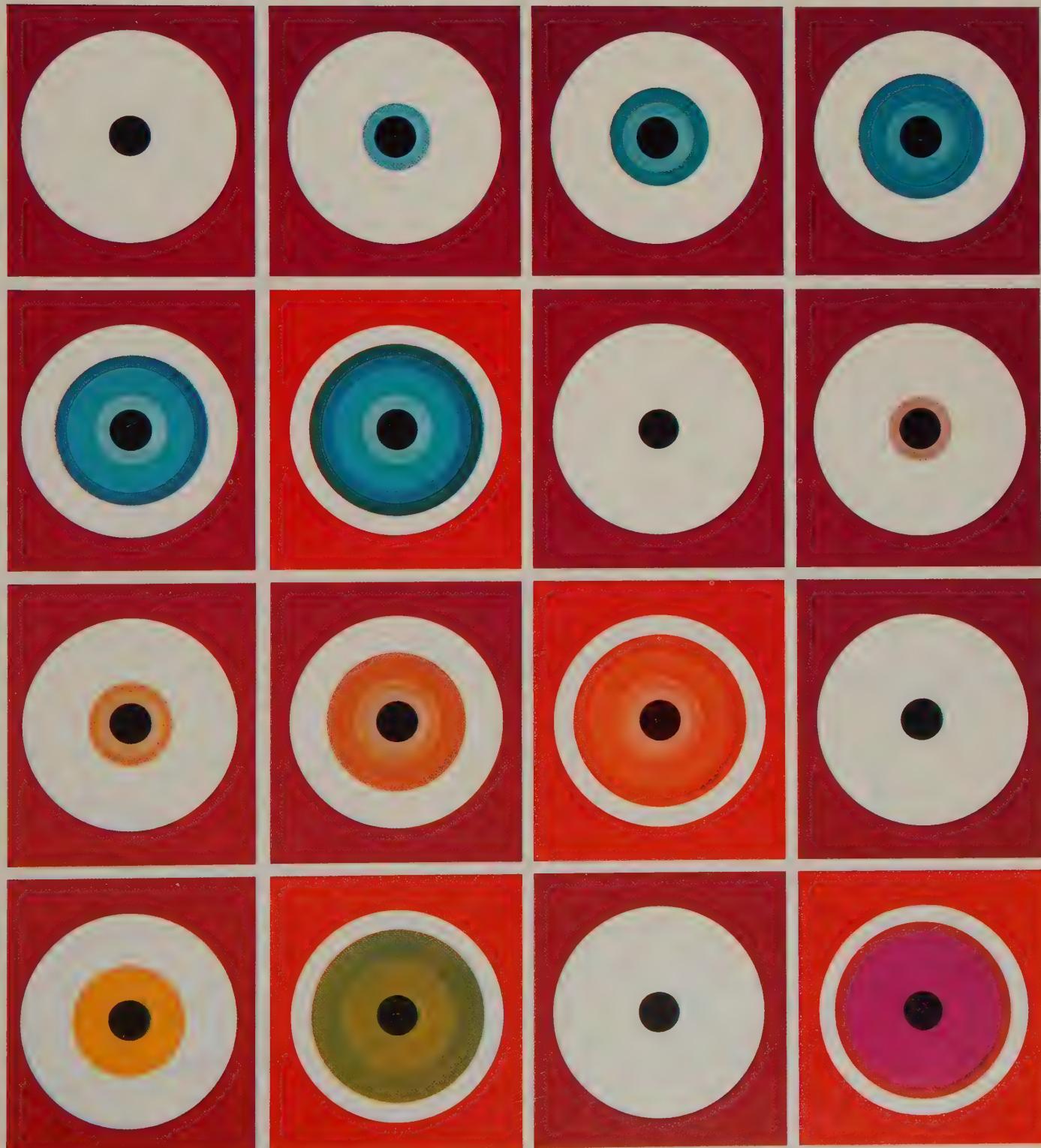
Some common-cold viruses and other respiratory viruses, such as influenza, induce weaker immunity. These viruses can reinfect the body after a period of months or years. This weak immunity may be partly explained by the fact that antibody concentrations are much lower in nose and throat fluids than in the bloodstream. In addition, influenza viruses are extremely versatile infecting agents because they are able to withstand drastic mutation of the parts of the viral RNA that code for membrane protein without losing their infectivity.

Every few years, a new virulent mutant of influenza virus arises in the human population. This mutant has a membrane protein that does not combine strongly with the antibodies previously produced. Therefore, the antibodies already present in the body do not immediately neutralize an invasion of the new mutant virus strain. If the influenza mutant is almost totally resistant to existing antibodies, a pandemic sweeps the world. Such a pandemic of influenza has occurred about every ten years, and as the human population becomes larger and denser, the likelihood of more frequent influenza pandemics increases.

Many viral diseases can be prevented by vaccination. Edward Jenner's vaccination against smallpox involved the injection of cowpox viruses into the human body. These viruses are so similar to smallpox viruses that they cause the production of antibodies that combat both kinds of viruses. The cowpox virus, however, causes relatively little damage in the human body. The antibodies produced to combat the cowpox infection are effective enough to handle most future invasions of either cowpox or smallpox viruses.

In dealing with most viral diseases, biologists have not been lucky enough to find a closely related, relatively harmless virus that can be used as a natural vaccine. Instead—as Louis Pasteur did in producing rabies

Figure 9.11. An artist's interpretation of a pandemic. Every few years, a new mutant strain of an influenza virus arises in the human population. If this virulent mutant is resistant to antibodies, a pandemic occurs. Such a pandemic has been occurring about every 10 years.



vaccine—they have been forced to produce forms of the disease virus with reduced infectivity. Biologists can produce this type of vaccine by physically or chemically treating the normal virus or by searching for mutant strains with the desired properties.

Vaccines have now been developed for many of the human viral diseases, but vaccination against influenza viruses is not yet completely successful. Because influenza viruses mutate so drastically, a new vaccine must be developed for each major new strain of virus. Often, an influenza pandemic is well under way before a specific vaccine can be prepared in quantity. Vaccination does little or no good after the body has already been infected by viruses. Furthermore, influenza vaccines are sometimes ineffective, or they may produce relatively severe illnesses in a significant proportion of the population.

INTERFERON

It has been known for nearly 40 years that when an animal cell is infected with a virus, it becomes resistant to further infection (*superinfection*) by the same or different viruses. This phenomenon is called *interference*. Culture fluid taken from infected cell cultures can confer resistance upon uninfected cell cultures. The active principle was shown to be a protein and was named *interferon*.

Interferon probably plays a natural role in the body's defense against viral infection. It appears soon (12 to 18 hours) after infection, long before the immune system can produce significant amounts of antiviral antibodies (about 5 days). The cells that are first infected may be killed by the virus, but they will produce and release interferon, which is transported via the circulatory system to other cells, which then become resistant to infection.

Research on interferon has centered upon two questions. First, what is the mechanism by which cells are stimulated to produce interferon? Not only viral infection but also bacterial *endotoxins* and synthetic substances can induce animal cells to produce and release interferon. The most potent of these *inducers* has turned out to be double-stranded RNA (dsRNA). Under the right conditions, minute amounts of dsRNA can confer resistance to viral infection.

Second, how does interferon cause cells to become resistant to viral infection? Although the answer is not yet clear, most of the evidence suggests that interferon-treated cells can distinguish viral mRNA from cellular mRNA; the cells refuse to translate the viral message into protein, but translation of cellular messages is unimpaired.

Because viruses take over the metabolic (genetic) machinery of the host cell, until now it has been impossible to find a drug that interrupts the viral replication cycle without also disturbing the normal expression of cellular genes. Antibiotics are effective against bacterial (but not viral) infections because they interfere with aspects of bacterial metabolism that are not employed by animal cells. Thus, penicillin or sulfa drugs are effective as antibiotics because they block synthesis, respectively, of the polysaccharide cell wall and of certain coenzymes—processes that are not required by animal cells.

Interferon-treated cells seem to be resistant to most types of virus, yet interferon does not seem to interfere with normal cellular metabolism. Original attempts to use interferon as an antiviral drug were fraught with difficulty. First, interferon is one of the most active proteins known; con-

Figure 9.12. The life cycle of a lysogenic bacterial virus. Printed by permission from J. D. Watson, Molecular Biology of the Gene, © 1965, J. D. Watson.

versely, the amounts of activity that can be isolated represent extremely minute amounts of protein. Second, interferon's action is species-specific—it is active only on cells from the same species as the cells that produce it. It is not feasible to use humans as a source of commercial interferon. Third, when interferon is injected into the bloodstream, it is cleared from the circulatory system fairly rapidly. In spite of these difficulties, interferon has been used to confer resistance upon humans to some viruses—for example, the cold-causing rhinoviruses. Interferon also causes resorption of the fetus in pregnant mammals; it thus has possibilities for use in solving overpopulation problems.

Inducers of interferon, notably dsRNA, have been used to stimulate production of interferon by the organism itself. These drugs also pose problems; they are moderately toxic and induce fever. Nevertheless, inducers of interferon have provided remarkable protection against viral infection and have caused regression of established infections. Moreover, there is ample evidence that some forms of cancer are correlated with the occurrence of viral chromosomes in the cancer cells, and interferon has been shown to cause regression of viral tumors. The discovery of interferon thus has great potential in the prevention and cure of viral disease.

LYSOGEN

Certain other aspects of virus activity are of enormous significance for medicine and of value for the study of gene action. In many cases, viral DNA will break into segments during viral reproduction. These segments may

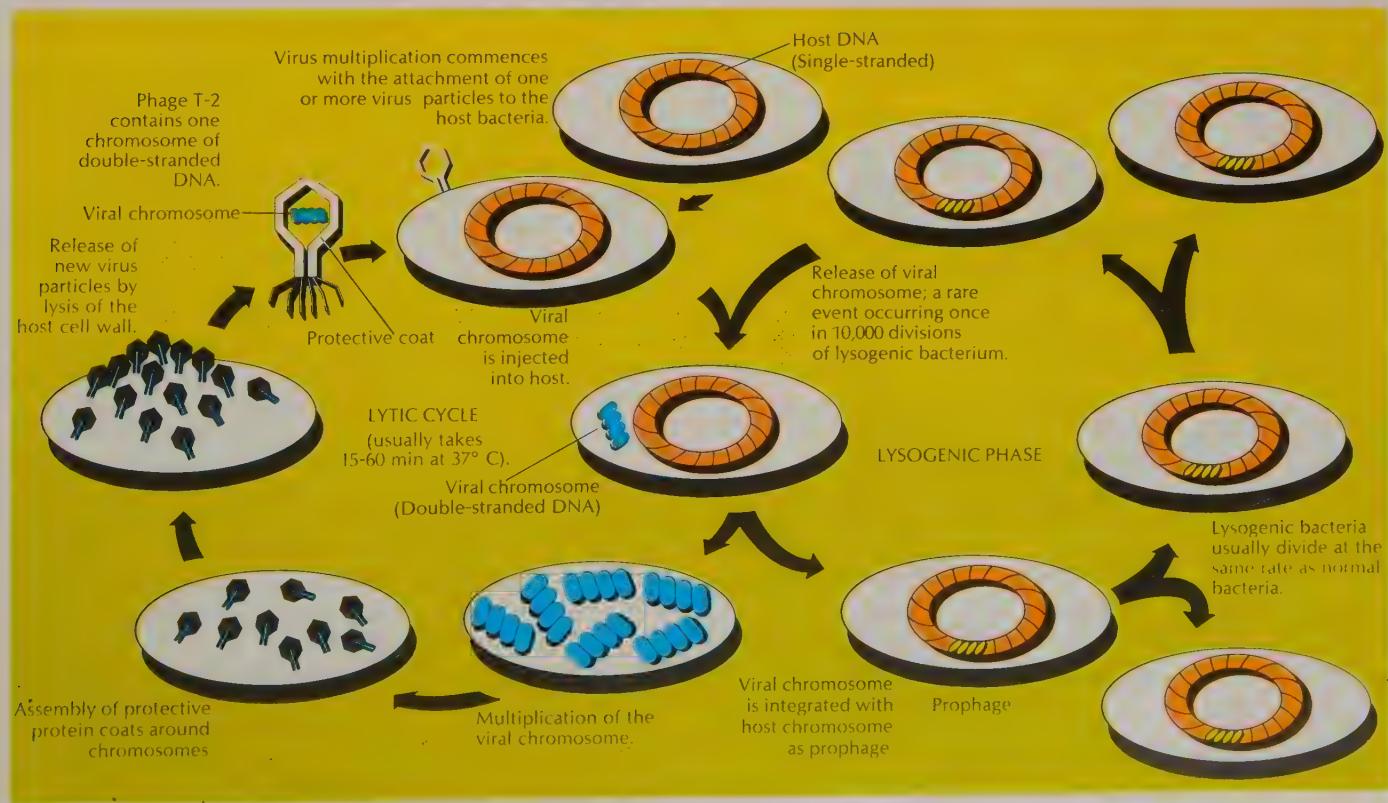
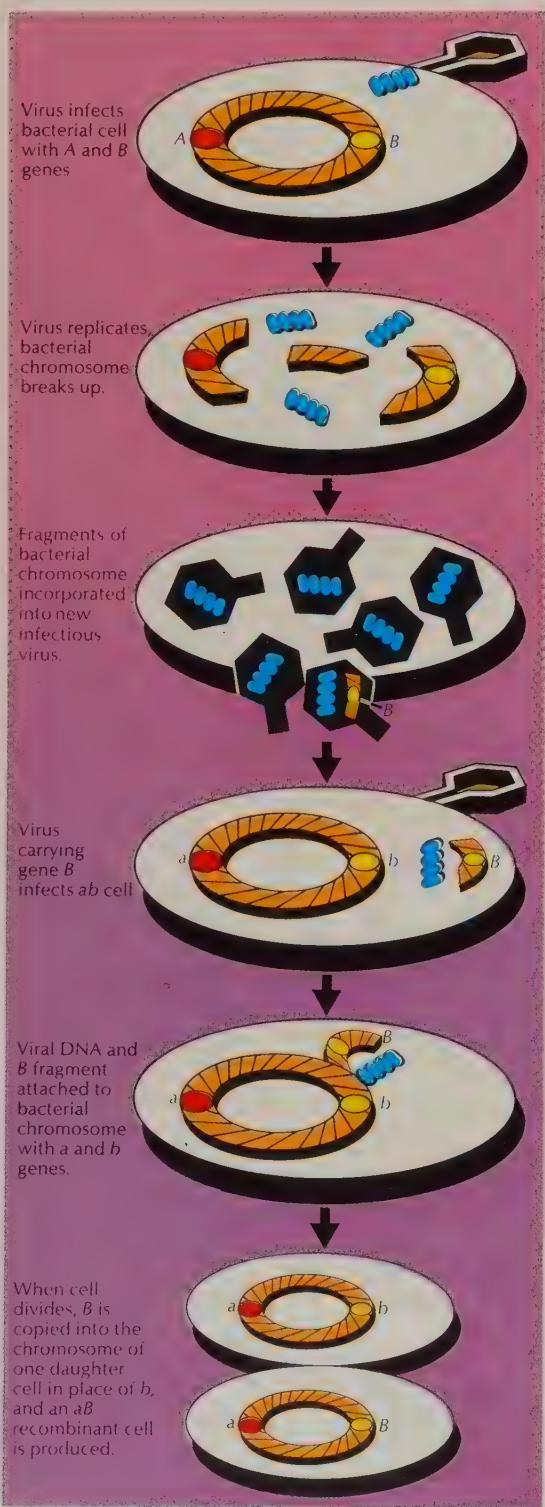


Figure 9.13. The process of transduction by a bacteriophage.



then recombine to form new genes or gene sequences. If a single cell is simultaneously infected with more than one virus, important new combinations of genetic material can occur from the various recombining DNA segments.

Some very small viruses—called *satellite viruses*—can reproduce only when they infect a cell that also is infected by a larger virus. Apparently, the satellite virus does not carry all the genes needed for its reproduction but must use some proteins coded by the other virus' genetic information. Sometimes the satellite virus is so small that its nucleic acid probably carries only a single gene coding for its own coat protein.

In bacteria infected with certain phage, such as phage lambda, a most unusual phenomenon occasionally takes place. These phage normally reproduce their DNA as small loops, wrap the DNA in a protein coat, and then burst the cell. In a certain proportion of the infected bacteria, however, the viral DNA does not replicate. Instead, its small loop of DNA attaches itself to the large loop of bacterial DNA. Nucleases then break both DNA loops at the point of their attachment, and the broken ends are joined to form one large loop that contains both bacterial and viral DNA. Thus, the viral DNA becomes an integral part of the host cell chromosome and is replicated every time the host cell divides.

This integrated state, or *lysogen*, may persist for thousands of generations, with each pair of daughter cells carrying the viral DNA within the chromosomal loop. Information in the integrated viral DNA produces protein, which prevents independent replication of the viral DNA. This protein also prevents replication of any new viral DNA entering the lysogenic cell. The bacterial cell thus gains immunity from further infection by related viruses.

On rare occasions, a lysogenic bacterium suddenly separates the viral DNA from the integrated chromosome. The released viral DNA then begins to replicate in its usual fashion, producing new viruses and killing the host cell. In this way, the lysogenic viral infection becomes activated into a virulent viral infection. Agents such as x-rays, ultraviolet light, and some chemicals can cause most lysogenic cells to release their integrated viral DNA.

In some cases, the viral DNA comes out of the integrated chromosome imperfectly. A part of the original viral DNA is left behind on the bacterial chromosome, and an equal piece of original bacterial DNA is included in the released viral DNA loop. After this abnormal viral DNA multiplies, it is wrapped in a protein coat just as normal viral DNA would be. When this virus infects another cell, the viral DNA may be missing so much of its information that it is unable to replicate and kill the host cell. The section of bacterial DNA carried along with the viral DNA may recombine with the DNA of the new host cell. Thus, the virus acts as a carrier of genetic information from one bacterium to another. This transfer of genetic information from one cell to another by a virus is called *transduction* (Figure 9.13). The technique has been used widely in genetics and molecular biology for many years and has been crucial in a number of important experiments clarifying the mechanisms of gene action.

Some kinds of viruses do not kill the cell that they enter. Instead, these *oncogenic viruses* cause a *transformation* of the animal cell, and the cell behaves in a fashion very similar to a cancer cell. Evidence suggests that the DNA of the oncogenic virus, like that of the lysogenic phage, is integrated into the DNA of the host cell. There is mounting evidence that study of re-

combinations of viral DNA, satellite viruses, and lysogenic viruses may provide important clues to the processes that cause cancer and other human diseases.

SYNTHESIS OF VIRUSES

Because the genetic information carried by a virus is relatively simple, viruses were logical choices for the first attempt at synthesis of a biological system. Viral nucleic acids were the first major components of biological systems to be synthesized in the laboratory. Viral RNA was replicated many thousandfold, using viral RNA as a template for the action of purified viral replicase acting on RNA precursors. When these newly synthesized RNA molecules were exposed to bacterial cells stripped of their rigid cell walls, the viral RNA replicated in the cells and killed them, producing thousands of viruses complete with protein coats (thus demonstrating the accuracy of the synthesized RNA information). In this experiment, the viral RNA was replicated outside of a living cell, but an initial supply of viral RNA was used as a template as well as a supply of purified viral RNA replicase. Thus, this experiment is a long way from the complete synthesis of viral RNA from simple precursors.

A similar replication of viral DNA outside the cell was later accomplished. In this case, DNA precursors, DNA polymerase, and a ligase enzyme (to close the newly synthesized DNA loops) were used to produce fully infectious synthetic DNA from a viral DNA, which acted as a template.

ORIGIN OF VIRUSES

Because viruses are unable to replicate without using the mechanism of a cell, they could not have existed as primitive living systems that evolved before the existence of cells. Despite the simplicity of their structure, some scientists believe that viruses are relative latecomers in the evolutionary story, developing from cells that became parasitic on other cells and eventually lost most of their cytoplasmic machinery.

The simplicity of viruses has been of great value to scientists in their attempts to understand basic principles of living systems. In the interactions of viral systems with almost every kind of cell, biologists have found many clues to the basic nature of gene action. It is ironic that much of current knowledge about genetic mechanisms of living systems has come through the study of viral systems, which cannot fully be classified as living. Yet scientists often find that study of the unusual or odd phenomenon leads to important insights into the nature of the normal world.

FURTHER READING

Vaccination and immunity are discussed in greater detail in Chapter 22. Among the many books on viruses, particularly good introductions to the field are those by Fraenkel-Conrat (1962), Luria and Darnell (1968), and Stanley and Valens (1961). For more detailed information on the molecular biology of viruses, see books by Burnet and Stanley (1956–1959), Fenner (1968), and Fraenkel-Conrat (1968).

Bacteriophage are described in greater detail by Hayes (1969) and Stent (1963). For further information about oncogenic viruses, see the books by Gross (1961) and Hafris (1964).

10

Membranes



Cell biologists of the late nineteenth century thought that the cell membrane, or plasma membrane, was simply an invisibly thin film that held together the living contents of the cell. Information gained from biochemical studies and electron microscopy has greatly changed this view of the membrane. It is now known that every cell—prokaryotic or eukaryotic—is surrounded by a membrane about 100 Å thick. This membrane has a regular molecular structure and plays an active role in the life of the cell. Within the eukaryotic cell, similar membranes form the boundaries of the organelles and provide sites for many enzymatic reactions required by the cell.

The plasma membrane surrounding the cell serves as a passive barrier that holds together the contents of the cell and protects them from the conditions of the external environment. Yet a living cell must continually interact with its environment, obtaining metabolites and discarding waste products. The plasma membrane serves as an active envelope that regulates this vital flow of materials to and from the cell interior. Within the eukaryotic cell, membranes play a similar role in maintaining the integrity and specialized conditions of each compartment of the cell, while simultaneously regulating and facilitating the necessary interchanges among compartments.

Many of the membranes inside the eukaryotic cell play another important role. Membranes of the endoplasmic reticulum (ER), Golgi apparatus, mitochondria, and plastids—and even parts of the plasma membrane itself—provide structural sites for the regular organization of multienzyme complexes (Chapter 4). Substrate molecules are passed from one enzyme to another along the membrane in highly organized and efficient biochemical reaction sequences.

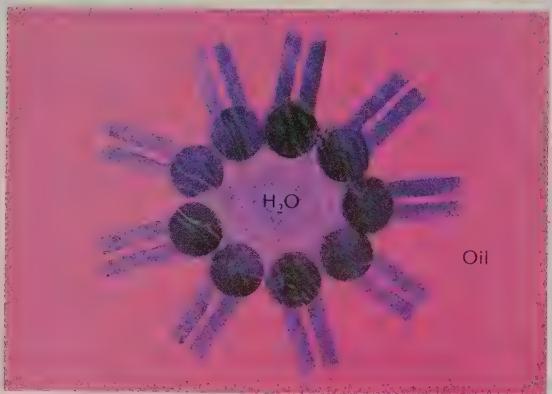
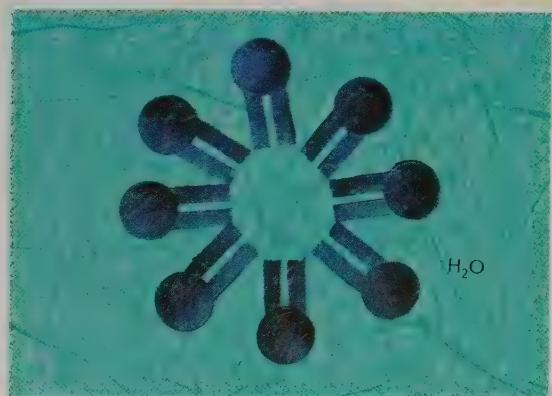
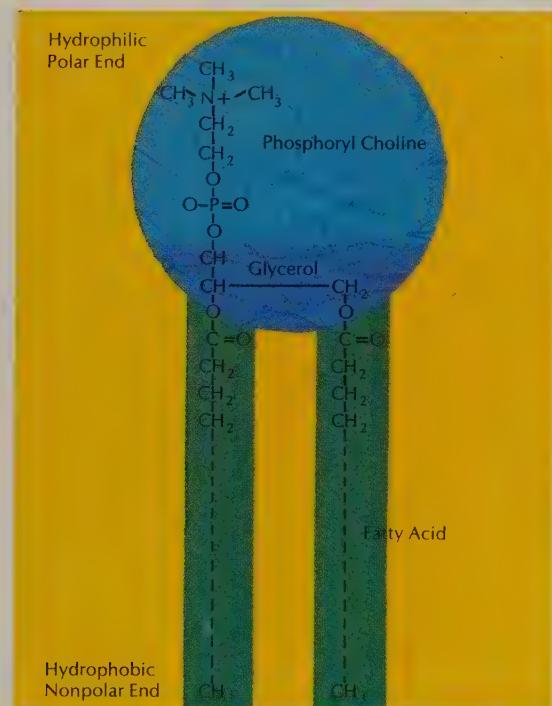
Protein synthesis is carried out on membrane-associated ribosomes, which apparently carry out this process while floating freely in the cytoplasm. Membranes, however, also appear to be important in protein synthesis, for ribosomes tend to cluster along the nuclear membrane, the rough ER, and the plasma membrane. In prokaryotic cells, which lack the ER and a nuclear membrane, some of the ribosomes are distributed along the inner surface of the plasma membrane. There is growing evidence that the process of DNA replication normally occurs at a site on a membrane, with the long DNA molecule moving past a stationary enzyme site as it is replicated.

One of the better-studied, membrane-mediated processes is photosynthesis (Chapter 5). Enzyme and cofactor molecules involved in photosynthesis are arranged in a precise pattern within the complex membrane system of the chloroplast—sequentially transferring electrons and substrate molecules from one step in the process to the next, much like a factory assembly line. The photosynthetic pigments, enzymes, and cofactors are arranged side-by-side on these membrane stacks to facilitate an efficient assembly-line process. When the appropriate molecules involved in photosynthesis are mixed in a test tube, without the ordering provided by membranes, the photosynthetic process is weak and inefficient.

Biochemists have had to develop new techniques and theories for the study of these membrane-mediated reactions, for traditional chemical techniques and theories deal largely with the study of reactions in solutions. A cell's membranes are only a few macromolecules thick. Clearly, an understanding of the properties of membranes will be attained only through a full knowledge of membrane molecular structure. Although much has been learned about membrane structure, many important questions have been

Figure 10.1 (above). Phospholipid structure. Phospholipids are complex molecules built around the three-carbon alcohol glycerol. An organic base is attached to one of glycerol's three carbon atoms and fatty acids to the remaining two carbon atoms. A variety of bases and fatty acids are found in membrane phospholipids. In the symbol used here to represent a phospholipid molecule, the polar head is represented by the circle, the hydrocarbon tails by the two sticks.

Figure 10.2a (middle). Schematic diagram of a micelle



in water. Phospholipid molecules form various stable configurations in different environments. In water, the molecules are arranged with the hydrophobic tails shielded from the water by the hydrophilic ends.

Figure 10.2b (below). Schematic diagram of a micelle in oil. The hydrophilic ends are interacting with each other (and with any water that happens to be in the environment), and the fatty acid ends are interacting with the surrounding oil.

answered only tentatively or not at all. Membrane structure is an area of active research at the present time, and almost every month brings new experimental findings.

COMPOSITION OF MEMBRANES

Nonpolar molecules, soluble in fatty or oily solvents, pass relatively freely through cell membranes. Polar molecules, soluble in water but insoluble in fatty solvents, move much less freely through the membranes. For these reasons, it was suggested early in this century that the plasma membrane consists of a thin layer of lipids. But more recent chemical analyses have shown that cell membranes are composed of lipids, proteins, and carbohydrates. All three components contribute to membrane properties. The lipids make the membrane relatively impermeable to ions and polar molecules. Long-chain protein molecules apparently give the membrane strength, elasticity, and the ability to expand and contract. Other proteins have enzymatic functions, including active transport of molecules across the membrane and metabolic reactions carried out on the membrane surface. The carbohydrates play an important role in chemical interactions between the cell and its surroundings. Thus, the plasma membrane is far more than a simple envelope; it is a complex molecular system that performs many intricate chemical and physical processes essential to the survival of the cell.

Membrane *lipids* are mainly of a class of compounds called phospholipids. The structure of a *phospholipid* is built around the three-carbon alcohol, glycerol. A polar phosphate group is attached to one of glycerol's three carbon atoms, and nonpolar fatty acids are attached to the other two carbon atoms (Figure 10.1). Because of the attached polar group, this end of the phospholipid molecule is *hydrophilic*—that is, it has a strong attraction for water molecules. The fatty acid groups, however, are nonpolar, making the other end of the phospholipid molecule *hydrophobic*—that is, it is more strongly attracted to other nonpolar molecules than to water molecules.

Small droplets called *micelles* are formed when a phospholipid is added to a water solution. The arrangement of individual phospholipid molecules within a droplet has been determined by thermodynamic calculations and by various physical measurements. These studies reveal that the most stable arrangement assumed by most phospholipid molecules is that in which the hydrophobic ends of the molecules are shielded from the surrounding water and the hydrophilic ends are in contact with the water (Figure 10.2a).

If any fat-soluble, nonpolar compounds are present in the water solution, they tend to collect within the micelle at the hydrophobic ends of the lipid molecules. Soaps and detergents also are molecules with hydrophobic and hydrophilic ends. They act in a fashion similar to the phospholipids in that they isolate small amounts of oily substances within micelles, which move freely through a water solution. Thus, an insoluble oily film can be converted into a large number of micelles, which are easily washed away by water. Phospholipid molecules are oriented in the opposite direction if a micelle is formed in a nonpolar solvent such as oil. In this case, polar substances tend to collect within the micelle (Figure 10.2b).

Phospholipids are one of three major classes of lipids; the other two are triglycerides and steroids. A *triglyceride* molecule consists of glycerol in which a fatty acid group is attached to each of the three carbon atoms. Triglycerides serve as a major form of long-term energy storage in animal cells but appear to play no part in membrane structures. A *steroid* is a complex

Figure 10.3 (above). A negative-contrast micrograph of a lipid micelle. ($\times 74,000$)

Figure 10.4 (below). Protein structure. The linear structure of amino acids in a protein is referred to as its *primary structure*. Interchain disulfide bridges and hydrogen bonds determine its *secondary structure*. The three-dimensional structure is referred to as the *tertiary structure* of the protein.

alcohol containing a four-ring carbon structure. Cholesterol is a steroid found in the membranes of many kinds of animal cells. In various glandular cells, cholesterol is converted into steroid hormones—substances that are circulated through the organism, altering the rates of metabolic processes in many kinds of cells. The role of cholesterol in the many other kinds of animal cells where it is found is not yet known, but the rigid, planar cholesterol molecules may add strength to the lipid portion of the membrane.

Proteins are the second major type of molecule in cell membranes. Because of technical problems in isolation and purification of membrane proteins, relatively little is known about the types and properties of protein molecules in the membrane. A number of nonmembrane proteins have been thoroughly investigated, and their three-dimensional arrangements, or conformations, fall into four major categories (Figure 10.4). Although the conformational states of most membrane proteins have not been determined, there is evidence that they share some of the conformations of the better-studied proteins. Membrane proteins also may assume certain unusual conformations as a result of lipid-protein interactions within the membrane.

The carbohydrate groups of the plasma membrane may be linked either to proteins, forming complexes called *glycoproteins*, or to lipids, forming *glycolipids*. Both glycoproteins and glycolipids appear to confer unusual properties on the cell surface. Parts of these molecules coat the outer surface of a cell, forming chemical configurations that make the surface of that cell unique—different from one type of cell to another, different from one

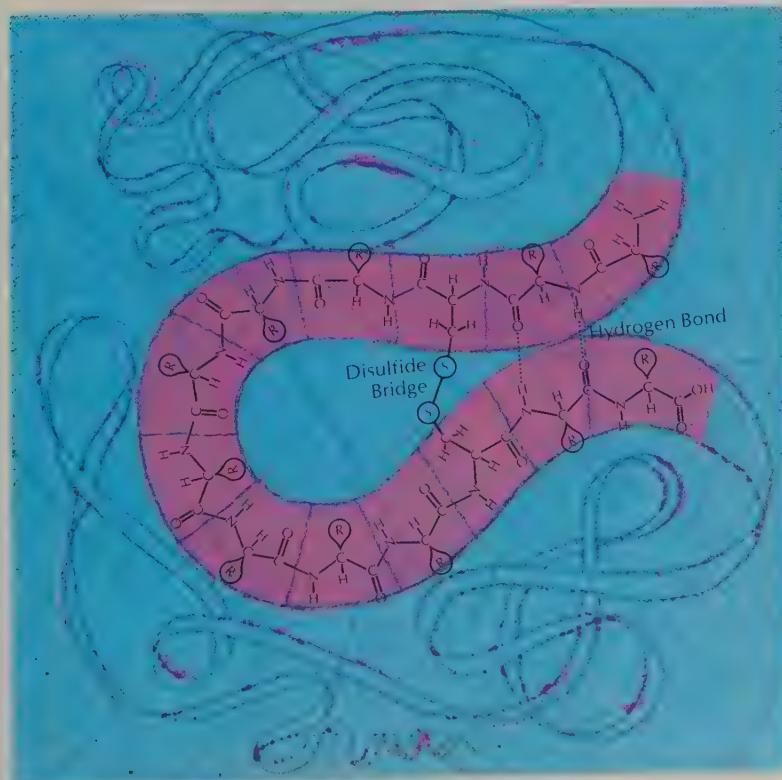
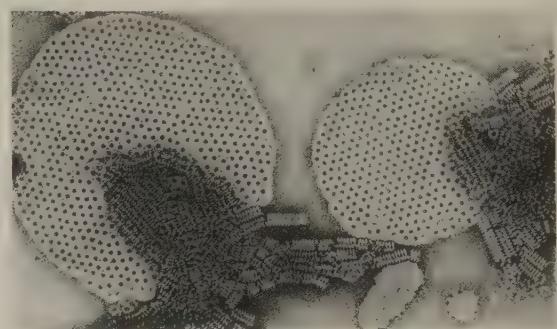


Figure 10.5. Lipid monolayer. When applied to a water surface, extracted membrane phospholipids will spread out to form a layer one molecule thick (monolayer). This lipid monolayer can be compressed to its minimum surface area, this area measured, and the results compared with calculated surface areas of the original cell membrane. These data suggest that the possible configuration of lipids in the cell membrane is a bilayer.

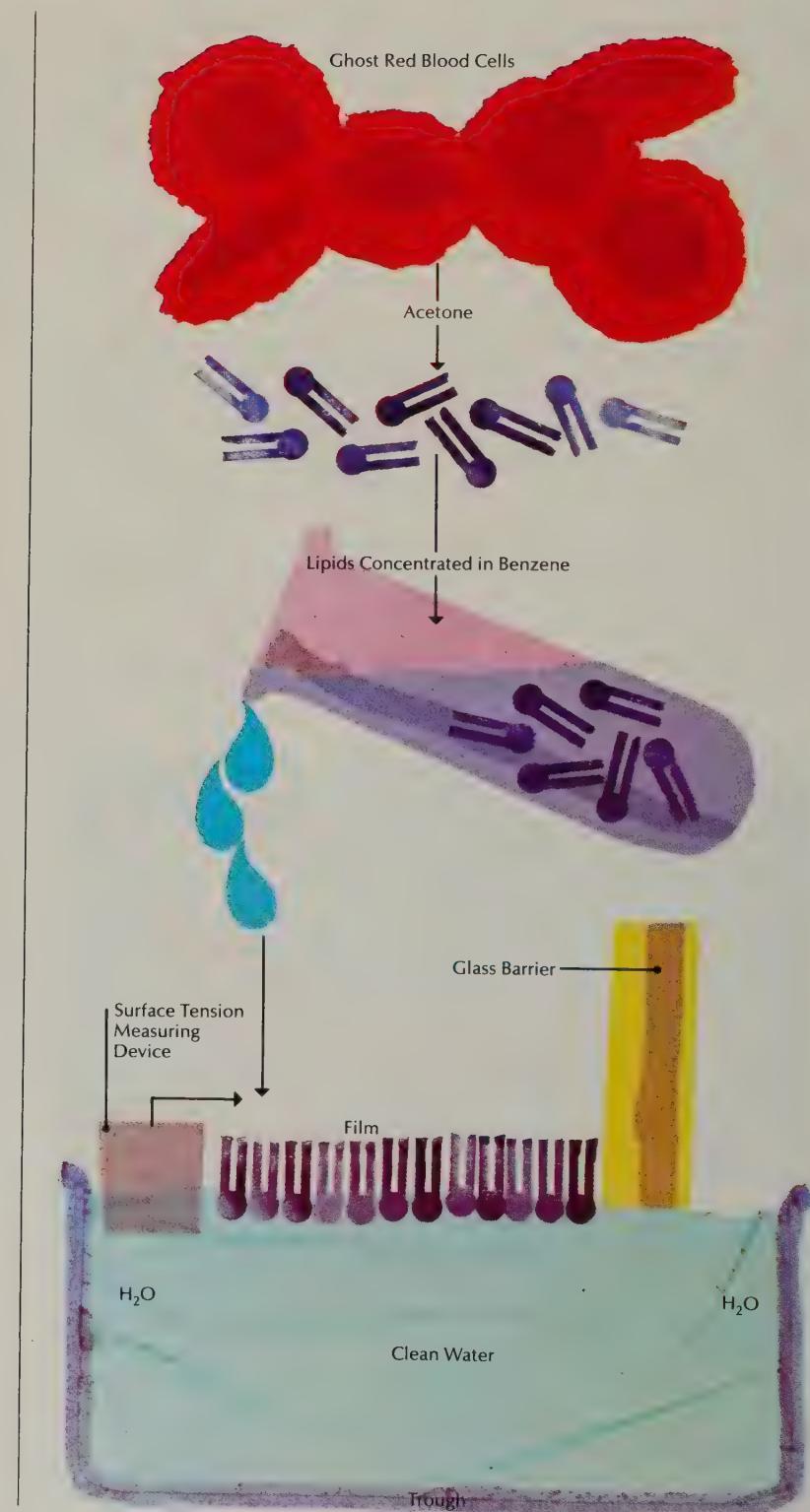


Figure 10.6. Red blood cell ghost membranes seen in an electron micrograph of a thin section of several red blood cell membranes. The triple-layer structure is seen most clearly when the section cuts directly across a membrane. This triple-layer configuration is represented by two highly visible electron-dense lines, separated by a lightly stained space. Each of the three layers in this structure is about 20 to 30 Å in thickness.

species to another, and even different from one individual organism to another. These surface chemical configurations are intimately involved in interactions among cells. Cells react in certain ways to other cells bearing surface groups similar to their own; they react in quite different ways to cells bearing different groups. Surface groups that are unique to an individual organism are responsible for the rejection reactions that often follow organ transplantsations.

Electron microscopy shows that most of the internal membranes have a structure similar to that of the plasma membrane. This observation and the available chemical data suggest that the internal membranes are composed of similar lipids, proteins, and carbohydrates, although the ratios of these substances probably vary from one kind of membrane to another and from one kind of cell to another.

Molecular Structure of Membranes

When a polar-nonpolar substance such as phospholipid is placed on a water surface, it spreads out to form a layer only one molecule thick (Figure 10.5). Within this monomolecular layer, or *monolayer*, the molecules are oriented with their polar, hydrophilic ends in the water and their nonpolar, hydrophobic ends pointing into the air. In a device called a film balance, a monolayer can be formed on the surface of a shallow trough of water and then compressed to align all of the molecules in the layer. The study of lipid monolayers with this device offered the first clues about the possible structure of cell membranes.

The mammalian red blood cell has been used for many membrane studies. The red blood cell is a highly specialized cell that lacks a nucleus and has a relatively simple internal structure. When these cells are placed in distilled water, the water floods and the cell contents escape, leaving so-



Figure 10.7. Three possible structures for a lipid bilayer.

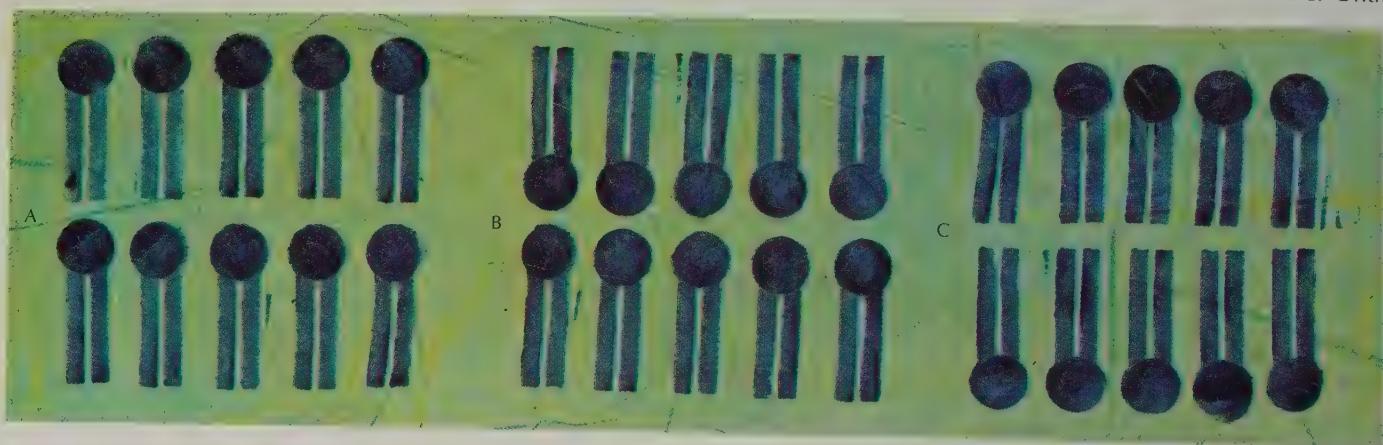
called red cell ghosts, which consist of the plasma membranes. (Analysis of quantities of such ghosts provided the best chemical information about plasma membrane composition.)

In a classic experiment, the lipids from ghosts were dissolved in acetone, concentrated by evaporation, and redissolved in a small amount of benzene. The benzene-lipid solution then was used to form a monolayer in a film balance; after the benzene evaporated, a lipid monolayer remained. The area of this lipid monolayer was compared with the calculated surface area of the original cells. The data are consistent with the theory that the lipid in the cell membrane is evenly distributed in a bimolecular layer.

At least three structures could be imagined for such a lipid bilayer (Figure 10.7). Because the cell membrane is bounded on both sides by water solutions, the early researchers concluded that the most likely structure for the lipid bilayer in the cell membrane is one with the hydrophilic ends facing outward and the hydrophobic ends together in the center of the bilayer.

J. F. Danielli and his coworkers (1935) measured the surface tension of cell membranes and found it to be much lower than the measured surface tension of lipid monolayers and bilayers. They suggested that the outer surfaces of the lipid bilayer are coated with protein molecules. The polypeptide chains of the proteins, lying at right angles to the fatty acid chains of the lipids, add strength to the membrane but reduce its surface tension. Nonpolar groups on the protein chains extend into the lipid bilayer toward the hydrophobic ends of the lipid molecules. Polar groups on the protein chains are directed in the opposite direction toward the water solution. These specific attractions hold the membrane structure together without the formation of covalent bonds among the various molecules.

During the 1950s, techniques were developed for the study of cell membranes with the electron microscope. The membrane, which is far too thin to be visible with even the most powerful light microscope, could be observed for the first time. Using a special stain and very high magnification, electron microscopists observed the membrane as a triple-layer structure, formed by two electron-dense lines separated by a lightly stained space (Figure 10.8). Each of the three layers in this structure is about 20 to 30 Å in thickness. Because this structure was found to be characteristic of all cell membranes, J. D. Robertson (1959) suggested that it be called the unit membrane configuration. The structures observed are consistent with a model similar to the Danielli model of cell membrane structure. Until



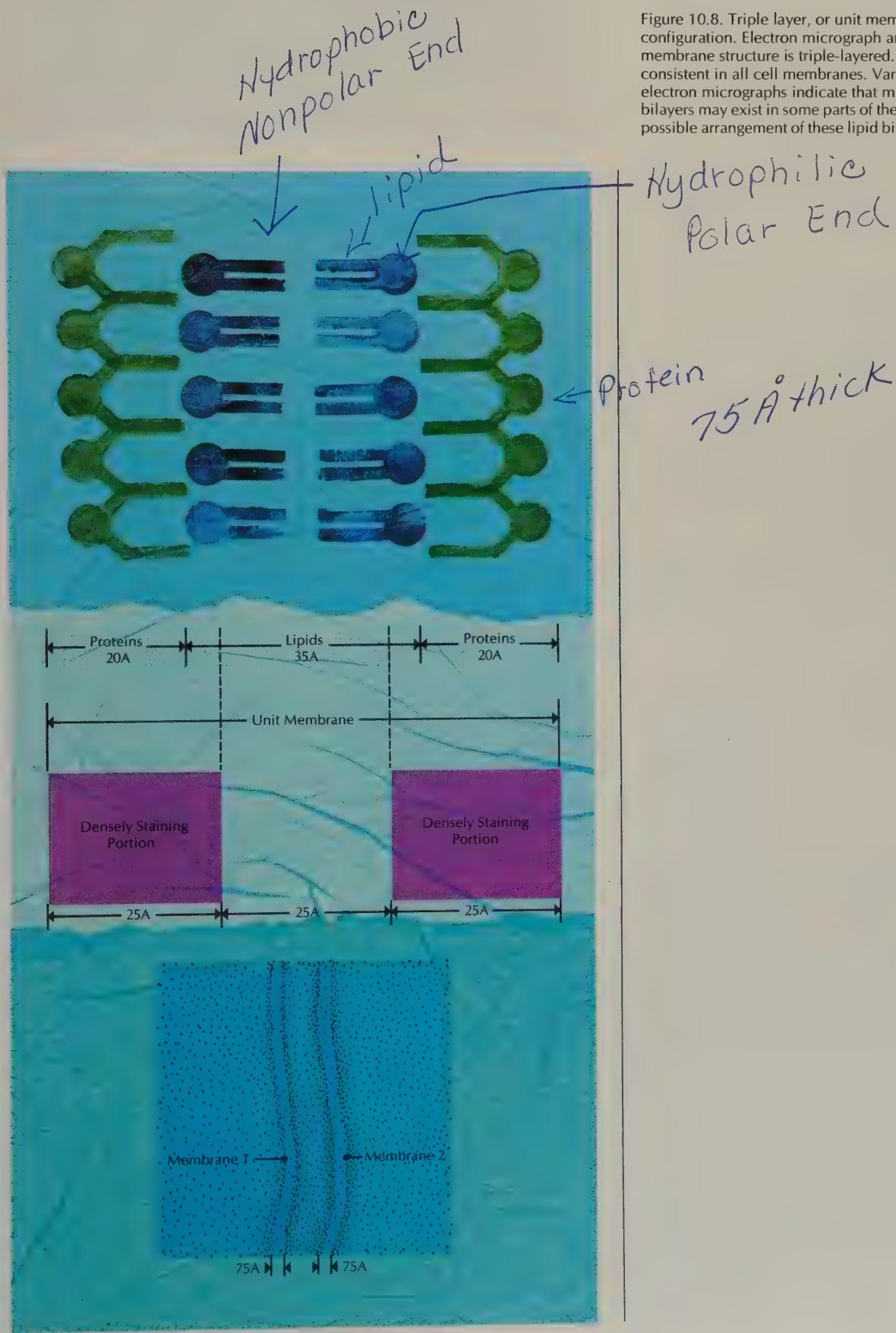
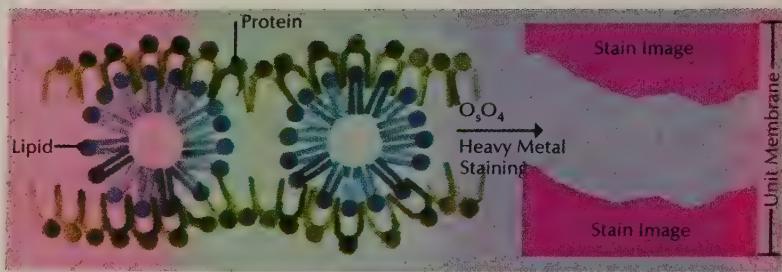


Figure 10.8. Triple layer, or unit membrane, configuration. Electron micrograph analysis indicates membrane structure is triple-layered. This structure is consistent in all cell membranes. Variations visible in electron micrographs indicate that multiple lipid bilayers may exist in some parts of the membrane. A possible arrangement of these lipid bilayers is illustrated.

Figure 10.9 (right). The lipid micelle–protein configuration is an alternative to the Danielli model but is also consistent with experimental data.

Figure 10.10 (below). Indirect evidence suggests that pores lined with polar groups may exist in the membrane. If present, they would facilitate the passage of ions and polar molecules. However, pore structures are too small to be visible in electron micrographs, and their existence remains a matter of conjecture.



recently, this Danielli model of membrane structure has been widely accepted (Davson and Danielli, 1943). It remains one possible structure for cell membranes, but recent research has emphasized other possible structural arrangements.

Alternatives to the Danielli model that are also consistent with the experimental data have been proposed. The triple-layer image could be produced by a structural arrangement of lipid molecules such as that shown in Figure 10.9. Even though the lipid molecules are arranged in micelles, the image seen in a cross-sectional electron micrograph would be difficult to distinguish from that of a lipid bilayer.

Although the unit membrane structure is remarkably constant in biological membranes, there are some variations visible in electron micrographs. Variations in spacing between the dark bands suggest that multiple lipid bilayers may exist in some parts of the membrane (Figure 10.10). Indirect evidence suggests that pores lined with polar groups may exist in the membrane. Ions and small polar molecules would pass more readily through these pores. Such structures are too small to be visible in electron micrographs, and their existence remains a matter of conjecture. There also is evidence that the nature of the proteins and lipids varies from place to place over the membrane, probably creating a mosaic pattern of varying properties.

The position of the glycoproteins and glycolipids within the membrane structure is unknown. Most of the sugar groups of these molecules are along the cell surface, but there is some evidence that other parts of the molecules may extend into the lipid portion of the membrane.

FREEZE-CLEAVAGE STUDIES

Recently, interesting new discoveries about cell membranes have been made with a technique of electron microscopy called *freeze-cleavage preparation*. Instead of chemically fixing and staining the cell, biologists use a process of rapid freezing. This technique greatly reduces the possibility of rearrangement of the membrane components during preparation. The sample is then sectioned in a very cold, evacuated chamber. Instead of cutting a smooth section, a blade cleaves the sample along natural lines of weakness. The sample is then coated with a heavy metal to make the surface visible* in the electron microscope.

The process is diagrammed in Figure 10.11a as if it were being carried out on a single ghost embedded in ice. In practice, the procedure involves the cleavage of hundreds of randomly oriented ghosts by a single fracture. Close inspection of the membrane surface revealed in these electron micrographs shows that the surface is covered with globular units spaced at regular intervals (Figure 10.11b). Further studies have shown that these units are entirely absent from some membranes—for example, the membrane sheaths surrounding some nerve cells—and are extremely abundant in others—such as the membranes of red blood cells.

At first, it was assumed that such micrographs show the inner or outer surface of the membrane. However, D. Branton suggested that the cleavage plane passes down the middle of the membrane. This hypothesis has been confirmed by the addition of an etching step to the freeze-cleavage process (Figure 10.12b). In a photomicrograph produced with the freeze-etching technique, two surfaces can be seen—one produced by cleaving and one by etching (Figure 10.12a). The cleaved surface is covered by the globular

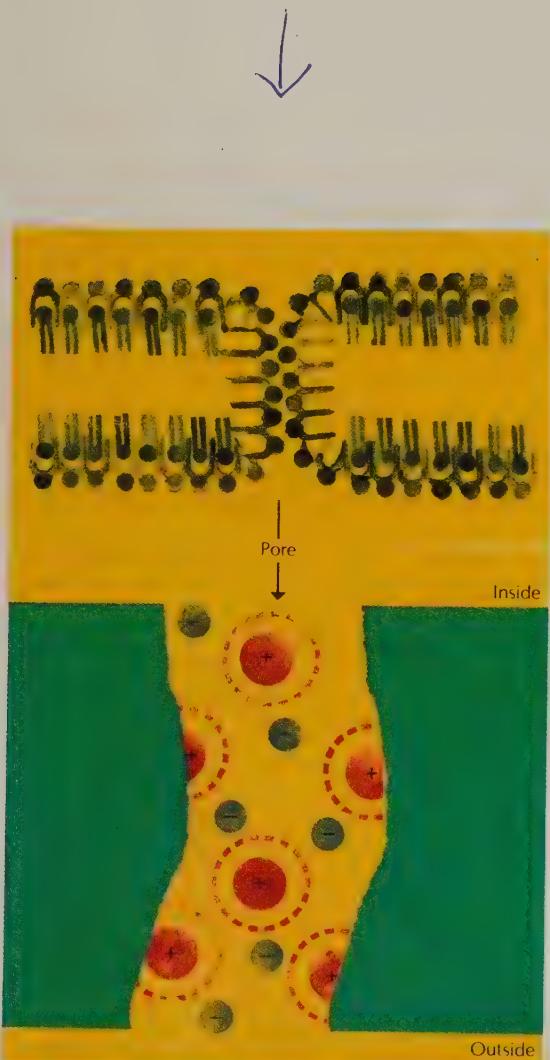


Figure 10.11a (upper left). The effect of the freeze-cleavage technique on cell membranes. This technique involves the rapid freezing of the cells, presumably to prevent alteration of membrane components during preparation. The cells are then sectioned in a cold, evacuated chamber. Samples are prepared for the electron microscope by coating them with a heavy metal.

Figure 10.11b (upper right). Electron micrograph showing a platinum replica of a red blood cell membrane prepared by freeze-cleaving. Note the textured appearance of the surface illustrated. Analysis of the membrane surface revealed in these electron

micrographs shows that the surface is covered with globular units spaced at regular intervals.

Figure 10.12a (lower left). Electron micrograph of a platinum replica of a red blood cell ghost membrane prepared by the freeze-cleavage-etching technique. Comparative analysis of the freeze-cleavage surface and the etched surface is possible.

Figure 10.12b (lower right). The freeze-cleavage technique coupled with the etching process. With the addition of the etching process, both the cleavage surface and the outer surface layer of the membrane are exposed and available for comparative analysis.

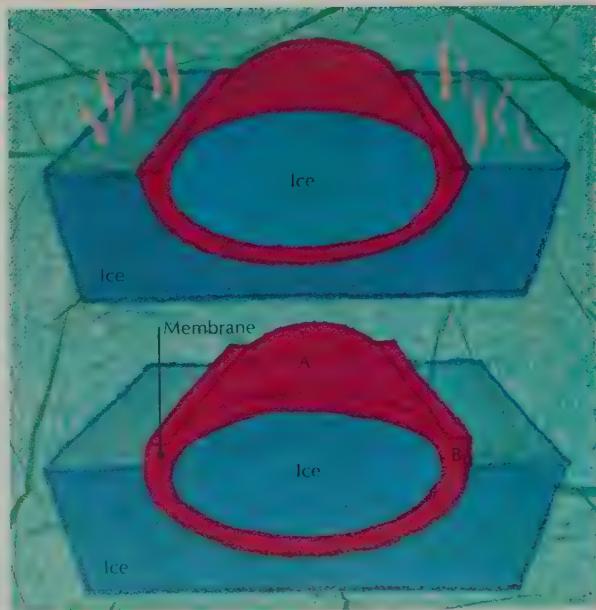
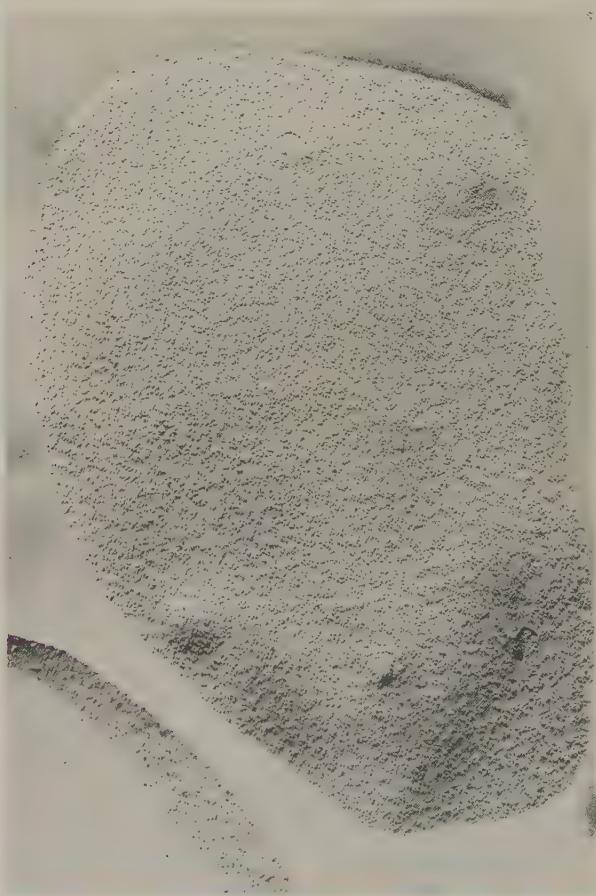
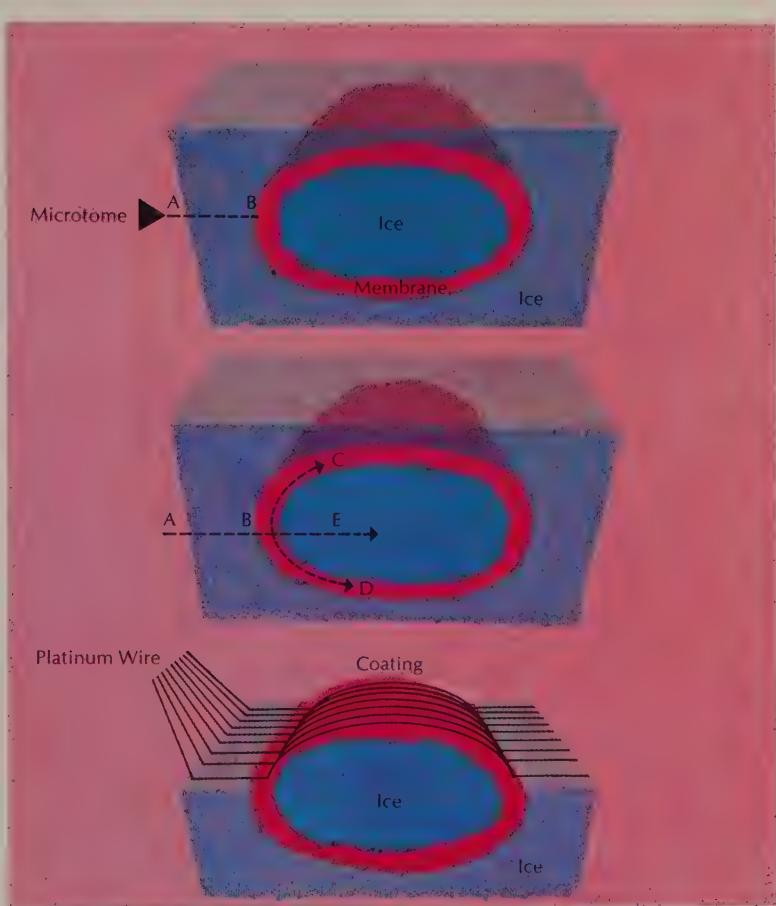
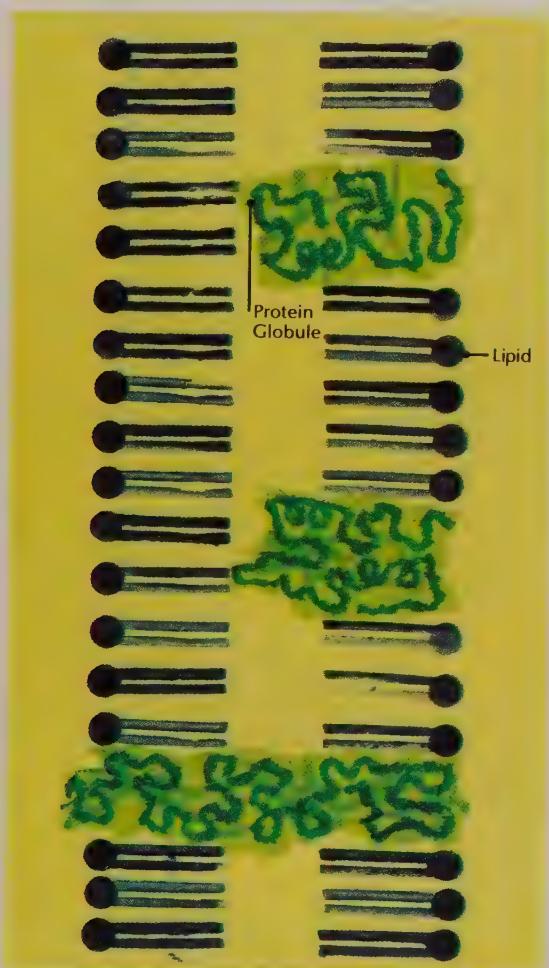


Figure 10.13. Lipid micelle–protein globule model. This diagram depicts the globule as a bilayer arrangement of lipids modified into a micelle configuration surrounding protein molecules. Glycoproteins may be anchored in the membrane by associating with these globule units.



particles, whereas the etched surface is relatively smooth. If the etched surface is the true outer surface of the membrane, the cleaved surface must lie at some level within the membrane. Because the experiment was done with ghosts, the globules cannot be internal structures of the cell.

Further confirmation that the globules lie inside the membrane was obtained by labeling the outside surfaces of the red blood cells with marker proteins. After the freeze-etching procedure, these marker proteins are observed on the relatively smooth, etched surface.

The nature of the globules is not yet known. One possible model of membrane structure incorporating these new findings is shown in Figure 10.13. The globules are indicated, but their molecular structure is unknown. Tentative evidence suggests that part of the globular unit may be composed of protein molecules surrounded by a lipid micelle. The bilayer arrangement of lipids is retained as the basic membrane structure in this model, although some scientists feel that the globules are evidence against the bilayer model. However, the cleavage through the middle of the membrane is similar to the cleavage that occurs when artificial lipid bilayers are subjected to the freeze-cleavage process. The variation in numbers of globules within various kinds of membranes suggests that these globules are specialized structures that are present only in membranes that carry out complex functions. The simpler membranes, such as those of the myelin sheath wrapped around some nerve cells, may be similar in structure to the simple bilayer model. The model shown in Figure 10.13 suggests that parts of the glycoproteins are anchored in the membrane in association with the globular units, but this suggestion also is a tentative hypothesis based upon slight evidence.

TRANSPORT OF MOLECULES ACROSS MEMBRANES

Membranes serve as barriers that separate different compartments within the cell and also separate the cell from its external environment. They are selective barriers, transporting needed substances into cells and unwanted substances or secretions out of them. The concentrations of ions and molecules within a cell are maintained at levels suitable for the processes of life, and the cell's volume is regulated by the amount of material kept within it. Because of their obvious importance to living systems, the mechanisms by which substances are transported across membranes have been under intensive study.

Molecules passing through a membrane may move by one of three basic mechanisms: diffusion, facilitated transport, or active transport. If the concentrations of any particular substance are unequal on the two sides of the membrane, more molecules will strike the membrane on the side of higher concentration than will strike it on the other side. Thus, the overall flow of a substance to which the membrane is permeable will be from the side of higher concentration toward the side of lower concentration, until eventually an equilibrium is reached and the two concentrations separated by the membrane are equal. Transport by diffusion is always passive—that is, it requires no expenditure of energy.

Most biological membranes are semipermeable—that is, permeable to certain molecules and not to others. All, however, are somewhat permeable to water. Because the cell cytoplasm enclosed by the membrane is a highly concentrated solution of various molecules (the membrane is impermeable to most of them), the concentration of water molecules inside

the cell is lower than the concentration of water molecules in pure water. For this reason, most animal cells placed in water burst because water diffuses into the cell in response to the difference in concentration. Diffusion of water or other solvent molecules across a semipermeable membrane is known as osmosis (Figure 10.14). Plant cells, unlike animal cells, have rigid cellulose walls outside their membranes and if immersed in water can withstand the osmotic pressure without bursting. Conversely, higher vertebrate cells shrink in size if placed in sea water, which is about three times saltier than blood. Water leaves these cells because it is more concentrated inside than out. Thus, a fresh-water fish dies of thirst if placed in the ocean.

Nonpolar inorganic molecules (such as oxygen and carbon dioxide gases) and lipidlike substances (such as hydrocarbon anesthetic drugs) can move across membranes at rapid rates and without any apparent selectivity. These nonpolar molecules probably penetrate the membrane directly at any randomly selected site, and their transport is diffusion. On the other hand, the great selectivity with which particular ions and polar molecules

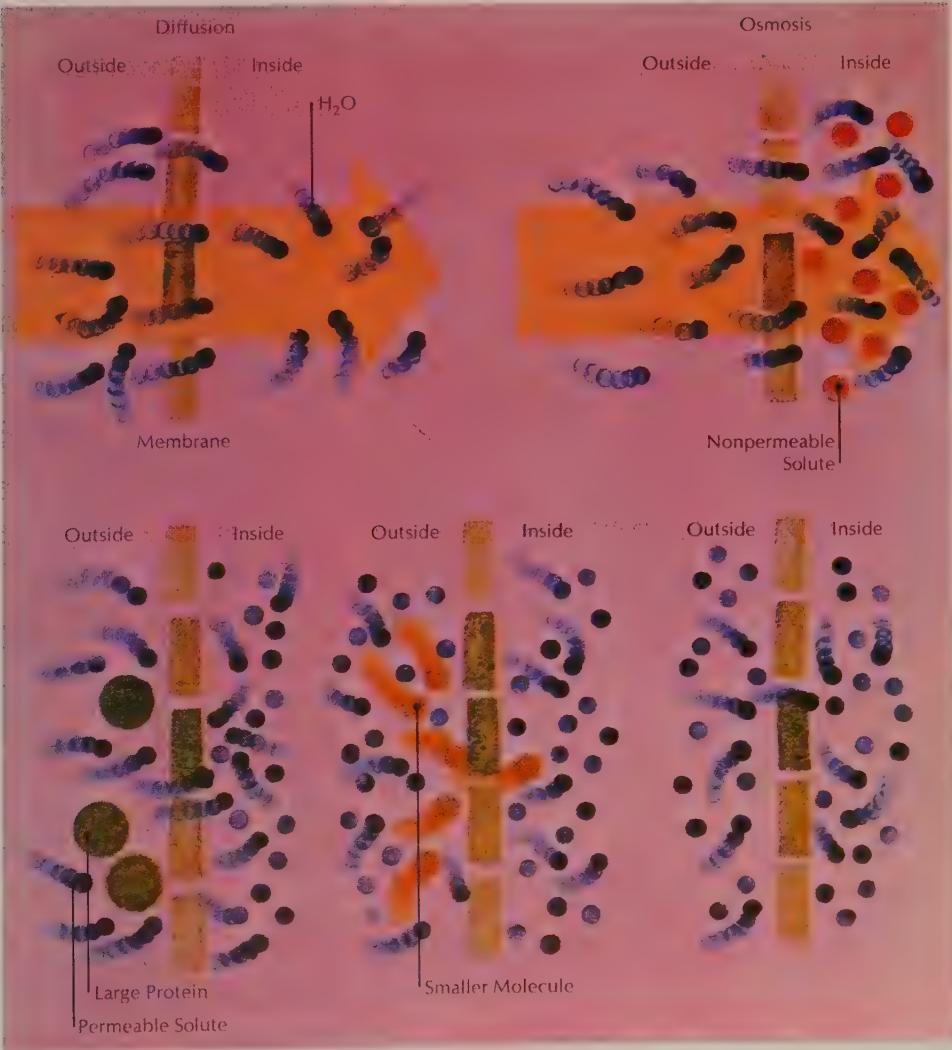


Figure 10.14 (left). A demonstration of osmosis in which a thistle tube filled with a colored sugar solution is immersed in water. The two solutions are separated by a semipermeable membrane. Water moves along its concentration gradient (as the membrane is impermeable to sugar) until an equilibrium stage is reached. This inward pressure raises the sugar solution in the tube.

Figure 10.15 (right). The upper portion of the figure compares diffusion and osmosis. In diffusion, a permeable solute or molecule such as water is able to freely diffuse across the membrane following a concentration gradient. In osmosis, a pressure is built up due to the presence of nonpermeable solutes, resulting in the flow of water across the membrane. The lower portion of the figure shows the effect of solute molecule size on osmotic pressure. A large molecule, such as a protein, inhibits the movement of smaller permeable molecules through the pores, causing a large difference in osmotic pressure across the membrane (inside relative to outside). A smaller solute is able to pass through the pore, resulting in a smaller difference in osmotic pressure. Small molecules (on the right) pass through easily, resulting in no osmotic pressure.

are moved across membranes and the rates at which transport of these molecules occurs are consistent with the view that there are relatively few sites on membranes specialized for the transport of ions and polar molecules.

The lipid layer of membranes behaves as if it has pores, which allow free passage of small polar molecules and through which ions pass in a controlled manner. However, most polar molecules do not diffuse across membranes except as mediated by *carriers*, which are specific for various types of molecules needed by the cell. Such carriers are not visible with the electron microscope, but their existence may readily be demonstrated by physiologists. Recently, biochemists have isolated membrane proteins that appear to be able to function as carriers; like enzymes, these proteins bind the molecules to be transported. It is postulated that molecules can move through the lipid portion of the membrane to deliver their bound substrates to the other side. Transport across a membrane is *passive* if it occurs only from a region of high concentration to one of low concentration and therefore requires no expenditure of energy. If carriers are utilized in passive transport, such transport is said to be facilitated.

Some substances, however, are moved across the membrane toward the side of higher concentration in a direction opposite that expected for passive transport. Some mechanism of *active transport*, utilizing chemical energy to move the molecules against the natural direction of diffusion, must exist. A mechanical model of such a mechanism is shown in Figure 10.16.

The molecular mechanisms responsible for active transport are currently being studied intensively. One mechanism that has been shown to operate for several cases of active transport of sugars involves the immediate phosphorylation by ATP of the sugar as it arrives inside the cell. The resulting sugar phosphate is not bound by the carrier protein, so that the free sugar concentration inside the cell is kept very low. The transport system responds to this difference in concentration by bringing more sugars into the cell. Thus, in a sense, this active transport is really passive.

ENDOCYTOSIS

Another transport mechanism is involved in the passage of very large molecules (such as proteins) or multimolecular particles (such as viruses and bacteria) into a cell. This process of *endocytosis* involves the infolding of the membrane to form a small vesicle within the cytoplasm. This vesicle contains materials trapped from the exterior of the cell. The plasma membrane then fuses across the opening, and the vesicle detaches from the membrane and moves into the cytoplasm.

This process occurs on a relatively large scale in amoeboid cells that feed upon bacteria and other relatively large particles. In such cases, the process is called *phagocytosis* (cell eating) and can readily be observed with the light microscope. The amoeboid cell flows around the particle until it has completely engulfed the particle. The cell membrane then fuses, and the vesicle containing the particle moves into the cytoplasm, where digestive enzymes are introduced into the vesicle. The process of phagocytosis is used by amoeboid cells (such as some white blood cells) in animal bodies; these cells remove many of the bacteria, viruses, and other foreign particles that find their way into the intercellular spaces of the body.

It has also been observed that some cells take minute droplets of extracellular fluid into the cytoplasm. Although the droplets are so tiny that they

Figure 10.16. A mechanical model of active and facilitated transport. Parts A and B are two hypothesized mechanisms for active transport. As the solute is being transported against a concentration gradient (more solute molecules on the inside than outside), energy in the form of ATP breakdown is required. In part A, a mobile carrier-solute complex moves the solute into the cell. In part B, a series of fixed carriers relay the solute into the cell. Parts 1, 2, and 3 are hypothesized mechanisms for facilitated transport. In this system, solute movement is along a concentration gradient (diffusion—no energy requirement) but polar molecules

require carriers because they do not readily diffuse across the membrane. Part 1 shows a carrier protein within the membrane in a globular conformation. An exposed terminal peptide complexes with the solute and, through a conformation shift followed by a dissociation of the complex, releases the solute into the cytoplasm. Part 2 shows a mobile carrier analogous to that in the active transport model. Part 3 depicts a carrier model in the form of a rotating mechanical device. Transportation of the solute involves a complexing and 180° rotation of the carrier into the cell, where the solute diffuses into the cytoplasm.

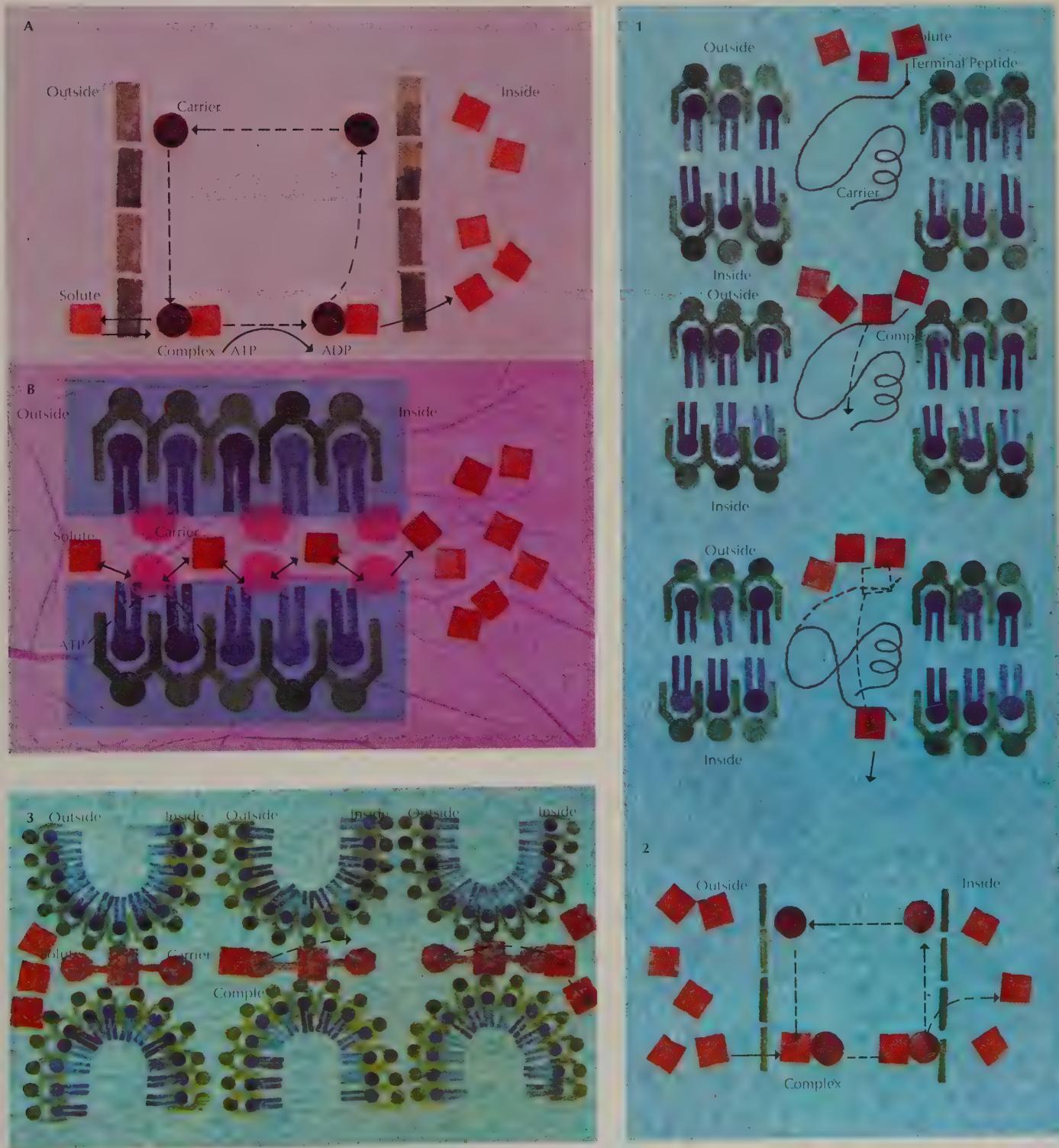
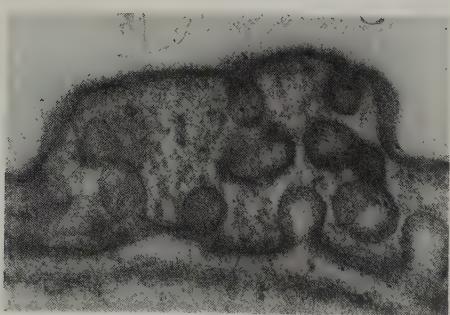
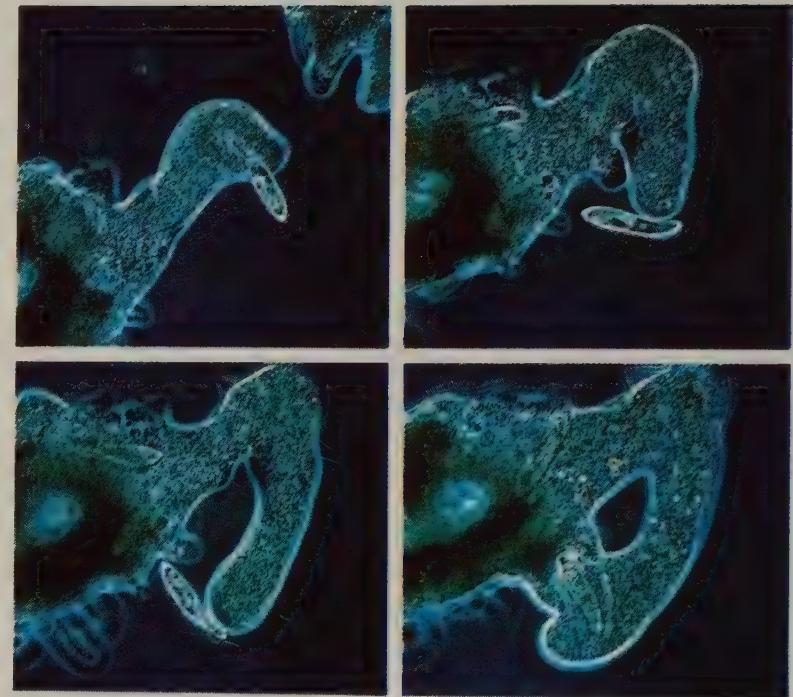


Figure 10.17 (above). Phagocytosis in the amoeba *Chaos chaos*. To capture its prey, the amoeba extends pseudopodia to encircle and engulf it. The cell membrane then fuses, and the vacuole containing the captured prey moves into the cytoplasm, where digestion takes place. (Eric Gravé)

Figure 10.18 (below). Electron micrograph of capillary endothelium. The invaginated membranes are forming pinocytic vesicles.



can barely be seen in the light microscope, what could be seen suggested that the mechanism of this process, called *pinocytosis* (cell drinking), is similar to that of phagocytosis. Electron micrographs have confirmed that pinocytosis does involve the infolding of minute vesicles and the subsequent pinching off of those vesicles into the cytoplasm.

Part of the membrane buckles in by some unknown mechanism, entrapping a portion of the external solution that may contain some large molecules. The pinched-in membrane forms a cuplike structure, which continues to deepen until the edges fuse to form a closed vesicle. The vesicle detaches from the membrane and moves into the interior of the cytoplasm with its contents. The large molecules may be broken down by enzymes to units small enough to pass through the vesicle membrane into the cytoplasm, or the vesicle membrane may be broken down to permit the large molecules to enter directly into the cytoplasm.

A similar process in reverse is used by certain cells as a way to excrete protein. Proteins produced for secretion in gland cells are packaged in vesicles by the Golgi complex. These vesicles move to the plasma membrane and pass through a series of stages similar to pinocytosis, but in reverse. The proteins thus are released outside the plasma membrane.

Processes of endocytosis presumably involve the use of ATP energy to rearrange the membrane conformation. There is evidence that the process may be triggered by the presence of protein molecules in the solution outside the cell, but the triggering and infolding mechanisms remain unknown.

MEMBRANES: THE NEXT CHALLENGE FOR MOLECULAR BIOLOGY

Within the cell, membranes function as parts of chemical factories—as floor space for the organized arrangement of systems of enzymes. They act

as phase separators, creating and maintaining various volumes of different chemical compositions. The cell membranes of nerve fibers act to transmit electrochemical signals.

In each of these situations, the membranes react with great sensitivity to physical and chemical factors in the environment. This sensitivity reaches its greatest development in the sensory receptor cells, which utilize membrane structures to convert various forms of energy from the external environment into nerve signals. These mechanisms will constitute the principal challenge for the next phase of molecular biology.

Current ignorance of receptor mechanisms and membrane structure and synthesis is comparable to the ignorance of the molecular basis of genetics 30 or 40 years ago, when biologists knew that there are genes, that the genes are located on chromosomes, and that the genes are arranged in a linear order. They also knew that the chromosomes contain proteins and nucleic acids, but for several decades biologists assumed that the proteins represent information storage and that the nucleic acids—with their apparently limited and monotonous structure—represent a structural backbone. With respect to membranes, there now exists a similar degree of uncertainty as to the relative roles of protein and lipid: Which one is primarily involved in structural support and which one is primarily involved in the sensitive reactions of the membrane?

Just as the deciphering of the molecular mechanism of the linear gene represented the major biological breakthrough of the first part of this century, the understanding of the molecular mechanisms of two-dimensional membranes may represent the next step for molecular biology.

FURTHER READING

The general references on eucaryotic cell structure listed in Further Reading for Chapter 7 will provide more information on membrane structure. Also of interest are articles by Danielli and Davson (1935), Dippell (1962), Fawcett (1958), Hokin and Hokin (1965), Holter (1961), Robertson (1959, 1960, 1962), and Solomon (1960). Bangham (1971) provides an interesting example of the current debate over various models of membrane structure. Korn (1966) offers a summary of objections to Robertson's unit membrane theory.

11

Specialized Cells and Tissues



Although unicellular organisms are extremely small, they exist in a great variety of shapes and structures. There are thousands of species of prokaryotes, unicellular algae and fungi, and protozoans, or protists. Each species differs from the others in structural details, metabolic processes, or life cycle. Autotrophic organisms require only inorganic nutrients and synthesize their own organic materials. Heterotrophic organisms survive in a wide variety of environments in which they can obtain various organic materials as food. Some organisms are encased in hard shells or slimy coatings. Others utilize flagella or amoeboid extensions for motility. Some are attached to fixed surfaces. Yet each unicellular organism must possess a full complement of structures or organelles if it is to survive independently.

Most plants, animals, and fungi larger than microscopic size are multicellular organisms, each made up of thousands, millions, or trillions of individual cells functioning cooperatively to maintain integrated processes throughout the entire organism. Therefore, most cells in such organisms are highly specialized. Various parts of the "typical" eucaryotic cell structure are modified, missing, or increased in size and number to adapt that particular cell to the performance of specific functions in the organism. These individual cells have lost their ability to survive independently (except in laboratory cultures, where they can be supplied with the nutrients and environment normally provided by other cells). Their structures have become so specialized that they must depend upon other cells in the organism for essential life processes.

The line that separates unicellular from multicellular organisms is not always sharp. Many kinds of organisms lie somewhere between fully independent single cells and fully interdependent cells of a multicellular organism. These intermediate cell forms are of particular interest to biologists. By studying them, biologists hope to learn what kinds of processes have led to the evolution of multicellular organisms.

Some unicellular organisms join together to form filaments or bodies, but the cells in these groupings retain their individuality. Any cell separated from the group can survive independently. Among some algae and fungi, the fusion of two or more cells forms a single, large, multinucleate cell—such organisms are called coenocytes (shared cells). The slime molds, or *Myxomycophyta*, are of particular interest because they can exist in various parts of their life cycle as coenocytic bodies, as multicellular structures with cellulose cell walls, and as independent cells (Figure 11.1).

Some protists, such as *Pleodorina* and *Volvox*, form colonies composed of large numbers of cells joined together. The individual cells of *Volvox* are linked by strands of cytoplasm (Figure 11.2). Most cells in a *Volvox* colony are flagellated, chloroplast-containing cells. The vegetative cells of *Volvox* are embedded in a matrix of extracellular material and are incapable of individual motility or of reproduction. Only a few reproductive cells in one part of the colony retain the capability to reproduce by either sexual or asexual processes. *Volvox* appears to be a very primitive form of multicellular organism with the simplest kind of cell specialization.

Of greater complexity are the sponges, which are composed of groups of cells with a variety of specialized functions: food-gathering cells, food-transporting cells, skeleton-making cells, maintenance and repair cells, reproductive cells, and cells that attach the entire creature to the surface on which it lives (Figure 11.3). The different kinds of cells in a colonial protist

Figure 11.1 (left). An adult slime mold with developing sporangia, or fruiting bodies. (Courtesy Carolina Biological Supply Company)

Figure 11.2 (right). *Volvox*, a colonial protistan, consisting of many flagellated cells. Cells are arranged in a single layer, with each cell in direct contact with the external environment. Located in the interior of the sphere are daughter colonies, produced by the fusion of gametes from specialized reproductive cells. (Courtesy Carolina Biological Supply Company)

Figure 11.3 (below). Photograph of a sponge taken at Cayman Island, British West Indies. Hidden in the body wall (diagram) are hundreds of tiny porelike openings, through which water is drawn into the internal cavity of the animal. Particulate matter in the water is trapped by the flagellated collar cells, and water passes on through the animal.

or a sponge are not completely interdependent. Any one kind of cell can become modified to perform the function of another. Because the cells retain some degree of independence, these organisms are regarded as simple or primitive multicellular organisms.

The corals, jellyfish, sea anemones, and other coelenterates represent a more complex or advanced form of multicellularity. They exhibit cell specialization of a fairly high degree—such as sensory cells of various types, a primitive sort of nerve cell, muscle cells, and the highly specialized stinging cells called nematocysts. In addition, the coelenterates show a simple form of tissue organization (Figure 11.4). The outer surface of the organism is covered with a layer of specialized cells that form an *ectodermal* (skin) tissue. The inner surfaces are lined with an *endodermal* tissue composed of different specialized cells.

In the plant kingdom, organisms also can be arranged in a sequence of increasing cell specialization and tissue development. Between the groups of unicellular algae and the simple multicellular plants are a variety of intermediate forms that can be regarded either as colonies of unicellular organisms or as simple multicellular organisms.

Among the coelenterates are other organisms that might be regarded as colonies of specialized multicellular individuals or as primitive examples of organisms with relatively independent organs. In the Portuguese man-of-war, for example, there are individual organs specialized to provide services of locomotion, flotation, feeding, and reproduction for the entire colony or single organism (Figure 11.5). Most of the more complex animals possess a body organization in which tissues make up a great variety of dif-

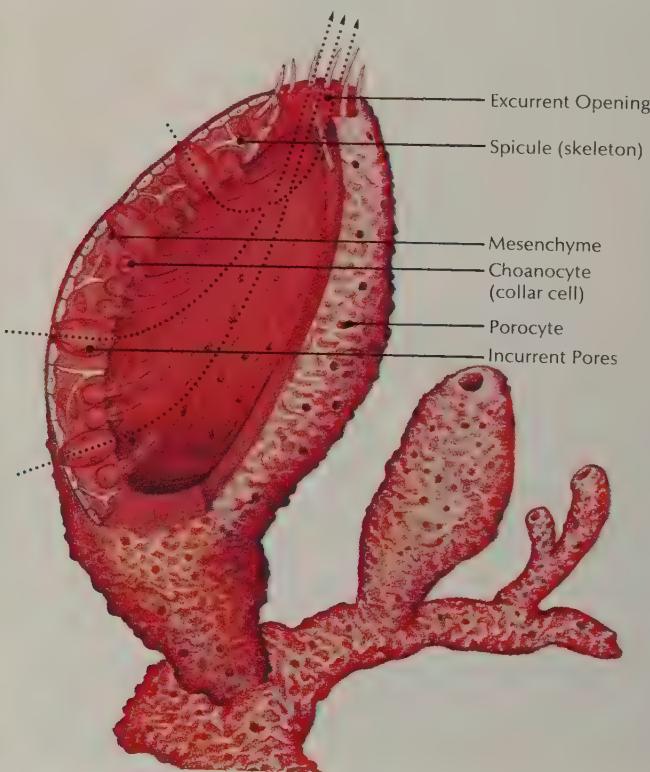


Figure 11.4 (above). The coelenterates show a simple form of tissue organization. The outer surface is covered with ectodermal cells, and endodermal cells line the inner surface.

Figure 11.5 (lower left). Portuguese man-of-war. This coelenterate colony is made up of hundreds of individuals in four distinct classes based on function: feeding polyps, protective or defensive polyps, float polyps, and reproductive polyps.

ferent organs, each specialized for a particular task in the life of the organism. In complex plants, on the other hand, there are relatively few organ structures, but many functions are carried out by specialized tissues.

Although the intermediate kinds of organisms are of great interest because they offer clues about the ways that cell specialization, tissues, and organs might have evolved from simpler organisms, it will be useful here to examine some of the specialized cells and tissues of more "advanced" multicellular plants and animals. Such an organism is a collection of cells of many different structural types and functions. Cells of similar type and function are organized into tissues. Various tissues may be coordinated to form an *organ* that carries out a more complex yet unified function. A survey of some major types of plant and animal tissues reveals some of the ways in which cell specialization makes multicellularity possible.

ANIMAL EPITHELIAL TISSUE

The epithelial tissue of an animal is an aggregation of cells that cover surfaces and thus "contain" the organism (Figure 11.6). Epithelial cells must adhere tightly to one another to form a continuous sheet. If they are damaged, they must replace themselves rapidly in order to maintain the integrity of the surface. The ways in which epithelial cells are attached to one another serve as good examples of the various forms of cell-cell attachment found throughout multicellular organisms.

Between adjacent cells is a thin layer of *intercellular cement*, whose major component is a mucopolysaccharide secreted by the epithelial cells themselves. The layer of epithelial cells rests upon a basal lamina, which

Figure 11.6 (lower right). Simple, cuboidal epithelium in the lining of a kidney tubule. Such cells are bound together by a thin layer of intercellular cement and an underlying basement membrane composed of collagen fibers embedded in a matrix. (Courtesy Carolina Biological Supply Company)

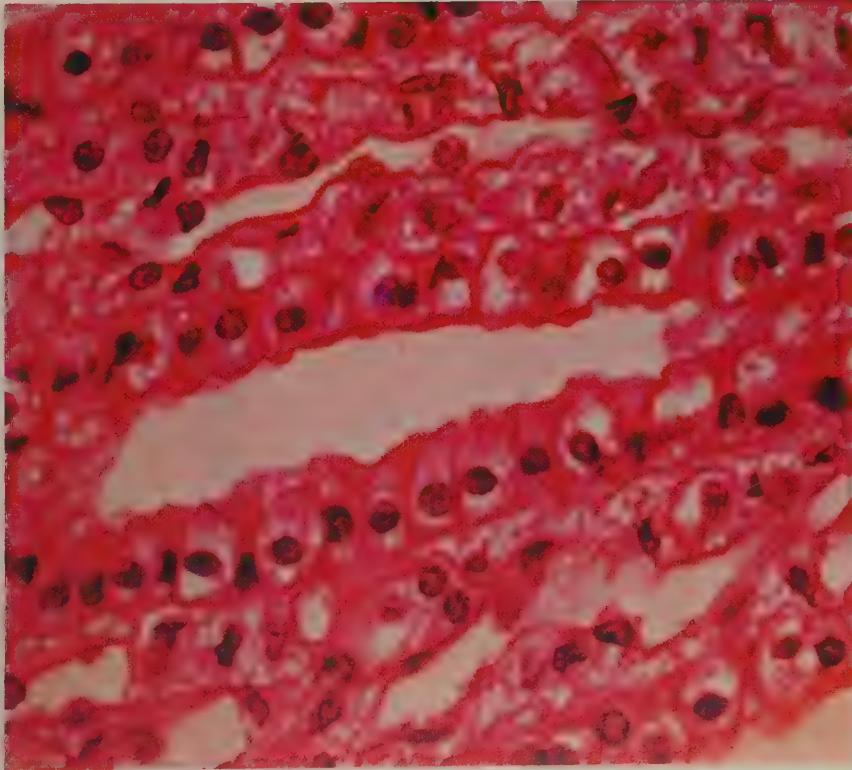


Figure 11.7. Photograph of the bioluminescent feathered sea star. Bioluminescence in this organism produces the golden glow effect and is due to cells that are able to convert chemical energy into light. The emission of light is dependent upon nervous stimulation of specialized cells in light-producing organs.

consists of collagen fibers embedded in a matrix. The collagen and the matrix also are secreted by the epithelial cells. The collagen fibers add strength to the tissue.

Many mechanical attachments exist between cells. The simplest of these attachments are *interdigitations*, or tongue-and-groove associations, of the membranes of adjacent cells. Because interdigitations increase the area of surface contact between cells, the amount of intercellular cement holding the cells together is also increased. Other forms of mechanical attachment are provided by specialized structures such as *desmosomes*, which are thought to act as attachments that anchor cells.

The skin that covers the outer surface of an animal is a good example of exterior epithelial tissue (Montagna, 1965). It serves primarily to hold the organism together and to protect it from the external environment, but its properties vary from place to place to meet particular needs of different parts of the organism.

Many epithelial cells are specialized to secrete substances onto the surface of the organism. One basic kind of secretion is *mucus*, a mucopolysaccharide that forms a protective covering over the outer cell surface. Certain cells in the skins of many organisms secrete poisons of various sorts; these cells apparently have evolved from mucus-secreting cells. Epithelial cells lining the interior of the digestive tract secrete enzymes that break down molecules of ingested food so that the material can be absorbed by other cells of the intestinal epithelium.

Sensory reception is another kind of specialization of epithelial tissues. Sensory receptor cells are triggered by various changes in the external or internal environment. Some sensory receptor cells are modified ciliated cells in which the cilia form the actual receptor organelles that respond to the stimulus from the environment. The modified cilia are instrumental in translating this stimulus into an electrochemical change that can be transmitted to the central nervous system (Chapter 26).

Some epithelial cells are specialized as pigment-bearing cells, which either protect the organism by absorbing harmful radiation or conceal it in



Figure 11.8 (left). Allium leaf epidermis. Note the thick, dark cell walls and the relatively large central vacuoles of these cells. Epidermal cells such as these typically secrete a waxy cuticle to prevent dehydration and to protect the plant from mechanical injury. Also visible in this photograph are numerous stomata-guard cell complexes, which serve to regulate plant transpiration. (Courtesy Carolina Biological Supply Company)

Figure 11.9 (right). Photograph of a partial section through a woody stem. The periderm layer consists of cork cells and their products, which comprise bark.

one way or another from predators (protective coloration). Bioluminescent cells, which are capable of converting chemical energy into light, are another example of epithelial specialization involving pigments (Figure 11.7).

Other forms of specialization exist within epithelial tissues, but these examples give some idea of the complexity of functions that epithelial cells serve and of the corresponding range of specialized cell structures that exist.

PLANT SURFACE TISSUE

Like the epithelial tissue of an animal, the surface tissue of a plant serves as a protective covering for the outer surface of the organism. *Epidermal tissue* covers the surfaces of roots, stems, and leaves in most plants (Figure 11.8). Like the skin of an animal, epidermal tissue is made up of a thin layer (usually one cell in thickness) of flattened, interdigitating cells. Most epidermal plant cells have thickened outer walls, relatively large central vacuoles, and a relatively small amount of cytoplasm. Many of the epidermal cells on the aerial parts of the plant secrete cuticle, a water-resistant, waxy substance that forms a surface layer, protecting the plant from dehydration and invasion by parasites.

The epidermal cells of the roots have no cuticle covering but are specialized to absorb water. Some of the root epidermal cells form long, hair-like extensions into the soil. Some of the epidermal cells of the aerial part of the plant may be specialized to form spines, hairs, or glands, all of which play roles in the protection or functioning of the plant.

In adult trees, the epidermis is replaced by another tissue, the *periderm*, which is composed of cork cells (Figure 11.9). Cork cells secrete a waterproof coating of suberin and then die, so that the surface of the periderm is a thick layer of hollow, water- and injury-resistant cork cells. The periderm forms the familiar bark of a tree.

ANIMAL SUPPORTIVE TISSUE

The body of an animal is supported by a number of different forms of tissue. Skeletal tissues such as chitin, cartilage, and bone provide a more-or-less

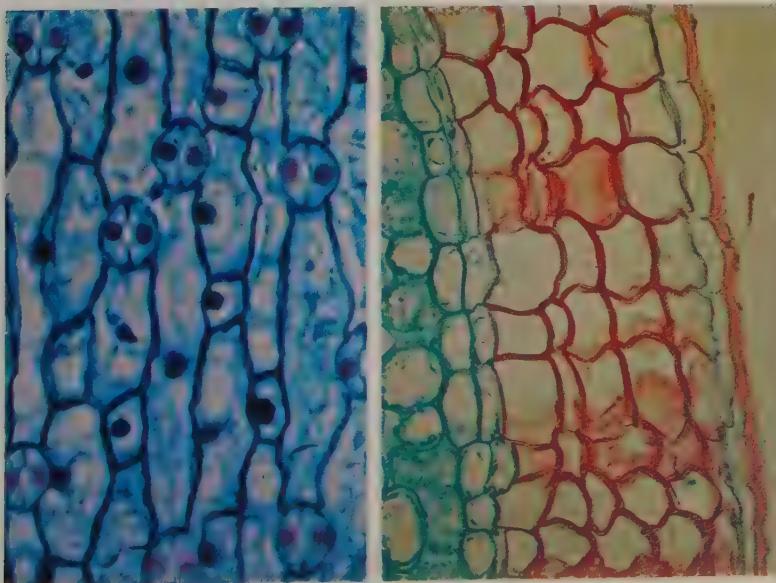


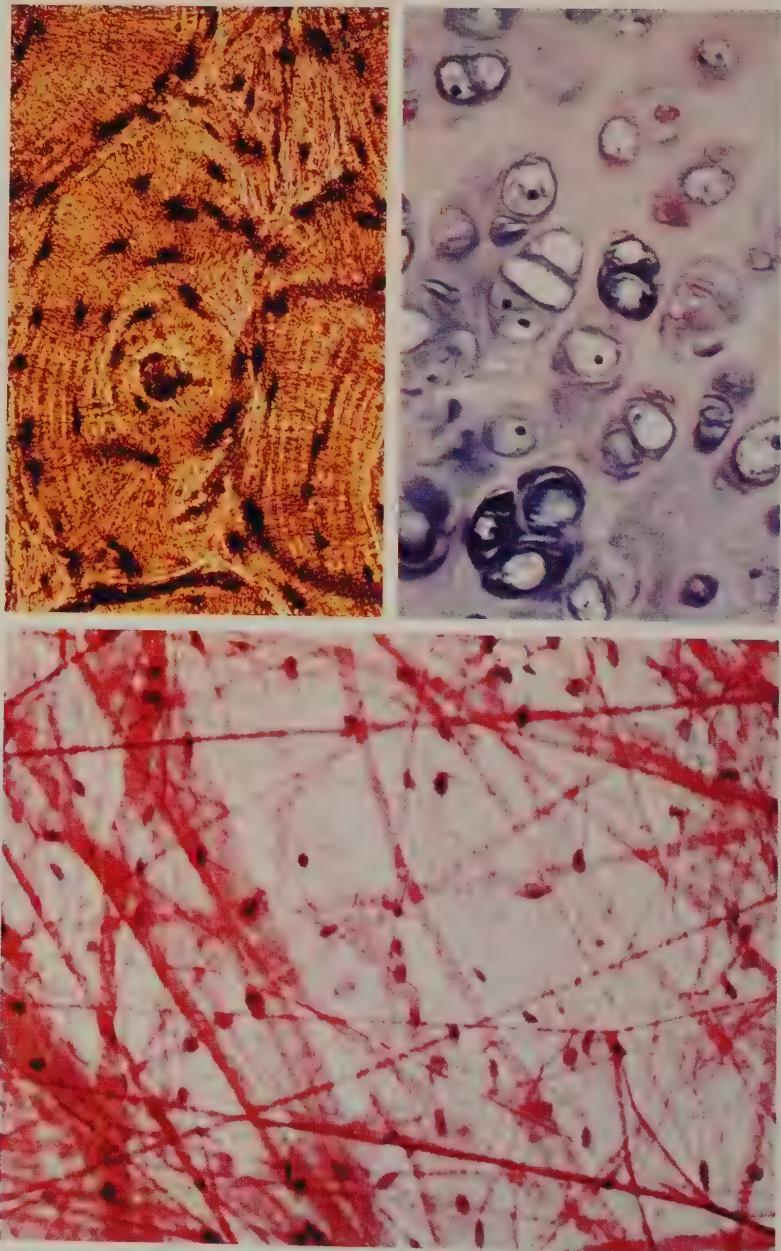
Figure 11.10 (left). Human bone cross section.
(Courtesy Carolina Biological Supply Company)

Figure 11.11 (upper right). Hyaline cartilage. Note the clear, almost transparent matrix in which are embedded lacunae, or cell spaces. Each lacuna contains one or more chondrocytes, or cartilage cells.
(Courtesy Carolina Biological Supply Company)

Figure 11.12 (below). Fibroelastic connective tissue.
The most conspicuous components of this tissue type
are the large number of threadlike fibers, some of them
tough and strong (collagen) and others elastic and
flexible (elastin). (Courtesy Carolina Biological Supply
Company)

rigid framework that gives structural support and provides a system of levers by which the organism can move parts of its body. The cells of skeletal tissues are specialized in the secretion of particular kinds of extracellular materials (Figure 11.10).

Structurally, connective tissues are made up of rather generalized cells, called *fibroblasts*, which synthesize extracellular fibers such as collagen and elastin (Figure 11.12). These fibers bind tissues together, provide support for the tissues and for the organs formed from them, and join skeletal members to each other and to the muscles that move them. These



connective fibers are an extremely important part of the structure of an animal. Collagen, in fact, is the most abundant single protein in the human body and the most common protein in the entire animal kingdom.

PLANT MERISTEMATIC AND FUNDAMENTAL TISSUES

The body of a plant contains a number of regions of nonspecialized cells that retain the capability of division to produce a continuous supply of cells that can specialize to form new tissues. The undifferentiated tissues that are sites of active cell division are called *meristems* (Figure 11.13). Although there is great variation in characteristics among meristematic cells, most tend to have thin walls and to be small, closely packed, and filled largely with cytoplasm. Meristematic tissues form the growing tips of roots and shoots. In many plants, there are layers of meristematic tissue near the surface of branches and stems. These lateral meristems enable the plant body to continue to grow thicker throughout its life. The existence of meristematic tissues that continue to produce new cells (and therefore new tissues and organs) throughout the life of a plant is one of the major differences between plants and animals. In most higher animals, the vast majority of tissues and organs are formed early in life, and most of the cells of the body lose the ability to divide.

Most of the plant body is made up of *fundamental tissues*, each of which is composed largely of a single kind of specialized cell. *Sclerenchyma* is a tissue composed of cells that secrete thick cell walls rich in lignin and then die. Thus, the mature sclerenchymatic tissue is composed of a network of lignified walls with minute pores that were once the cell interiors. Some sclerenchymatic cells become elongated into fibers such as those of flax and hemp. *Sclerenchyma* serves primarily as a supportive tissue for the plant body. *Collenchyma* is another supportive tissue with thickened cell walls, but the collenchymatic cells remain alive through most of the plant's life span.

Much of the body of a plant—particularly of the lower plants—is made up of a tissue composed of living, nonspecialized, thin-wall, large-vacuole cells that may occasionally begin meristematic activity or cell specialization to form other tissues. This *parenchymatic* tissue gives support and rigidity to the plant body and serves as a site for storage of nutrients and water.

ANIMAL NERVOUS TISSUE

As an animal embryo develops, some epithelial cells specialize to form nervous tissue. Comparison of more primitive organisms suggests that nervous tissue evolved through further specialization of epithelial tissue. Nervous tissue, however, has acquired such a distinctive and specialized function that it is regarded as a tissue in its own right. The individual cells of nervous tissue serve to integrate the organism's activities at all levels.

Nervous tissue is made up of two types of cells, *neurons* and *interstitial cells*, both of which are characterized by fairly extensive fiberlike projections. Neurons create the electrochemical signal called the *nerve impulse* and transmit this impulse to other cells—neurons, muscle cells, or gland cells. Interstitial cells play a supportive role—binding neurons together, forming insulating coverings for the nerve fibers, or providing the neurons with certain necessary nutrients.

Neurons represent an extreme example of cellular specialization. By the time a vertebrate animal is born, nearly all its nerve cells have been formed.

Figure 11.13. Photograph of a longitudinal section through a shoot apex, showing leaf buds and the apical meristem region. Meristematic tissue is characterized by its ability to undergo repeated mitotic divisions, thereby accounting for plant growth.



Neurons retain their nuclei, and their DNA continues to code for the production of enzymes and other proteins needed in various neural activities. Other portions of the DNA message are somehow irreversibly "switched off," so that a fully specialized neuron is incapable of mitosis and therefore cannot divide (Chapter 13). If a nerve cell dies, it can never be replaced. An individual human acquires his greatest number of neurons within the first few years of his life. Thereafter, the number of neurons in the body decreases steadily. Fortunately, the neurons of the central nervous system are well protected from physical and metabolic damage by the skull and vertebral column and by a peculiar physiological barrier called the blood-brain barrier, so that neuron degeneration probably does not become a significant phenomenon until late in life. Aging processes that damage the heart and the blood vessels can cause the death of neurons as a secondary effect because neurons require a constant supply of oxygen and glucose, and interference with these supplies brings about neuron death. The death of significant numbers of neurons may bring about progressive senility or paralysis.

A typical neuron consists of a cell body and one or more cytoplasmic extensions (Figure 11.14). The cell body contains the nucleus, mitochondria, Golgi apparatus, ribosomes, smooth and rough ER, and clusters of microtubules, which often appear in the light microscope as cytoplasmic fibers called *neurofibrils*. Also, there are structures called *Nissl bodies*, which are layers of rough ER cisternae.

The extensions from the cell body are of two types: the *axon*, which is relatively long; and the *dendrite*, which is relatively short and branched. Most neurons have multiple dendrites and a single axon. In most neurons, the axon extends from one side of the cell, and dendrites extend from many parts of the cell body. In sensory neurons, the cell body is located near the center of the axon, and the dendrites are attached to the end of the axon where impulses originate (for example, at sensory receptors in the skin). The transmitting end of the axon bears a number of fine branches called the *axonal arborization*.

The axons of many neurons are wrapped in a *myelin sheath*, another example of cell specialization. The myelin sheath is composed of the membranes of interstitial cells and is wrapped around the axons to form several concentric layers. The cells that wrap around peripheral nerve fibers—that is, nerve fibers outside of the brain and spinal cord—are called *Schwann cells* (because they were first described by Theodor Schwann). The cells that wrap around axons within the central nervous system (brain and spinal cord) are called *oligodendrocytes*. The axon, with its surrounding sheath, is called a *nerve fiber*.

Some axons are exceedingly long. For example, a nerve terminating in a blood vessel in the foot of a giraffe has its cell body and dendrites in the spinal cord. Its axon is a single, continuous fiber, perhaps eight or nine feet in length, extending from the spinal cord to the end of the foot. Thousands of Schwann cells are required to wrap a myelin sheath around such a long nerve fiber. Between each pair of successive Schwann cells is a gap called a *node of Ranvier*. Such nodes are not as conspicuous between the oligodendrocytes of the central nervous system.

ANIMAL MUSCLE TISSUE

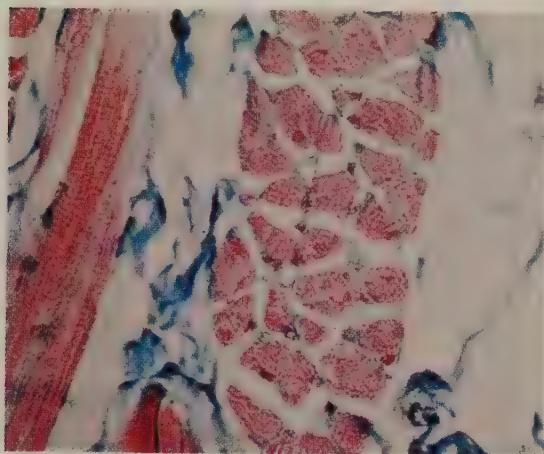
Muscle cells are specialized for the function of contraction. The simplest muscle cells are found in the coelenterates, where certain epithelial cells

Figure 11.14. Diagram of a typical neuron.



Figure 11.15 (above). Skeletal muscle. Cross striations (alternate dark and light bands), resulting from myofilament arrangement, may be seen in the longitudinal sections. (Courtesy Carolina Biological Supply Company)

Figure 11.16 (below). Electron micrograph and diagram of skeletal muscle. Note the dark A-bands that alternate with the light I-bands. In the center of each A-band is a light H-band, and in the center of each I-band is a thin, dark Z-line. Also visible is the sarcoplasmic reticulum.



have two extensions from the cell base running parallel to the surface of the organism. Within these extensions are fibrils that respond to nervous stimulation by contracting.

Three different types of muscle tissue are found in higher animals: skeletal, cardiac, and smooth muscle. Skeletal and cardiac muscle cells are characterized by transverse lines, or *striations*, which are visible in the light microscope. Smooth muscle lacks these striations.

The specializations of muscle cells are so extreme and of such fundamental importance to animal organisms that they merit rather detailed consideration.

Skeletal Muscle Cells

The cells of skeletal muscle appear as fibers in the light microscope. A single fiber measures about $100\ \mu$ in diameter and a few millimeters to a few centimeters in length. Each fiber shows a banded pattern of transverse striations with dark bands, or *A-bands*, alternating with light bands, or *I-bands* (Figure 11.15). In the center of each I-band is a thin, dark line (*Z-line*), and in the center of each A-band is a light *H-band*. The repeating unit of this pattern is called a *sarcomere*. Each fiber is a single cell with several nuclei surrounded by a thin but tough cell membrane called the *sarcolemma*. When a nerve impulse triggers the muscle cell, the cell contracts. Biochemical analyses show that about 20 percent of the weight of a muscle cell is protein; the balance is water and dissolved substances.

The light microscope reveals each cell to be composed of a number of *myofibrils* about $1\ \mu$ in diameter. The myofibrils also are striated; in fact, the striations of the fiber are due to the aligned striations of its myofibrils. Between the myofibrils in the cell are mitochondria and a complex membrane structure called the *sarcoplasmic reticulum*. The nuclei lie near the edge of the cell, adjacent to the sarcolemma.

Electron micrographs of myofibrils reveal that they are made up of still smaller *myofilaments*. There are two kinds of myofilaments in each myofibril; the thicker filaments are about 100 \AA in diameter and $1.5\ \mu$ in length; the thinner filaments are about 50 \AA in diameter and about $2\ \mu$ in length.

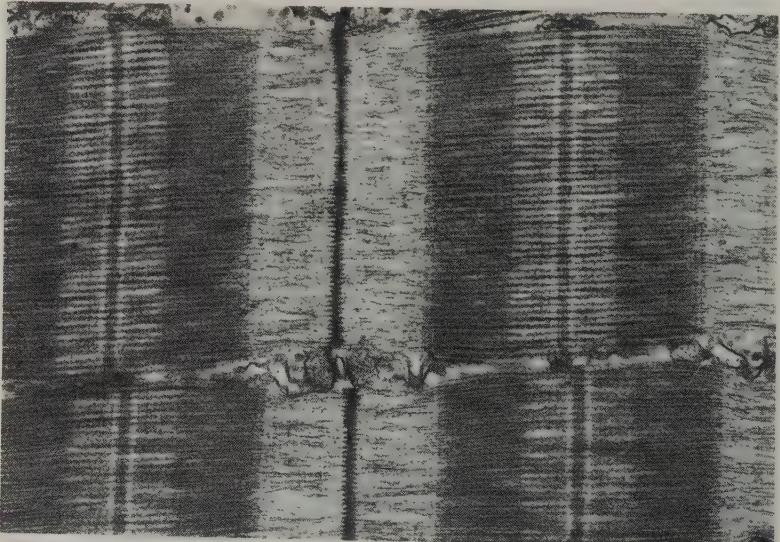
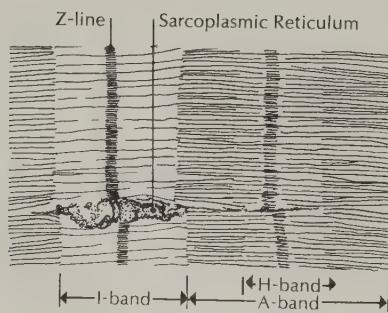


Figure 11.17 (above). Human cardiac muscle. Note the conspicuous cross striations and intercalated discs (opaque cross bands) characteristic of this type of muscle tissue. (Courtesy Carolina Biological Supply Company)

Figure 11.18 (below). Smooth muscle. Note the obvious lack of cross striations and the large, centrally placed nuclei, typical of this type of muscle tissue. (Courtesy Carolina Biological Supply Company)

The dark A-band is made up of both thick and thin filaments; the light I-band is made up of thin filaments alone, whereas the light H-band is made up only of thick filaments. The Z-line is a narrow zone of very dense material not arranged in filaments (Figure 11.16). In a section cut across the A-band, the thick and thin filaments can be seen to be arranged in a regular pattern.

Electron micrographs at high magnification show that the thick and thin filaments are linked by cross-bridges, which extend from the thick filaments at intervals of about 60 Å. These cross-bridges probably play an important role in the process of contraction (Chapter 25).

The protein in the myofibrils is composed chiefly of myosin and actin, with smaller amounts of tropomyosin. Myosin makes up about one-half of the protein in the myofibril. When myosin is extracted from the myofibril, this protein forms filaments about 0.2μ in length and 100 Å in diameter. These filaments, similar in appearance to the thick filaments of the myofibrils, have numerous side projections that appear similar in spacing to the cross-bridges of the myofibrils (H. E. Huxley, 1965). The hypothesis that the thick filaments are composed of myosin is confirmed by the observation that the A-bands disappear from a myofibril when the myosin is extracted from it.

The other major protein of myofibrils, actin, forms a globular molecule in pure solution. When placed in a solution with salt and ATP concentrations similar to those of the muscle cell, actin forms long fibers. The assumption that actin forms the thin filaments is confirmed by the observation that the structures of the I-band disappear when actin is extracted from the muscle cell.

Less than 3 percent of the protein in the myofibril is tropomyosin. This protein has a molecular weight much smaller than those of actin and myosin; it is thought to make up the structure of the Z-lines. Under the electron microscope, crystals of tropomyosin have structures similar to that of the Z-line (H. E. Huxley, 1965). The sarcoplasmic reticulum, a modified type of ER, forms a regular structure related to the myofibrils. Tubules of this structure lie along the surface of the A-band region, with small, saclike structures in the I-band region.

Cardiac Muscle Cells

Cardiac muscle, found only in vertebrate animals, forms the bulk of the heart. It also may extend a short distance along the walls of the large arteries that emerge from the heart. A modified type of cardiac muscle constitutes the heart's so-called neuromuscular tissue, which functions as an internal impulse-conducting system within the organ.

Cardiac muscle is intermediate in some respects between skeletal muscle and smooth muscle. It is striated like skeletal muscle but is not under voluntary control.

Smooth Muscle Cells

Smooth muscle tissue contracts or relaxes very slowly. In vertebrates, smooth muscle is found in internal organs such as the stomach, intestines, and blood vessels, and its contraction is involuntary.

This muscle tissue is called smooth because it lacks the striated pattern formed by the orderly array of thick and thin filaments in skeletal and cardiac muscle cells. Smooth muscle cells are about 10μ in diameter, tapering

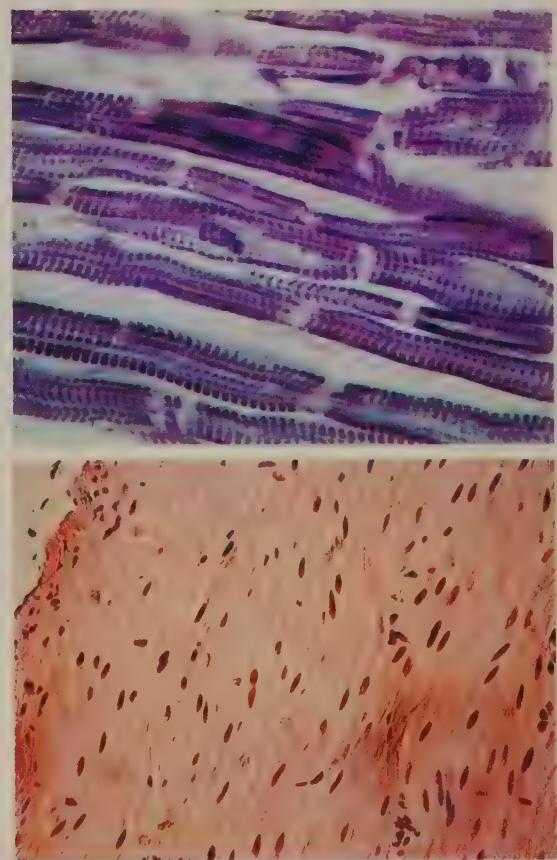


Figure 11.19 (below). Photograph of a cross section through a *Ranunculus* root. Note the cytological difference between the heavy-wall xylem cells and the small, thin-wall phloem cells.

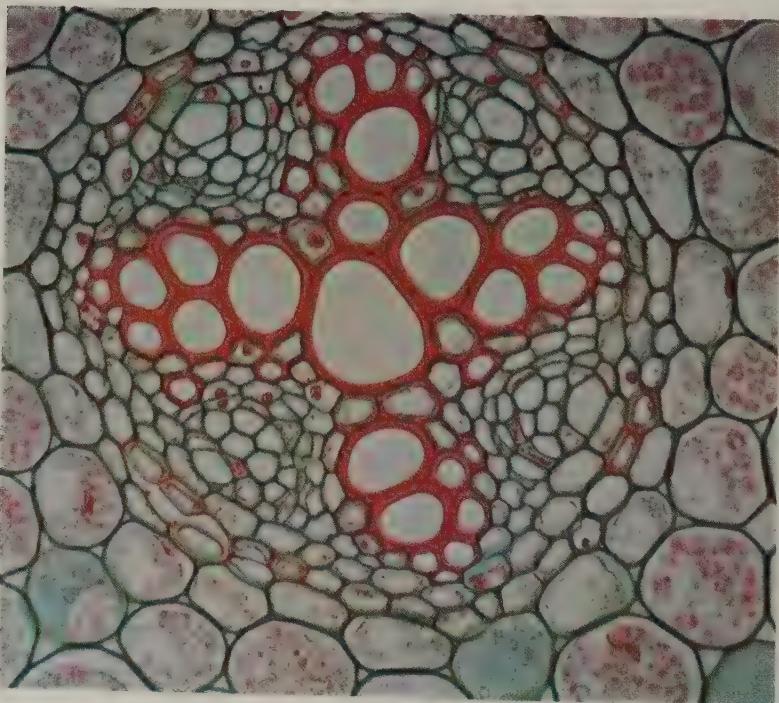
with a single, centrally located nucleus. Smooth muscle cells prepared for electron microscopy appear to contain only thin (actin) filaments. Recent results with different techniques of fixation, however, show that smooth muscles may also have a thick type of filament. The "true" ultrastructure of smooth muscle remains to be discovered.

PLANT VASCULAR TISSUE

Just as nervous and muscle tissues are characteristic of higher animals and essential to their way of life, so vascular, or conductive, tissue is characteristic of higher plants and plays a major role in the functioning of those organisms. Some of the cells of vascular tissues are specialized to serve as tubes through which fluids can be moved from one part of the plant body to another. The two major kinds of vascular tissues, xylem and phloem, are both complex tissues made up of a number of different specialized cells.

Xylem forms a conducting system that extends from the roots to the tips of all the shoots, leaves, and other appendages of the plant body (Figure 11.19). Through this tissue, water and dissolved nutrients and minerals move from the soil to all the cells of the plant. The fluids move through hollow tubes that are formed by specialized cells called *tracheids* and *vessel elements*, which form thick cell walls and then die to leave these walls as hollow tubes. Thick-wall fibers, formed by the death of sclerenchyma cells, help to support the xylem tissue. The only living cells in mature xylem are parenchyma cells scattered between the thick-wall, dead cells. Xylem is the wood of the plant, and it provides support for the plant body as well as transport of fluids.

Phloem forms another conductive system that extends through much of the plant body. In the phloem, the organic materials produced through photosynthesis or cell secretion are moved from one part of the plant body



to another. The fluids move through specialized *sieve cells*, which have relatively thin walls and which retain their cytoplasm at maturity, although the nucleus disintegrates. Closely associated with the sieve cells are companion cells, which retain both cytoplasm and nucleus at maturity. Like the xylem, the phloem also contains sclerenchymatic and parenchymatic cells, which provide support and nutrient storage.

OTHER SPECIALIZED CELLS

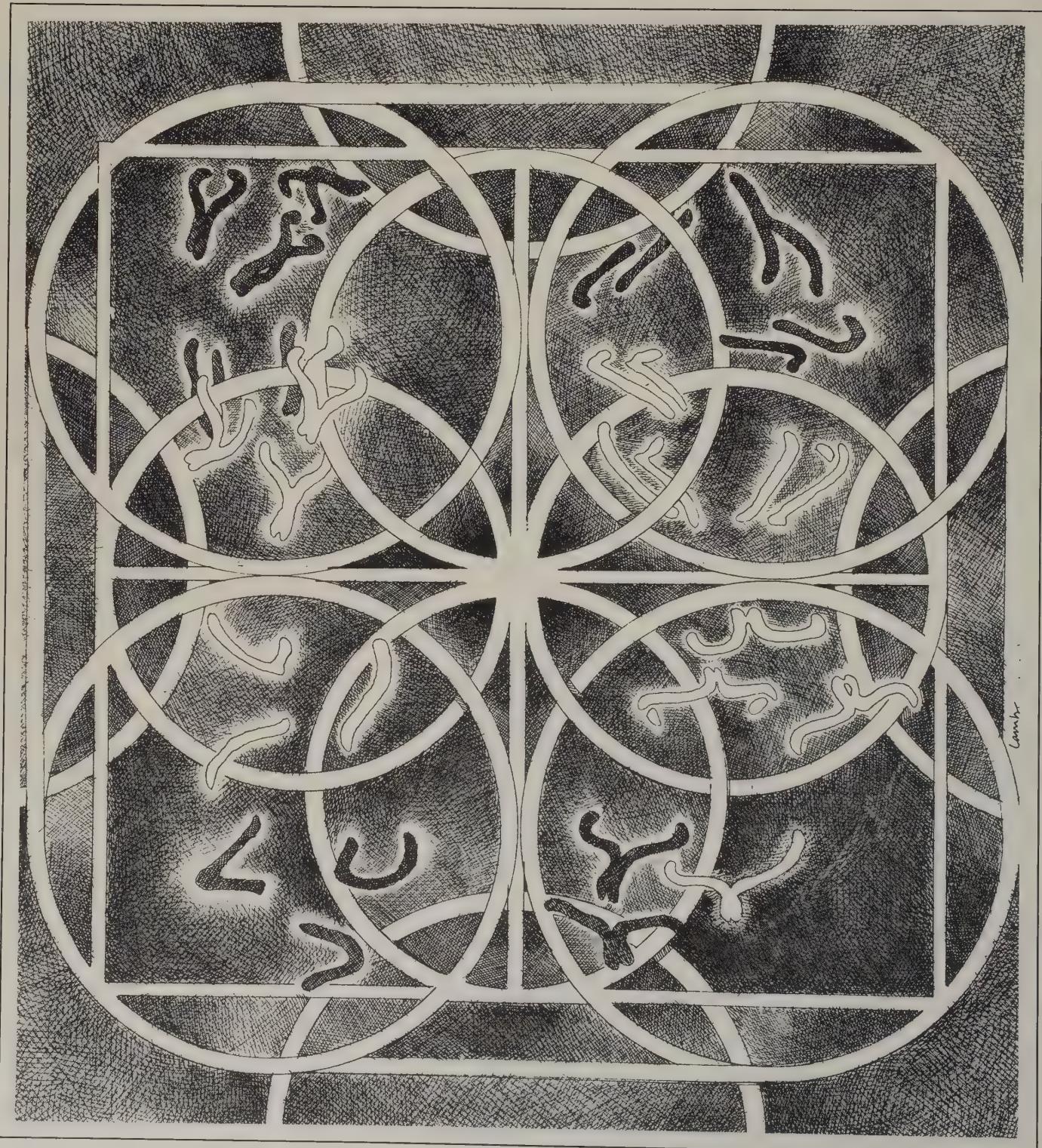
The examples discussed in this chapter by no means exhaust the catalog of specialized cells of higher animals and plants. The body of an average human contains about 100 trillion (10^{14}) cells of many thousands of different specialized types. Some cells are specialized for the synthesis of various necessary substances. Others are specialized for the transport of materials from one solution to another. Still others are specialized to play various roles in the integration or the reproduction of the organism. Some of these other specialized cells in animals will be discussed in Chapter 16 and in Unit Five. The process of differentiation, through which cells become specialized, is discussed in Chapters 17 and 18.

Only a cell biologist or a histologist (student of tissues) is apt to be familiar with the structural details of all the many different types of specialized cells or to be fluent in the use of the many names coined to describe specialized structures. It is important, however, to realize that the basic structure of the eucaryotic cell is modified in many ways in multicellular organisms, thus producing highly specialized cell structures with highly specialized functions. Each specialized cell, in turn, is a complex mechanism of highly ordered macromolecules. The molecular approach to biology has not stolen the wonder or beauty from the contemplation of life; rather, it has increased the sense of awe at the precision with which living systems are constructed and operate.

FURTHER READING

For further information on tissues and specialized cells in higher animals, see books by Arey (1963), Bloom and Fawcett (1962), and Patt and Patt (1969). Similar information about higher plants will be found in books by Eames and MacDaniels (1947), Esau (1965), and Jensen (1964). For more general discussions of specialized cell structures, see books by Brachet and Mirsky (1961), DeRobertis, Nowinski, and Saez (1965), Loewy and Siekevitz (1963), Stern and Nanney (1965), and Swanson (1969).

Among the *Scientific American* articles that discuss various specialized cells and tissues are those by Allen (1962), Comroe (1966), H. E. Huxley (1958, 1965), Kennedy (1967), Miller, Ratliff, and Hartline (1961), D. S. Smith (1965), H. W. Smith (1953), Speirs (1964), and Wurtman and Axelrod (1965).



N

The Continuity of Life

"Genetics" is a twentieth-century word and a twentieth-century science.

The word was coined by an English biologist, William Bateson, to designate that branch of biology which deals with the underlying causes of inherited resemblances and differences between individuals, and hence with the evolution of all living things.

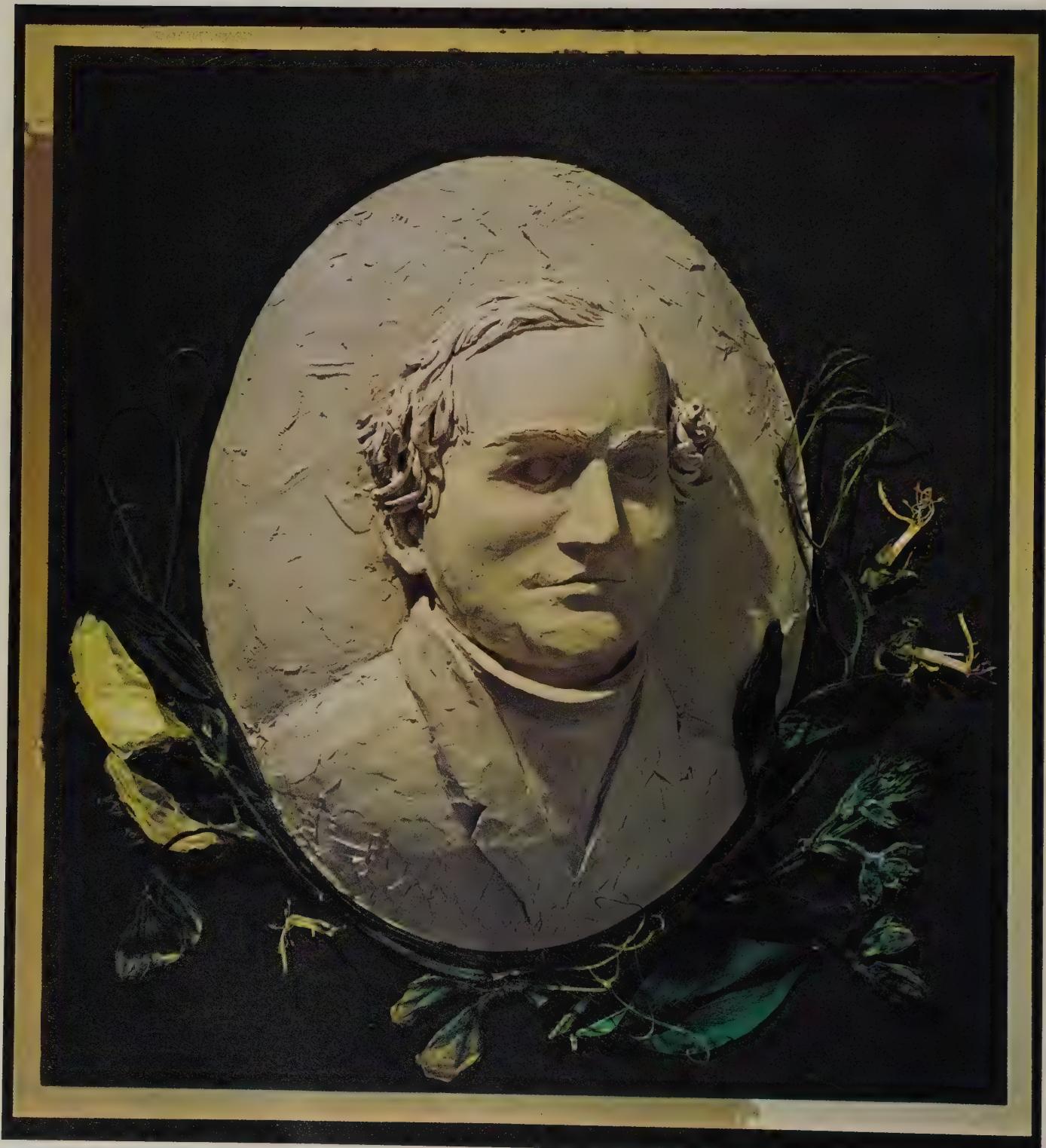
Asserting that "the essential process by which the likeness of the parent is transmitted to the offspring . . . is as utterly mysterious to us as a flash of lightning to a savage," Bateson in 1902 exhorted his fellow biologists to engage more actively in the experimental study of heredity. He promised them that "an exact determination of the laws of heredity will probably work more change in man's outlook on the world, and in his power over nature, than any other advance in natural knowledge that can be clearly foreseen."

. . . In the sixty-some years that have intervened since Bateson's quoted remarks appeared in the *Journal of the Royal Horticultural Society*, the process by which the genes (units of hereditary material) are transmitted from parent to child has become so well understood that man, alone among species, now possesses the ability to control his own evolution.

—George and Muriel Beadle (1966)

12

Mendelian Genetics



Gregor Johann Mendel is usually described as an obscure Moravian monk. According to the traditional legend, the research results of this cloistered priest long held the key to inheritance and evolution. He had published his account of the laws of heredity in 1866, but only in the proceedings of an obscure natural history society. A third of a century later, after Mendel's death, three botanists chanced upon his paper and—the legend has it—all the pieces of the evolutionary puzzle fell into place.

Almost every line in this legend is misleading, if not plainly false. A different picture emerges from an examination of Mendel's life and researches in their historical context.

Two hours' drive north of Vienna is the town of Brno, Czechoslovakia. The little town of Hyncice, where Mendel was born in 1822, lies to the northeast a few hours from Brno. The whole area was then part of Austria. Mendel's hometown was called Heizendorf and lay in the province of Silesia. Brno—then Brünn—was in the province of Moravia. The Mendel family had lived in Heizendorf since the late seventeenth century. Like many others in the district, they were of German descent, and Mendel spoke German as his first language.

His parents named him Johann, but on his admission as a novice to the Augustinian monastery at Brünn in 1843 he was renamed Gregor. Mendel's sharp mind already had been evident to his teachers, both in school and at the University Philosophical Institute of Olmütz. Because he was not a particularly churchy young man, his joining the monastery was as much a step in his academic career as a response to a devout calling. Many monastery members were full-time teachers, either at the Philosophical Institute in Brünn or at the local Gymnasium (preparatory school). From time to time others would leave for a few years to serve as professors in universities. Mendel himself was a substitute teacher of science, first at the Gymnasium in Znaim and then, until his election as abbot in 1868, at the Technical High School in Brünn. In 1850, at the request of the high school, Mendel took an examination to qualify as a regular science teacher. He was essentially self-taught in the natural sciences, but his colleagues apparently convinced him that he was ready to take this difficult examination, which normally followed several years of university study. Although Mendel performed surprisingly well on the examination, his inadequate training was obvious, and the examiners refused to qualify him as a teacher but complimented him for his industry and talent. To provide Mendel with the education he needed, the monastery sent him in 1851 to the University of Vienna for two years of studies in science and mathematics.

On his return to the monastery, Mendel not only undertook his arduous plant-hybridization experiments but made full use of the excellent monastery library to keep abreast of developments in other fields. He took several trips abroad, traveling to Italy more than once and in 1862 joining a group that visited the Industrial Exhibition in London.

In addition to being an alert, intellectual, and energetic man of the world, Mendel was a highly practical person. He had spent his childhood on a farm, and later he was a founding member of the Moravian and Silesian Agricultural Society, winning awards for the development of new fruit and vegetable varieties. He was active in the fire brigade in Heizendorf and was elected chairman of a bank in Brünn. Stocky, kind, and quietly genial, the monk was dearly loved by his pupils and fellow clerics. Yet there was an underlying intensity and nervousness about him. Like many college

students, he wrote bad poetry and got sick from worry over examinations. Later, as abbot, he fought a stubborn but futile resistance to new taxes on monasteries. There are reports of his cigar consumption hitting 20 a day and his pulse 120 a minute in these closing years, before he finally fell victim to Bright's disease in 1884.

MENDEL'S PAPER

Mendel's fame rests upon a single paper. He read it to the Brünn Natural Science Society at two evening meetings, on February 8 and March 8, 1865. The next year it appeared in the fourth volume of the proceedings (Mendel, 1866; Kříženecký, 1965; Stern and Sherwood, 1966).

The core of Mendel's paper comprises three major generalizations: the principle of dominance, the principle of segregation, and the principle of independent assortment, or recombination. These principles may be summarized as follows: (1) When parents differ in one characteristic, their hybrid offspring resemble one of the parents, not a blend of the two characters—the principle of *dominance*. (2) When a hybrid reproduces, its reproductive cells are of two kinds—half transmitting the dominant character of one parent, and the other half transmitting the recessive character of the other parent—the principle of *segregation*. (3) When parents differ in two or more pairs of characters, each pair shows dominance and segregation independently of the other pairs, so that all possible combinations of the various pairs occur in their chance frequencies in the reproductive cells of the hybrid—the principle of *independent assortment*, or *recombination*.

These fundamental and important principles were not the work of a naive priest puttingter with some peas to pass the time between morning and evening prayers. Mendel had received a comprehensive scientific education at the University of Vienna. After returning to Brünn, he crossed many species of plants, undertook microscopic investigations, kept bees and mice, studied the effects on plants of environmental changes, and statistically examined various meteorological data.

Two crucial features of Mendel's researches on heredity can be traced to his training at the University of Vienna. First, he analyzed his results with a mathematical sophistication rare among the naturalists and horticulturalists of his day. Second, his interpretation of his observations emphasizes the reproductive cells as the link between parents and offspring. Mendel's professors at Vienna had included Christian Doppler (of Doppler-effect fame) and Andreas von Ettinghausen—two physicists with a penchant for applied mathematics. Mendel studied botany with the eminent Franz Unger, whose contributions to cell theory included the identification of the male reproductive cells of mosses. Furthermore, Unger was a speculative theorist who, in a book published in 1852, denied the constancy of species and suggested in rather vague terms that the plant kingdom developed through natural processes. The pantheistic tone of Unger's book provoked the Catholic press to call for his dismissal. The students responded with a petition for his retention. The effect of this affair on Mendel is unknown, but the mutability of species and the history of the plant kingdom were hot topics at the time, and Mendel possibly undertook his investigation of heredity in the hope of shedding some light on the issues. Within a few months of his return from Vienna, Mendel had established some 34 pure strains of peas, obviously planning for some kind of hybridization experiments. The experi-

Figure 12.1. Photograph of Gregor Mendel (third from left) and members of the monastery.



ments reported in Mendel's famous paper were begun in 1856 and virtually completed by 1863.

Mendel was not the first person to cross plants, nor was he the first hybridizer trying to discover the mechanism of inheritance. Previous investigators who had crossed species or varieties had noted how the dominance of some characters produces a uniformity in the first-hybrid generation. They also had noticed the greater variability of the generation produced by breeding the first-generation hybrids among themselves. However, no one before Mendel had studied large numbers of single-character crosses with the intention of developing a general theory that would describe the statistical distribution of single characters among successive hybrid generations.

Just how Mendel reached his theoretical conclusions will always remain obscure. The core of his interpretation is the assumption that the hereditary elements or factors present in a hybrid are not irreversibly blended together but can reappear separately in the next generation. It is far from clear what first led Mendel to this assumption. There is agreement that most of his experimental results are too good to be true. That is, the close fit between his recorded results and the frequencies expected from theoretical calculations is extremely improbable. Both repetitions of Mendel's experiments and modern statistical analysis make it very unlikely that Mendel could have observed results so close to his theoretical expectations. This evidence does not necessarily imply deliberate dishonesty; the improbably close fit is more likely due to the unconscious bias in scoring plants or to termination of counts when ratios were close to the predicted number. In any case, it appears that many if not all of Mendel's experiments were performed to confirm his hunches about what the laws of inheritance must be, not to discover those laws.

Mendel's initial thinking may have gone something like this: Often it makes no difference to the results of a cross whether a male with the character being investigated is bred with a female lacking it or vice versa. Parents, then, transmit equal and equivalent contributions to their offspring. Because the offspring receives equal contributions from both parents, the reproductive cell of each parent must contribute half of the normal adult

Figure 12.2. Mendel's microscope.



hereditary factors for each character. Without such halving in the reproductive cells, there would be an indefinite build-up of the hereditary material. When the half-doses of hereditary factors meet in a fertilized egg, they must either mix (like red and blue ink) or remain effectively discrete (like red and blue billiard balls). The ability of characters to skip a generation and reappear in the next indicates that there is no irreversible mixing. There are thus discrete and permanent hereditary factors responsible for visible characters. The frequency of various characters in any generation is determined solely by the chances for each of the possible combinations of hereditary factors in fertilization.

There is no way of knowing whether Mendel reasoned his way to his basic principles by the route described above. It is easy to see, however, that the explanation Mendel gave for his crucial experiments follows directly from these few premises. Further description of the experiments will be facilitated somewhat by using terminology developed long after Mendel's paper was published. The original parental generation is designated the P generation; the offspring of P make up the F₁ (first filial) generation; the offspring of F₁ are the F₂ generation; and so on.

MENDEL'S EXPERIMENTS

Many observable characters of pea plants are influenced by heredity—characters such as the length and color of the stem; the size and form of leaves; the position, color, and size of flowers; the form and size of seeds; and the color of seed coats and seed contents. After preliminary work, Mendel chose for further study the seven pairs of characters outlined below.

1. *Characters related to the form of ripe seeds.* The seeds may be *round* (or roundish, with only shallow surface depressions), or they may be irregularly angular and deeply wrinkled.
2. *Characters related to the color of seed contents.* The contents of ripe seeds may be yellow to orange or may have a more-or-less intense green tint.
3. *Characters related to the color of the seed coat.* The seed coat may be *white* (such a seed produces a plant with white flowers), or it may be gray to brown, with or without violet spots (such a seed produces a plant with reddish-violet flowers).
4. *Characters related to the form of ripe pods.* The ripe pods may be *inflated* with a smooth surface, or they may be deeply *constricted* between the seeds and more-or-less wrinkled.
5. *Characters related to the color of unripe pods.* The unripe pods may be light to dark green, or they may be vividly yellow.
6. *Characters related to the position of flowers.* The flowers may be *axial* (distributed along the main stem), or they may be *terminal* (bunched at the top of the stem).
7. *Characters related to the length of the stem.* Stem length varies greatly in different strains of pea plants, but Mendel chose one pure-breeding strain with *short* stems (9 to 18 inches) and another with *long* stems (6 to 7 feet).

For each pair of characters, Mendel obtained two pure-breeding strains, differing only in that single pair of characters. He chose these characters for study because in each pair the two contrasting characters are always distinct; no intermediate characters appear in crosses between the two pure-breeding strains.

Mendel began his famous experiments by cross-pollinating plants for each pair of strains differing in a single character. For example, he dusted flowers of plants from the pure-breeding, round-seed strain with pollen from plants of the pure-breeding, wrinkled-seed strain. Other wrinkled-seed plants were fertilized with pollen from round-seed plants. Similar

Figure 12.3a (above). A monohybrid cross between pea plants differing in seed types. One parent is homozygous dominant for round seeds (RR), and the other parent is homozygous recessive for wrinkled seeds (rr). The phenotype of the F_1 offspring is round, but note that the genotype is Rr, or heterozygous. If two of these round F_1 plants are mated, their offspring (F_2) will show a 3 to 1 phenotypic ratio and a 1:2:1 genotypic ratio.

cross-fertilizations were performed for each pair of pure-breeding strains. In each case, the pure-breeding plants are the P generation for the crossing experiment.

Mendel carefully collected the seeds that were produced on the P-generation plants, recorded their characters, planted them, and recorded the characters of the resulting adult plants. These seeds and the plants into which they develop are the individuals of the F_1 generation. Mendel called them hybrids, but modern geneticists prefer to use this term only for individuals produced by cross-breeding between two different species. In each cross, Mendel found that all the F_1 individuals resemble one of the contrasting parental characters. For example, all the F_1 seeds collected after the cross of wrinkled-seed and round-seed plants show the round-seed character. Thus, Mendel called round seeds a *dominant* character and wrinkled seeds a *recessive* character. In a cross between pure-breeding strains with dominant and recessive characters, all the F_1 individuals show the dominant character. The recessive character is not expressed in the F_1 generation, but it must be present in some form because it is passed on to the F_2 generation. Mendel found the following characters to be dominant: round seeds, yellow seed contents, gray seed coats, inflated pods, green pods, axial flower distribution, and long stems.

The characters related to shape of seed and to color of seed contents are observed as the seeds are collected from the parental plants. The other characters can be observed in the offspring only after the seeds have been planted and have developed into mature plants in the year after the cross was made. Thus, Mendel's experiments required a great deal of time as well as careful record keeping.

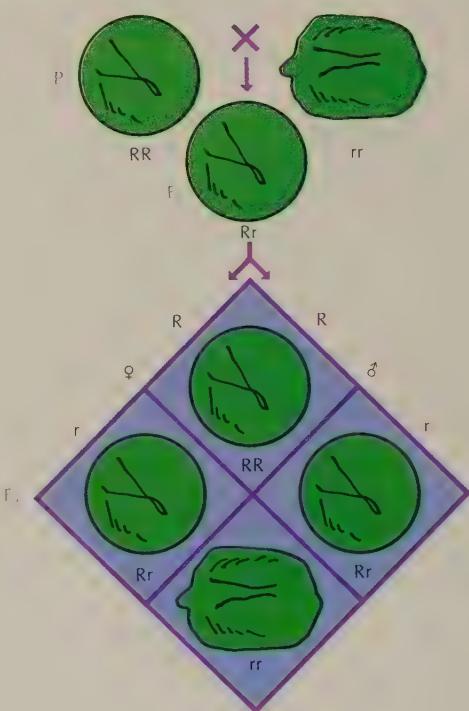
The F_2 generation for each cross was produced by self-fertilization of the F_1 plants. In each case, some of the F_2 individuals showed the recessive character that had disappeared in the F_1 generation. For each cross, the ratio of individuals showing the dominant character to individuals showing the recessive character averaged about 3:1. None of the F_2 individuals showed characters intermediate between the characters of the original parental strains.

For example, the cross between round-seed and wrinkled-seed parents produced 253 F_1 seeds, all of them round. When the plants grown from these seeds were self-fertilized the next year, 7,324 seeds were collected. Of these F_2 seeds, 5,474 were round and 1,850 were wrinkled, a ratio of 2.96 to 1. Table 12.1 shows the characters observed in the F_2 generations of the various crosses. The ratios of dominant to recessive varied from 2.82:1

Table 12.1
Ratios of Characters in F_2 Generations
of Mendel's Seven Crossing Experiments

Dominant Character Character	Number of F_2 Individuals	Recessive Character Character	Number of F_2 Individuals	RATIO
Round seed	5,474	Wrinkled seed	1,850	2.96:1
Yellow seed contents	6,022	Green seed contents	2,001	3.01:1
Gray seed coats	705	White seed coats	224	3.15:1
Inflated pods	882	Constricted pods	299	2.95:1
Green pods	428	Yellow pods	152	2.82:1
Axial flowers	651	Terminal flowers	207	2.14:1
Long stems	787	Short stems	277	2.84:1

Figure 12.3b (below). The same F_1 cross—diagrammed in a Punnett square—shows in more detail the origin of the 3 round to 1 wrinkled seed phenotypic ratio and the underlying 1:2:1 genotypic ratio—that is, 1 homozygous round (RR), 2 heterozygous round (Rr), and 1 homozygous wrinkled (rr).



		Male Gametes	
		R	r
Female Gametes	R	RR (round seed)	Rr (round seed)
	r	Rr (round seed)	rr (wrinkled seed)

to 3.15:1. Like any statistician, Mendel concluded that these ratios really represent a constant 3:1 ratio. To put the matter less flippantly, any statistically minded inquirer will not try to explain the exact ratio actually obtained in a particular experiment but will try to discover why similar experiments always yield a ratio of about 3:1. That is exactly what Mendel did.

The results expected from various crosses can readily be predicted according to Mendelian principles by use of a simple checkerboard diagram. Each column represents a different kind of male gamete, or reproductive cell. Each row represents a different kind of female gamete. Each box within the diagram represents one possible kind of zygote that can result from fusion of gametes. If there are four boxes in the diagram, then one-quarter, or 25 percent, of the offspring may be expected to be of the kind described by that box.

In the characters related to seed form and color of seed contents, Mendel found that most pods contained seeds of both characters. Similarly, the plants grown from seeds collected from a single pod usually show both characters studied in the other crosses. The ratio between dominant and recessive characters in seeds from a single pod or even in seeds from a single plant may be quite far from the average 3:1. For example, one of the F_1 plants in the first cross yielded 43 round F_2 seeds and only 2 wrinkled seeds. The 3:1 ratio is obtained only as an average of the results of many individual crosses.

The next year an F_3 generation was produced by self-fertilization of the F_2 plants. The F_2 individuals that showed recessive characters produced only F_3 individuals showing recessive characters. However, the F_2 individuals that showed dominant characters proved to be of two kinds. One-third of these dominant-character F_2 individuals produced only dominant-character F_3 individuals. The other two-thirds of the dominant-character F_2 individuals produced F_3 individuals with both dominant and recessive characters in the ratio 3:1. In other words, all of the recessive-character and one-third of the dominant-character F_2 individuals proved to be pure-breeding. The other two-thirds of the dominant-character F_2 individuals showed the same pattern of offspring as the F_1 generation had shown.

For example, all the wrinkled F_2 seeds grew into plants that yielded only wrinkled seeds when self-fertilized. Of 565 round F_2 seeds, 193 grew into plants that yielded only round seeds when self-fertilized. The other 372 round F_2 seeds grew into plants that yielded round and wrinkled seeds in a ratio of about 3:1 when self-fertilized.

The distribution of characters in the F_3 generation makes it clear that the 3:1 ratio of dominant to recessive characters in the F_2 generation actually reflects a 2:1:1 ratio. Among the offspring of the F_1 hybrids, one-half are F_2 hybrids showing the dominant character, one-quarter are pure-breeding dominants, and one-quarter are pure-breeding recessives. At the time he wrote his paper, Mendel had carried most of his crosses through four or five generations and found that the 2:1:1 ratio continues to appear among the offspring of hybrids in each generation, whereas the pure-breeding lines continue to produce offspring like their parents.

Mendel explained his observations by assuming that each seed contains two hereditary factors, or elements, affecting a particular character. One of these factors is obtained from each parent. For example, in the characters related to seed form, let R represent a factor for the round-seed character and r represent a factor for the wrinkled-seed character. The pure-breeding plants of the P generation can be represented as RR and rr . Cross-breeding

Figure 12.4a (above). A dihybrid cross, illustrating independent assortment, between a round, yellow pea plant ($RRYY$) and a wrinkled, green pea plant ($rryy$) carried through the F_2 generation. Those members of the F_1 generation are completely heterozygous ($RrYy$) for both traits and, when mated, yield 16 possible genetic combinations in four classes with the following phenotypic distribution: 9 yellow round, 3 yellow wrinkled, 3 green round, and 1 green wrinkled.

produces an F_1 seed that obtains one factor from each parent and thus is Rr . The seed becomes round because the dominant R is expressed despite the presence of the recessive r .

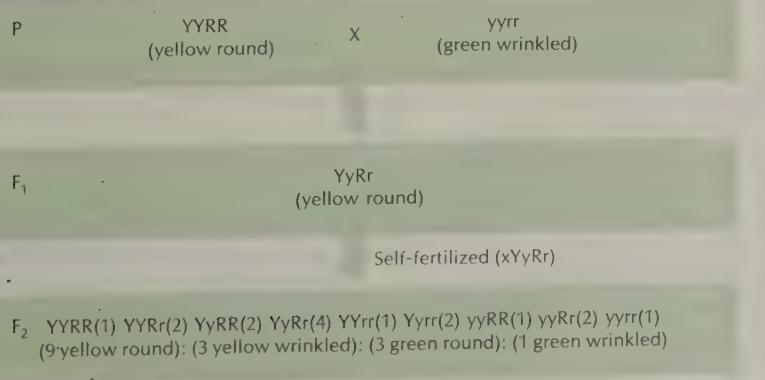
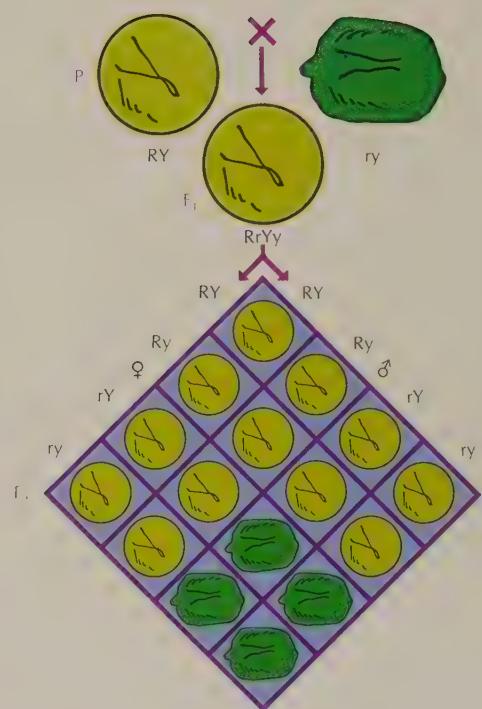
When the Rr seed grows into a plant, it will produce, in equal numbers, reproductive cells carrying R and cells carrying r factors. Four combinations are thus equally likely to be found among the F_2 seeds produced by self-fertilization: RR , rR , Rr , and rr . Thus, the F_2 generation will consist of pure-breeding dominants, hybrids, and pure-breeding recessives in the ratio 1:2:1, just as Mendel had found experimentally. Because half of the F_2 individuals possess exactly the same Rr factors that the F_1 generation possessed, further self-fertilizations of these individuals should produce the same ratios of offspring. Mendel's hypotheses can best be visualized through the simple checkerboard diagram that was developed by later workers in this field.

Next, Mendel carried out crosses involving two or more pairs of characters. In one experiment, for example, pure-breeding plants with round yellow seeds were crossed with pure-breeding plants with wrinkled green seeds. The plants of the P generation may be represented as $RRYY$ and $rryy$. All of the reproductive cells from one strain carry the factors RY and all of those from the other strain carry ry . Thus, all the F_1 plants will possess the factors $RrYy$. As expected, all the F_1 seeds were round and yellow. If the factors are assorted randomly in the production of reproductive cells (assuming, however, that each reproductive cell gets only one of each kind of factor), four kinds of reproductive cells are possible: RY , Ry , rY , and ry . The checkerboard diagram shows that the F_2 individuals will have four different combinations of characters in the ratio 9 : 3 : 3 : 1. Mendel's experimental results confirmed this prediction.

Crosses involving more than 2 independently assorting factor pairs can be diagrammed in the same way, but the situation rapidly becomes more complex. With 3 pairs of factors, the predicted ratio of distinguishable kinds of individuals in the F_2 generation is 27 : 9 : 9 : 9 : 3 : 3 : 3 : 1. With 4 factor pairs, there are 16 possible kinds of individuals, and with 5 factor pairs the number of kinds is 32. With only 23 different, independently assorting pairs of factors, the offspring of a cross between multihybrid individuals could be of about 8 million different observable types. It is easy to see why Mendel was impressed with the ability of his simple hereditary factors to account for variability in nature.

In fact, his theoretical principles could account for the results Mendel obtained in numerous experiments. He counted over 10,000 plants in the 8

Figure 12.4b (below). Only the dihybrid cross is outlined here, but the distribution of F_2 genotypes is illustrated.



years he gathered data for his paper. Unfortunately, those biologists of his time who did read Mendel's paper were skeptical of his results and theories, and Mendel was unable to convince them to repeat his laborious experimental work in order to check his conclusions. Mendel's powerful principles, however, gave rise in the early years of this century to many of the basic ideas of genetics, as the study of inheritance then came to be called.

Other useful terms were introduced early in this century. Individuals showing the same observable characters are said to be of the same *phenotype*; those possessing the same set of hereditary factors, or *genes*, are said to be of the same *genotype*. Pea plants of different genotypes (*RR* and *Rr*) both display the same round-seed phenotype. An individual whose genotype comprises identical factors (*RR* or *rr*) is called *homozygous*. An individual with the hybrid genotype *Rr* is called *heterozygous*. The word "gene" was not introduced until 1909 and has meant various things at various times, but one of its original uses was for the factors within cells that produce the characters studied by Mendel.

REACTIONS TO MENDEL'S PAPER

Mendel did not live to take part in modern genetics. In his own lifetime, no one showed even a slight understanding of his work. This failure was a bitter disappointment to Mendel. Contrary to legend, his paper was discussed at those two evening meetings of the Brünn society, but no one really understood the significance of his conclusions. In a letter written the next year, Mendel commented sadly: "I encountered, as was to be expected, divided opinion; however, as far as I know, no one undertook to repeat the experiments."

On publication the following year, the paper quickly became widely available. Of the 115 copies of the journal sent to subscribers, 8 copies went to Berlin, 4 to the United States, and a couple to the Royal Society and the Linnean Society in England. In addition, 40 reprints of Mendel's article were ordered and presumably distributed to biologists around the world. A standard bibliography on plant hybridization published in 1881 mentions Mendel's paper several times.

A number of scientists must have read Mendel's paper, but for several reasons they were not impressed. First, no agreement existed over the details of animal and plant reproduction. Darwin, for example, believed that more than one pollen grain is required for a single fertilization in plants. Second, anyone glancing at the title of Mendel's paper and then skimming over its contents could well get the erroneous impression that Mendel intended the conclusions to apply only to pea plants. Mendel did discuss the results of some crosses among bean plants, which generally supported his results with peas. However, the beans also presented some new phenomena that Mendel had tentatively explained by making some further assumptions about the combined effects of several factors influencing the same character. Third, mathematics—however elementary—was seldom used in discussions of plant breeding. Naturalists would have been baffled by the formulae, and those scientists who could have followed Mendel's mathematical arguments were unlikely to bother reading an article on pea hybrids. Fourth, there was no immediate follow-up from Mendel—no further articles on his research, much less a book. When another article by Mendel finally did appear in 1870, the news was bad. In experiments with hawk-

weed (*Heiracium*), Mendel found that both the F_1 and the F_2 generations of a hybrid cross showed only the dominant character. The recessive character failed to reappear in later generations, a result that was consistent with older theories of blending inheritance rather than with Mendel's theory of discrete hereditary factors. It is now known that hawkweed usually reproduces asexually and that many of Mendel's supposed crosses of hawkweed plants were not crosses at all.

With hindsight, one might bemoan Mendel's bad luck in choosing hawkweed plants for his later experiments. However, it would be more realistic to marvel at his good luck in choosing pea plants for his initial experiments. Modern knowledge of plant genetics reveals that few plants could be as suitable as peas for demonstrating simple genetic properties. Even with peas, Mendel was fortunate, for many genes are linked together on chromosomes and therefore do not assort independently. The chance of anyone happening to choose seven independently inherited characters in peas is only 1 in 163.

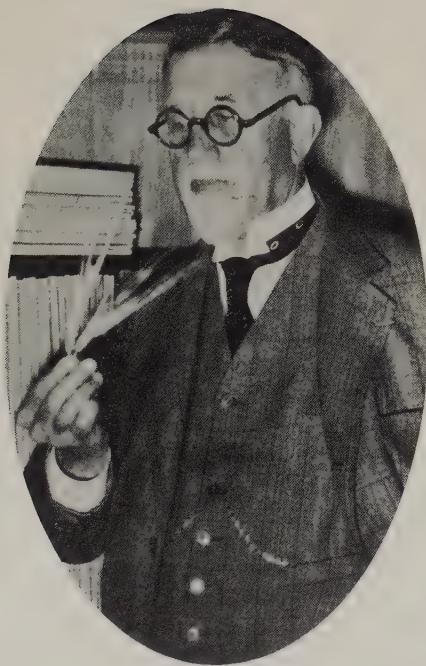
Mendel made a concerted effort to explain his discoveries to the famous Swiss botanist and cell theorist Karl von Nägeli. Mendel sent his paper to Nägeli and followed it with ten letters, but to no avail. Nägeli's replies are lost, but he never accepted Mendel's major generalizations. This rejection may have been partly due to Nägeli's own experience with hawkweed. At any rate, all Nägeli did for Mendel was to urge him to concentrate on hawkweed and therefore to lose confidence in his earlier conclusions.

Nägeli was an evolutionist, but Mendel's letters to him make only passing or indirect reference to the problem of the origin of species. The nature of Mendel's thought on this question is difficult to establish. A copy of the German translation of Darwin's *On the Origin of Species*, annotated in Mendel's handwriting, has been found among Mendel's belongings, so it is clear that Mendel carefully studied Darwin's theories. However, the markings do not indicate how far Mendel agreed with Darwin's proposals. Mendel's classic paper points out that any conclusions about inheritance will have important implications for hypotheses about evolution, but that is all he says. Some passages in Mendel's paper seem to allude to Darwin's book, but they may be a response to an 1849 book written by the German plant hybridizer Carl Friedrich von Gärtner.

It is often stated that Darwin would have recognized the significance of Mendel's paper if he had read it. Confidence in such speculations is always misplaced and especially so in this case. Darwin may well have seen an early reference to Mendel's paper, but he did not pursue it. Had he done so, he probably would have made little sense of Mendel's arguments. In the first place, Darwin had difficulty with even simple equations. Second, Mendel's conclusions were in opposition to a longstanding conviction of Darwin's—drawn from the study of domesticated animals and plants—that the variability in any species is due to the disturbing effects of external conditions on the reproductive system. Third, by the time Mendel's paper appeared, Darwin had developed his own highly comprehensive theory of inheritance, "pangenesis," a theory he constructed to explain a far wider range of phenomena than Mendel had considered. Darwin, then, would probably have tried to explain Mendel's results in terms of his own broader theoretical scheme.

Hindsight suggests that Mendel would have had better luck in interesting Darwin's cousin Francis Galton. Galton, a keen Darwinian, loved

Figure 12.5. Erich von Tschermark, one of the men who rediscovered Mendel's work around the turn of the century.



mathematics and was already applying it to the study of inheritance. In his later work, Galton came close to postulating the existence of independently segregating hereditary factors. He and Darwin together might have discussed Mendel's work and tried to incorporate Mendel's principles into a genetical theory of natural selection. However, there is no evidence that Galton ever read Mendel's paper.

A man less reticent than Mendel would have sent reprints and letters to men like Darwin, Galton, and other evolutionists. But that was not Mendel's way. Part of his reticence may have been due to prudence rather than to shyness. It may have been risky for a priest and teacher to take a stand on such issues. Mendel surely had memories of the attacks on his former professor Unger. Yet, on the other hand, Mendel was never one to dodge opposition.

The neglect of Mendel's work was a personal tragedy. His tragic flaw was his modesty. The truly effective theorist is humble before facts but just a little arrogant before his fellow scientists. Mendel's admirers often say that he alone founded the entire science of genetics—a sincere compliment to a deserving hero, but also an ironic misstatement of the historical facts. Mendel's whole life is a demonstration of the fact that it takes more than one man—however talented—to initiate a new field of science. Genetics was not established as a science until Mendel's principles were rediscovered by a number of other scientists around 1900, and there was a further delay of 30 years before Mendelian inheritance and Darwinian natural selection were coupled finally into a widely acceptable evolutionary theory.

REDISCOVERY OF MENDEL'S WORK

According to the usual account, Mendel's principles were independently rediscovered about 1900 by three men—Hugo De Vries, Carl Correns, and Erich von Tschermark. Each of these men found at the last minute before publication of his own paper that he had been scooped 40 years earlier. The republication of Mendelian theories supposedly shed light upon the nature of heredity and evolution and led to rapid and unified progress in the field of genetics.

The timing of this rediscovery was no chance affair. In the 40 years following Mendel's publication, a series of fundamental innovations in biological theory had prompted a general search for universal laws governing the inheritance of single characters. By 1900 the cell theory was generally established, the nature and role of male and female sex cells in fertilization had been clarified, and nuclear fusion had been identified as the crucial event in fertilization. Several diverse hypotheses had proposed the existence of subunits within the cell nucleus, and some theorists had suggested that these submicroscopic particles are involved in the transmission of hereditary characters. Many biologists were aware of the central role of chromosomes in cell division, and some had speculated that the chromosomes might be involved in heredity. In short, although Mendelian principles were far from being obvious consequences of the basic biological theories of 1900, these theories were compatible with Mendelian principles, whereas the prevailing theories of 1866 had not been.

Furthermore, the biologists studying heredity at the turn of the century were far more ready to accept mathematical concepts than had been the plant breeders of Mendel's day. Statistical analyses were being used in a variety of biological researches, and it is not surprising that a number of

investigators independently realized that a better understanding of heredity might come through statistical analysis of the offspring characters in crossing experiments.

However, rediscovery of Mendel's principles did not provide instant clarification of the problem of heredity. Although some investigators using Mendelian theories did make rapid progress toward an understanding of the nature of the gene, many biologists remained unconvinced of the validity of Mendel's principles for more than a decade after their rediscovery. Like Nägeli with his hawkweed, many biologists working with organisms other than the pea plant knew from firsthand experience that Mendel's principles in their simple form could account for only a few of the phenomena of heredity.

Nor did the rediscovery do anything to settle disputes among evolutionary theorists. Far from it. For various reasons, there were numerous rivals to the Darwinian theory of natural selection by 1900. Many of the Mendelians became vigorous opponents of Darwinian selectionists. De Vries' mutation theory was one attempt to explain the origin of new species by a mechanism other than natural selection.

Only in the 1930s did geneticists reach sufficient understanding of the distribution of genes in a population over many generations to permit a satisfactory synthesis of the theories of evolution and genetics. Thus, it was nearly two decades after the rediscovery before Mendelian principles of inheritance were generally accepted (in a modified form) and more than three decades before those principles were used to provide a convincing explanation of the mechanism of evolution.

Before turning to the developments in genetics research after 1900, it will be useful to examine the nuclear processes involved in cell division, many of which were first described during the period between Mendel's original publication and its later rediscovery.

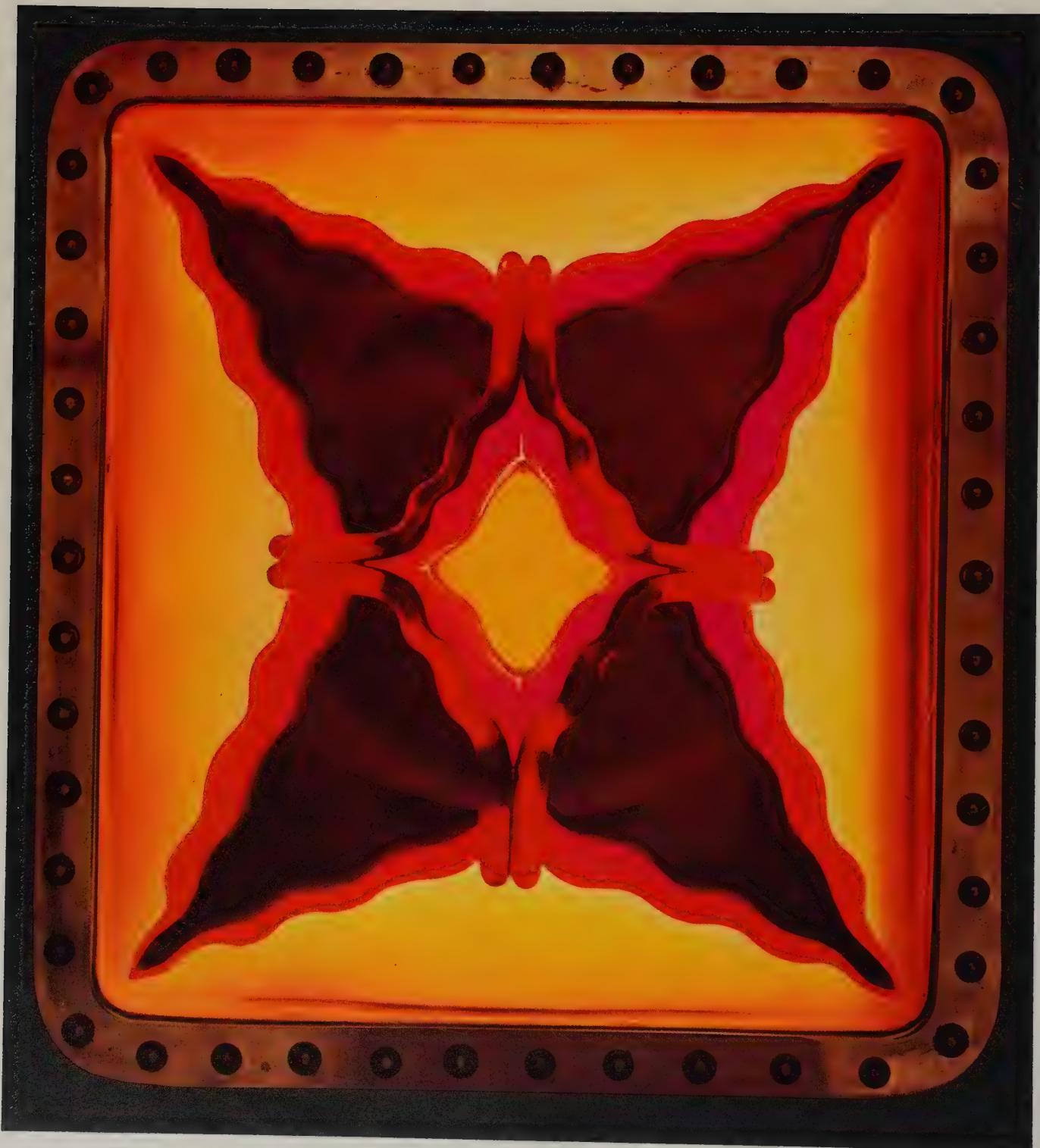
FURTHER READING

Moore (1963) in his first 18 chapters gives a brief and clear account of the development of genetics from Mendel through the early 1960s. Olby (1966) presents a full account of the hereditary theories of Darwin and other nineteenth-century biologists.

Stern and Sherwood (1966) and Voeller (1968) furnish useful collections of important historical papers in English translations. Interesting and contrasting early interpretations of Mendelian principles are given by Bateson (1913) and Morgan, et al. (1915).

13

Cell Division



In 1866 Mendel wrote of hypothetical hereditary factors carried by the pollen cell and the female germinal cell. Two factors affecting each trait are combined when pollen and germinal cells fuse during fertilization. The new plant that develops shows characters related to the pairs of factors that it has received. When it produces pollen and germinal cells, it contributes one factor for each trait to each of these cells. With the knowledge of cells and of reproductive processes available at the time, Mendel could not go much further in proposing a physical mechanism of heredity.

During the years following Mendel, cytologists made tremendous progress in filling out the details of cell theory. Against the new background of knowledge about cell and organism reproduction, Mendel's principles took on physical significance for their twentieth-century rediscoverers. Many twentieth-century geneticists shifted their attention toward an attempt to locate the hereditary factors within the cell and to understand how the factors are carried and assorted. This physical understanding proved crucial in the further extension of Mendel's laws to accommodate a number of observations that are not compatible with those laws as Mendel stated them.

The most detailed nineteenth-century descriptions of division in animal cells were made by the German anatomist Walther Flemming, who used the newly developed oil-immersion microscope lens. He showed that the threadlike structures split lengthwise and that half of each thread moves into each of the daughter nuclei (Figure 13.2). Flemming introduced the term *mitosis* (the Greek *mitos* means thread) to describe this orderly process of nucleus and cell division. He called the dark staining material within the nucleus "chromatin." The name "chromosomes" for the threadlike bodies composed of chromatin was suggested a few years later by another researcher.

During the 1880s, Flemming and other microscopists worked out most of the details of the mitosis process. These experimenters discovered that every cell of an organism contains the same number of chromosomes. This number is reduced during the formation of egg and sperm cells; fusion of sperm and egg nuclei during fertilization restores the full chromosome number in the offspring. The process by which chromosome number is halved during formation of sperm and egg cells was described in detail. This special kind of cell division is now called *meiosis* (the Greek *meioún* means to lessen).

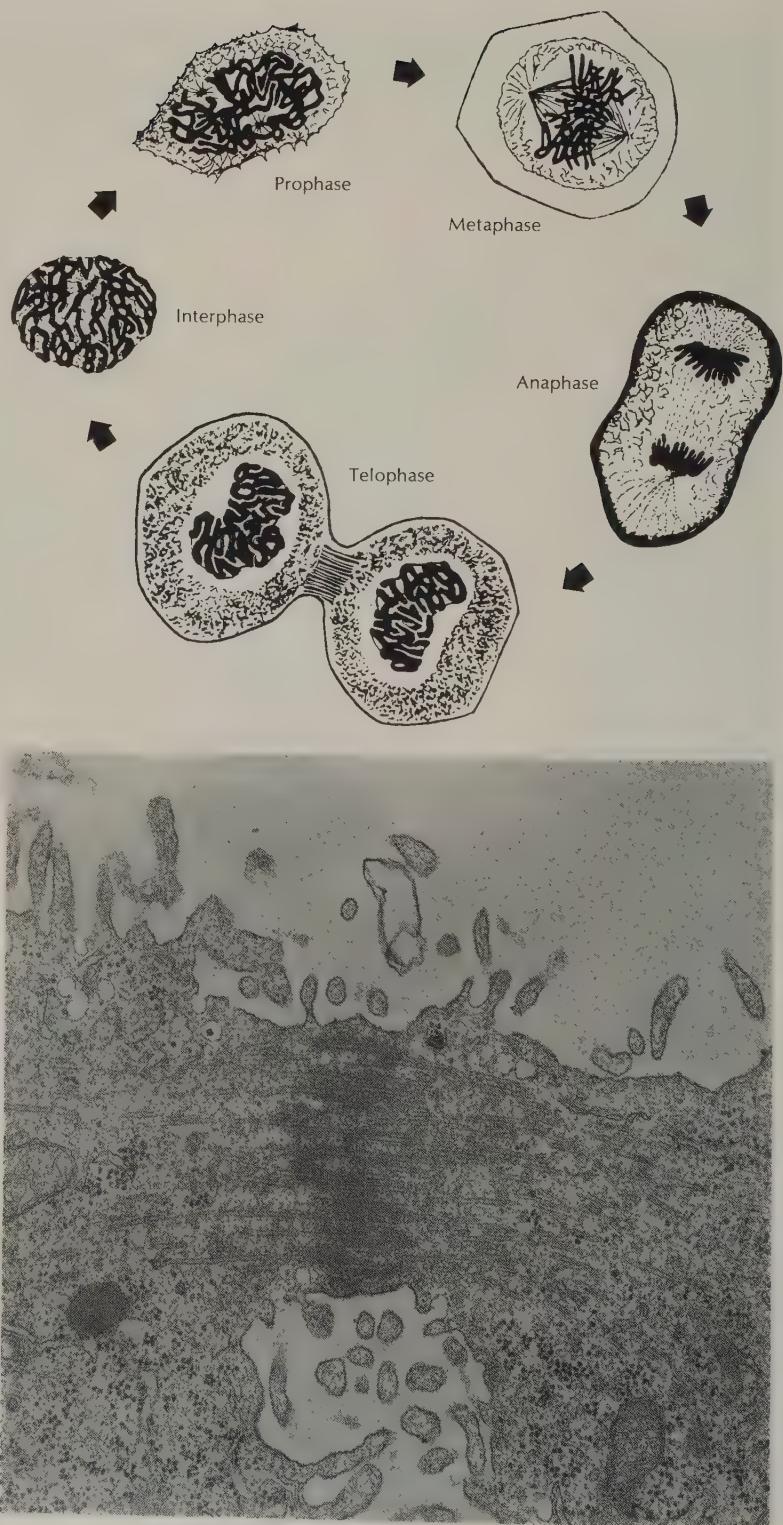
HAPLOIDY AND DIPLOIDY

Chromosomes are present in the nucleus of every eucaryotic cell. In a multicellular organism, every cell (except sperm and egg cells) contains exactly the same number of chromosomes as any other cell in the organism. Reproduction of most unicellular organisms and multiplication of cells within a multicellular organism are accomplished through the process of cell division called *mitosis*. In this process, each daughter cell receives a set of chromosomes that matches the set present in the parent cell. The special process of division called *meiosis* results in a halving of the chromosome number in the daughter cells. Such a process is necessary for sexually reproducing organisms if each generation is to have the same number of chromosomes in its cells as the parents had.

The processes of mitosis and meiosis do not occur in prokaryotic cells. These cells have neither a nucleus nor distinct, threadlike chromosomes.

Figure 13.1. Early drawings of the mitosis process.

Figure 13.2. The Flemming bodies shown in this micrograph were named after the German anatomist Walther Flemming, who introduced the term "mitosis."



The prokaryotic equivalent of a chromosome is a single molecule of DNA. This molecule is duplicated before cell division, and during fission one copy moves into each daughter cell. The mechanism by which the copies are properly divided between the daughter cells is not known.

Most eukaryotic cells contain a number of chromosomes that can be distinguished microscopically on the basis of size and shape. In 1900 it was thought that all chromosomes within a nucleus are equivalent, but cytologists soon showed that the chromosomes are of distinct kinds and that most cells contain a pair of each kind of chromosome. Meiosis results in the formation of sperm and egg cells with only a single chromosome of each kind. A cell with only one chromosome of each kind is called a *haploid* cell, and the number of chromosomes that it contains is called the *haploid number* (n) for the species. The zygote, or fertilized egg cell, is formed by fusion of sperm and egg cells and thus contains $2n$ chromosomes—a pair of each kind. This condition is called *diploidy*. In most higher plants and animals, body cells are diploid throughout the life cycle. Haploid sex cells are produced by meiotic divisions of certain specialized body cells. Diploidy is restored after fertilization.

Other patterns are found in the life cycles of many simpler plants and animals and of most protists. For example, the cells of many organisms are haploid through much of the life cycle. The fusion of haploid sex cells forms a diploid zygote, which quickly undergoes meiosis to restore the haploid condition in the new organism. In unicellular organisms reproducing asexually, the diploid condition may be produced briefly by a duplication of the chromosomes just before cell division. Nevertheless, the division involved in reproduction restores the haploid number of chromosomes in the offspring cells.

MITOSIS

The process of eukaryotic cell reproduction involves a complex mechanism that ensures the proper duplication and division of the chromosomes. For descriptive purposes, the process of mitosis has been divided into five stages, or phases (Figure 13.3). In living cells, mitosis proceeds smoothly and without sharp changes from one stage to the next; the stages are thus artificial descriptive aids, not inherent discontinuities in the process.

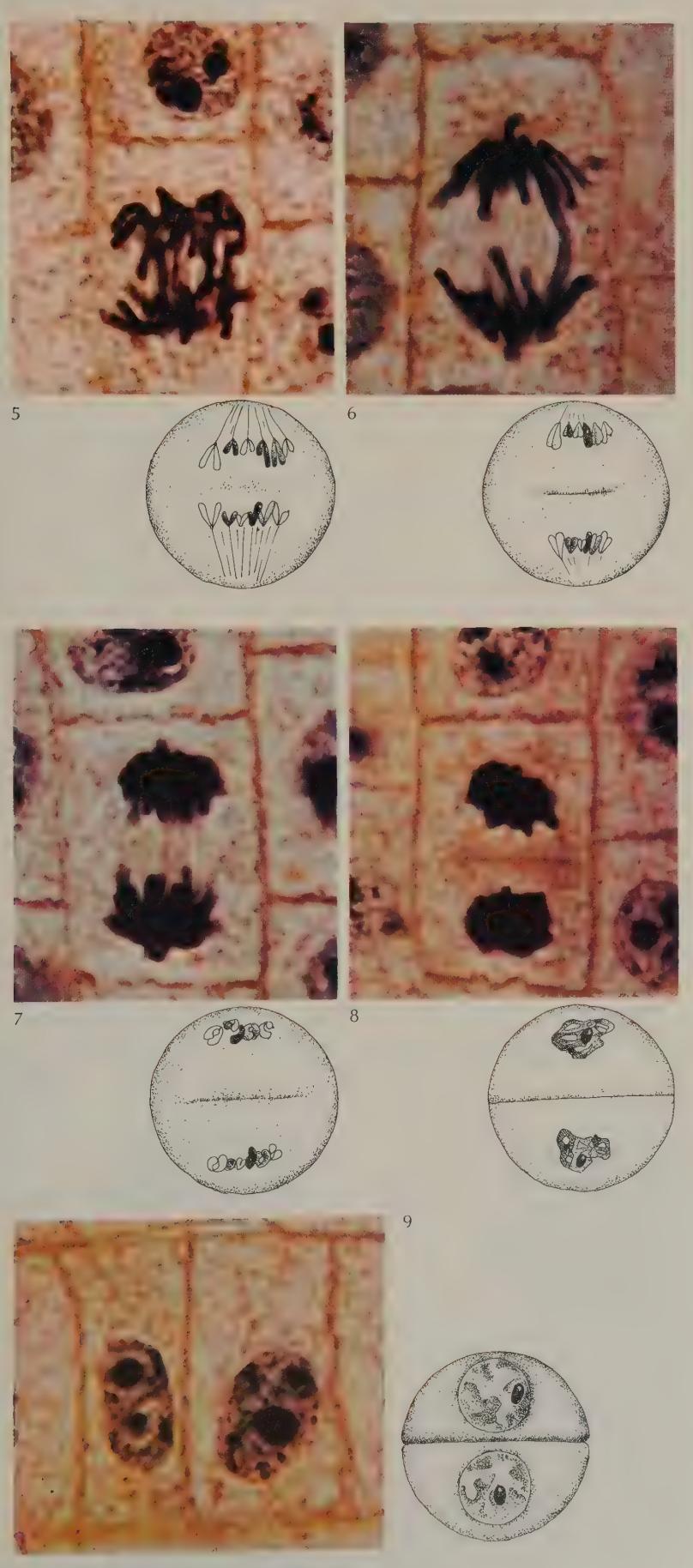
The nondividing phase in which no chromosomes are visible with the light microscope is called *interphase*. Under the light microscope, the chromatin appears granular or in the form of very thin, randomly coiling threads. Early investigators called this phase the resting stage, but subsequent research has shown that it is, in fact, a period of active preparation for the coming cell division.

Under the light microscope, the first visible signs of mitosis occur during *prophase*, as the chromosomes become distinctly visible. The chromatin appears to gather into long, thin, highly twisted chromosomes, each made up of two coiled filaments called *chromatids*, which lie side by side and are joined at a point called the *centromere*. Early in prophase, the cell becomes rounder, and the cytoplasm becomes denser and more viscous. As prophase progresses, the chromatids become shorter and thicker, the centromeres become more obvious, and the chromosomes move toward the nuclear membrane. The *spindle*—a structure made up of thin filaments stretching across the cell between two poles—begins to form. The nuclear membrane disintegrates, and the chromosomes move toward the center of the

Figure 13.3. Shown here is a sequence of light microscope photographs of mitotic stages in the division of an onion root cell. Mitosis is a continuous, dynamic process, and this sequence must be viewed in that context. (Courtesy Carolina Biological Supply Company)

1. Interphase. Note the darkly stained nucleus, containing what appears to be granular chromatin. Often called the "resting stage", this is a period of high molecular activity within the cell nucleus.
2. Prophase. Chromosomes are quite distinct and, as depicted in the drawing, are composed of coiled, threadlike filaments called chromatids.
3. Early metaphase. Chromosomes are beginning to become oriented on the equatorial plate with their "arms" pointing toward the poles.
4. Late metaphase. Chromosomes have become noticeably oriented on the equatorial plate.





5. Early anaphase. Chromatids are beginning to separate, with division of the centromere joining the two chromatids of each chromosome.
6. Late anaphase. Separation of chromatids continues with movement occurring toward the poles. The spindle filaments attached to each chromatid appear to be pulling them from the equatorial plate.
7. Early telophase. The clumping of daughter chromatids at the poles can be seen. Spindle filaments joining chromatids across the equatorial plate are also visible. These spindle filaments apparently pull the chromatids apart during their movement across the cell.
8. Late telophase. Cytoplasmic division is visible in this photograph, with a cell plate forming along the equatorial plate. Individual chromosomes have lost their definition, and the formation of daughter nuclei is in progress.
9. Interphase (daughter cells). Two new identical daughter cells have formed. These are conspicuous from surrounding cells by their reduced size.

Figure 13.4. Phase-microscope photograph of isolated mitotic apparatuses from sea urchin eggs magnified 1,000 diameters. In the center of the photograph is a mitotic apparatus composed of a spindle with asters at either end. The light area in the center of the asters represents the mitotic poles. ($\times 450$)



cell, where they arrange themselves on a plane perpendicular to the spindle axis about midway between the two poles. When the chromosomes have become noticeably oriented on the *equatorial plate* (the imaginary plane described above), the cell is said to be in *metaphase*. By metaphase, some of the filaments of the spindle have become attached to the centromeres of the chromosomes.

The next phase, or *anaphase*, begins with the separation of the chromatids. The centromere appears to split in two, freeing the two chromatids that make up each chromosome. The chromatids move to opposite poles of the spindle, where they gather into compact groups. During this movement, the chromatids look as if they are being dragged across the cell by the spindle filaments attached to the centromere. The filaments between the two groups of chromatids, or daughter chromosomes, look as if they were being stretched.

The gathering of the daughter chromosomes at the two poles marks the beginning of *telophase*, during which the chromosomes become longer and thinner, nuclear membranes form around the two groups of chromosomes, and finally the cytoplasm is divided to form two daughter cells, each containing one of the newly formed nuclei. The two daughter cells now return to the stage of interphase.

Mitosis is a continuous, dynamic process. The cycle of events has no beginning or end. Interphase offers a convenient starting place for description because the cell structure seems simplest in this stage and because most cells spend about two-thirds of their lives in interphase.

The Spindle

The spindle was so named because it reminded early investigators of the old-fashioned spindle used in making thread by hand. Its shape is similar to two cones joined together at their bases. The poles are the vertices of the cones, and the chromosomes at metaphase typically lie where the bases of the two cones make contact. Short chromosomes may lie entirely within the equatorial plate at metaphase; longer chromosomes usually have only their centromeres on the plate, with the arms extending into the cones or oriented more-or-less at random.

The structure of the spindle is not yet well understood. In many kinds of cells, filaments or fibers radiate into the cytoplasm from the poles, forming asters. The spindle, the asters, and the centrioles (in those cells that possess them) make up the *mitotic apparatus*. The mitotic apparatus has been isolated from some kinds of cells during division (Figure 13.4). Analysis of the isolated apparatus shows that the main component is a protein. Water, RNA, and ATP are also present in significant amounts (Mazia, 1955). As much as 15 percent of the protein in the cytoplasm may be utilized in the formation of the mitotic apparatus. The organization of the protein into fibers probably involves the formation of disulfide bridges that cross-link the various protein molecules. Microscopic studies suggest that the centromeres—and, in animal cells, the centrioles—play a role in the formation of the spindle fibers. It is not yet known whether the RNA plays a role in mitosis.

There is still considerable debate about how the chromosomes are separated in the spindle. The protein fibers of the spindle appear in electron micrographs as microtubules. Visual observation in the light microscope suggests that the spindle fibers contract between the chromosomes and

Figure 13.5. Micrographs of the various stages of mitosis in fixed and stained pollen grains (microspores) of a spiderwort plant (*Tradescantia*). (a) Pollen grain of a spiderwort plant (*Tradescantia*). (b) A cell with one interphase nucleus, containing many chromatin strands but no distinguishable nucleus. (c) A flattened cell with a nucleus in prophase. Thick, intertwined chromosomal threads are clearly visible. (d) Late prophase with six doubled chromosomes. Each chromosome can be clearly seen to be composed of two chromatids. (e) Late anaphase with two groups of

chromosomes clustered at the poles of the spindle (not visible). (f) Late telophase with nuclear membranes formed around the two sets of daughter chromosomes. Note the disparity in size between the two nuclei (see text for explanation).

the poles and then elongate between the pairs of chromosomes, with the fibers pushing or pulling the chromosomes apart. There is some evidence that ATP may be necessary for the contraction and elongation of the spindle fibers. Electron micrographs confirm that spindle fibers attach to the chromosomes at the centromeres. It has been postulated that the fibers leading from centromere to pole begin to be formed at the centromere and extend toward the pole as they grow. Some investigators have suggested that the fibers contract with expenditure of ATP energy, perhaps in a process analogous to the contraction of myofibrils in muscle cells. Others have suggested that the fibers between centromere and pole decrease in length by loss of protein molecules, whereas those between centromeres grow longer by addition of protein molecules. None of the many hypotheses under consideration has been confirmed experimentally, and the mechanism of chromosome movement during anaphase remains a mystery.

Chromosomal Changes During Mitosis

Figure 13.5 shows the various stages of mitosis observed in fixed and stained pollen grains (microspores) of a spiderwort plant (*Tradescantia*). In Figure 13.5a, the cell contains one interphase nucleus with many chromatin strands but no distinguishable chromosomes. Figure 13.5b shows a cell that has been somewhat flattened to make the structures more visible. The nucleus of this cell is in early prophase, with thick chromosomal threads that are still long, contorted, and intertwined. Figure 13.5c is a nucleus in late prophase with six separate chromosomes, each clearly double. Figure 13.5d shows a metaphase cell in a view looking toward one pole of the spindle, showing the six double chromosomes lying on the equatorial plate. Each chromosome can be clearly seen to be composed of two chromatids. The spindle itself is not made visible by the staining procedures used in preparing these cells. Figure 13.5e shows a cell in late anaphase with the two groups of chromosomes clustered at the poles of the spindle. Because of restricted space, the spindle is rather short in this cell. Figure 13.5f shows a pollen grain in late telophase, with nuclear membranes

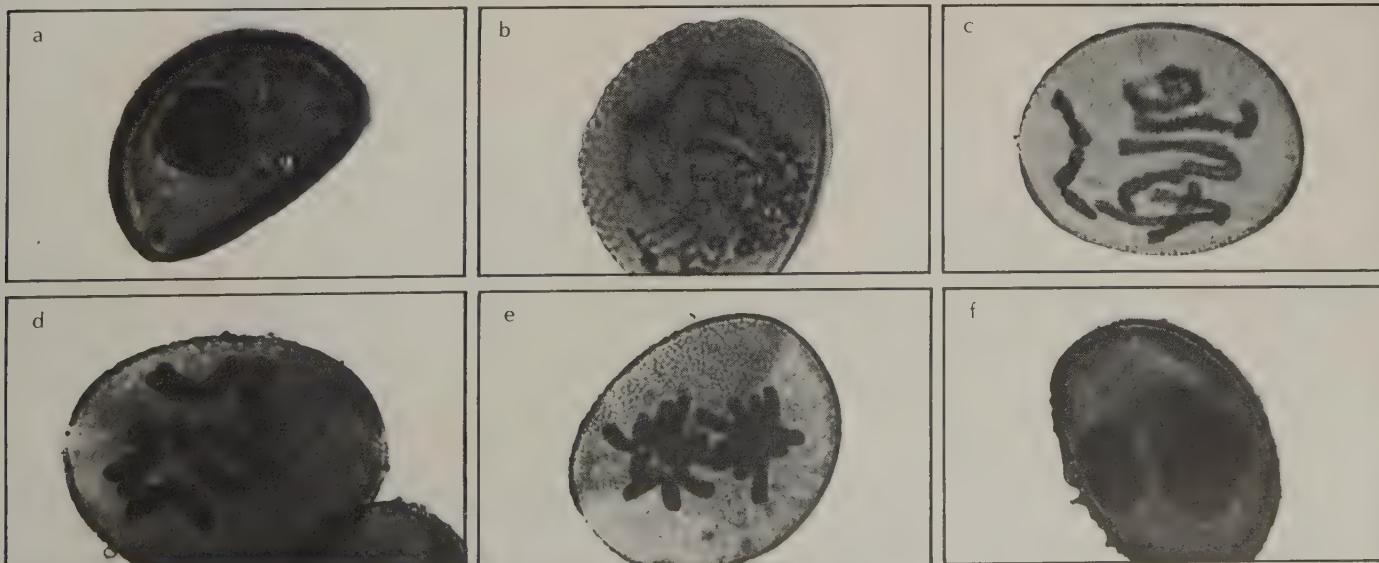
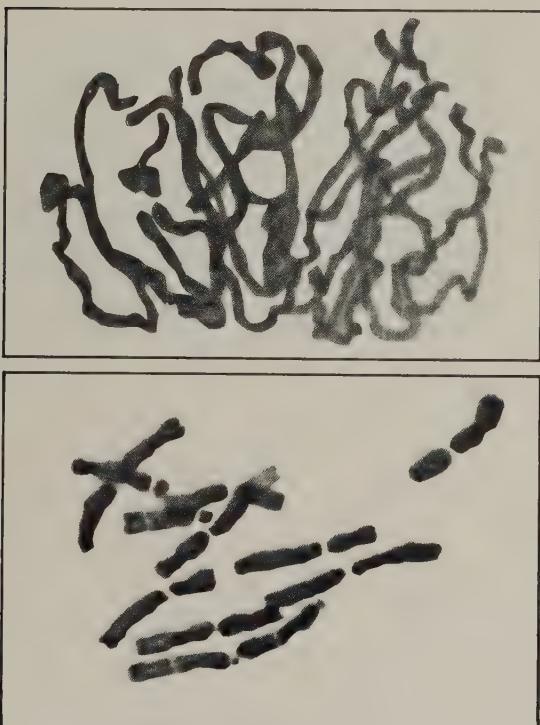


Figure 13.6 (above). Prophase chromosomes of a root tip cell from the May Apple plant (*Podophyllum*). Note the ribbonlike nature of the chromatid pairs.

Figure 13.7 (below). Metaphase chromosomes from a cell treated with colchicine. The spindle has been broken by the drug, and the metaphase position of the chromosomes has been altered somewhat by flattening the cell. Various morphological features of chromosomes can be identified by the colchicine-metaphase technique (see text for explanation).



formed around the two sets of daughter chromosomes. The two nuclei contain the same number of chromosomes, and, as far as can be determined, their two sets of chromosomes are identical. As can be seen, however, the two nuclei already show a difference in appearance and will perform different functions. The larger of the two nuclei will never divide again, but it has metabolic functions in programming the further development of the pollen grain. The smaller nucleus is in a separate cell, and it will later divide again and form the two sperm nuclei that can function in fertilization of an appropriate egg cell.

Figure 13.6 shows the prophase chromosomes of a cell from a root tip of the May apple plant (*Podophyllum*). The pairs of chromatids, which become more distinct in later phases of mitosis, can be seen in this micrograph. The group of chromosomes in Figure 13.7 are in a cell that has been treated with the drug *colchicine*, an alkaloid that blocks mitosis at metaphase. The chromosomes are in the metaphase position but freed from the spindle fibers and oriented by the cells having been flattened before the photograph was taken. This group of chromosomes at the colchicine-metaphase illustrates some of the morphological features of chromosomes.

Each chromosome has at least one constriction, which appears as a light spot in the chromosome. This narrow region is the centromere, or spindle attachment. The two parts on each side of the centromere are called the arms. The overall length of a chromosome and the ratio of its arm lengths are identifying features. In a diploid cell, such as this root cell, there are two chromosomes of each type. The pair of similar chromosomes are called homologous chromosomes, or simply *homologs*. In bisexual organisms, one homolog is inherited from each parent.

In some cells, each kind of chromosome can be distinguished from the others by its size and shape. Various species of mammals have from 17 to more than 40 kinds of chromosomes, and some of these may appear identical under the light microscope. On the other hand, the six homologs of the May apple cell are readily distinguishable. Four of the six different homologs have a single constriction, or nonstaining gap. The other two homologous pairs (four chromosomes, each with two chromatids) have two constrictions each. In this species, the two shortest chromosomes have two constrictions. One constriction is the centromere; the other is the nucleolar organizer region. The May apple nucleus may have four nucleoli, one at each organizer region, or in some cells two or more of the nucleoli may fuse; therefore, the number of nucleoli may vary from one to four. The nucleoli disappear during prophase and are re-formed during telophase. During metaphase and anaphase, the nucleolar organizer regions appear as nonstaining gaps in the chromosomes. Determination of which gap represents the centromere and which represents the nucleolar organizer can be made by observing the cells in true metaphase to see where the spindle fibers attach. In the two chromosomes at the lower right of Figure 13.7, the constriction near the middle is the nucleolar organizer, and the constriction near one end is the centromere.

STUDIES OF THE CELL CYCLE

A cell spends most of its time in interphase. Because few events associated with division can be observed with the light microscope in interphase cells, this stage was long thought to be a period of mitotic inactivity. However, the interphase stage now is known to be the active, metabolic stage when

Figure 13.8. The diagram illustrates the life cycle of a "typical" cell. Note the great disparity between interphase and the other cell division stages. The total time required to complete one cycle varies from one cell type to another and with varying environmental conditions, such as temperature.

most or all of the components of the cell are synthesized. In a cell preparing for division, nearly every component may be doubled in amount before prophase. The DNA molecules of the chromosomes are replicated during interphase, and the mitotic process may be regarded as an intricate mechanism that precisely divides the two sets of DNA between the daughter cells. The other parts of the cell are divided less precisely, and the two daughter cells may differ in size and in cytoplasmic components.

A technique called autoradiography provided the first tool for the study of DNA synthesis during interphase (Interleaf 13.1). This technique reveals that there is an interval, or gap (called the G_1 period), of several hours after the end of telophase during which no DNA but much RNA and protein is synthesized. In the bean root cells used in the original studies, the G_1 period lasts about six hours after the start of interphase. The G_1 period is followed by an S-phase of DNA synthesis (chromatin replication), which lasts for about six to eight hours. After the S-phase, there is another gap, the G_2 period, of about six hours during which no DNA synthesis occurs. The G_2 period ends with the beginning of prophase.

Studies of DNA synthesis in many kinds of plant and animal cells have shown the same general cycle of activity that was observed in the bean root cells (Figure 13.8). In prokaryotes, however, similar studies show that DNA synthesis proceeds almost continuously during the short life of the cell.

Autoradiographic studies have revealed a great deal about the events that occur during interphase, when few changes can be observed directly with the light microscope. It is now clear that "resting stage" is an extremely inaccurate description for interphase.

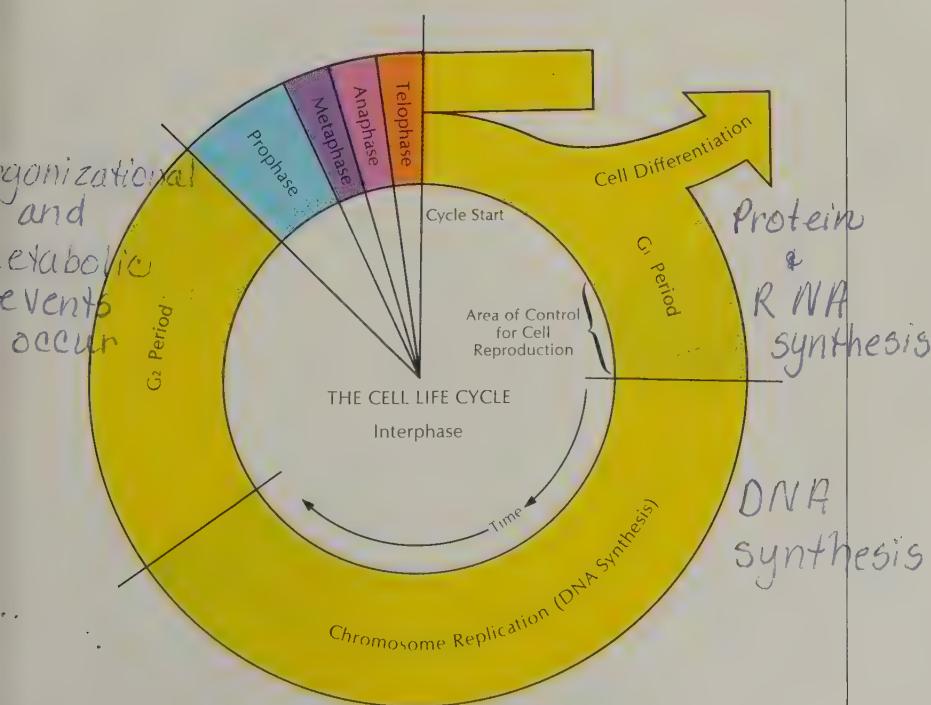


Figure 13.9. Autoradiographs of a cell labeled with H³-cytidine, which is utilized in the synthesis of both DNA and RNA. In the autoradiograph at left, note that only interphase nuclei are labeled, as cells in other stages of division do not incorporate cytidine into the nucleic acid structure. On the right, the cytoplasm is labeled.

Interleaf 13. AUTORADIOGRAPHY

In the technique of autoradiography, a cell is "labeled" by radioactive material that is incorporated into particular cellular structures. The cell is then placed in close contact with photographic film, which is exposed by the radiation. When the film is developed, it shows a picture of the cellular structures into which the radioactive material was incorporated.

The first available radioactive labeling material was phosphorus-32. P³²-phosphate supplied to a cell is incorporated into nucleotides, the building blocks of DNA. If the cell is synthesizing DNA, some of the radioactive nucleotides will be built into the new DNA molecules as they form. When the photographic film (which has been placed in close contact with the cell for an appropriate time) is developed, black silver grains will appear wherever radiation (beta rays) from decaying atoms of P³² struck the film. If the radioactive thymidine has been built into DNA molecules, the dark grains will lie in long lines. If no DNA is being synthesized, the dark grains will be scattered randomly through the cell.

The technique of autoradiography was further improved through use of carbon-14 and hydrogen-3 (tritium) as labels. The lower energy radiations from these isotopes penetrate the film for shorter distances, permitting more exact location of the radioactive structures. J. H. Taylor and his colleagues first prepared thymidine labeled with H³ and used this method to study DNA synthesis in cells. Since then, many other precursor compounds labeled with H³ have been used to study the synthesis of DNA, RNA, proteins, and other large molecules in the cell.

The simplicity and elegance of autoradiography have made this technique very popular among cell biologists. Many new features of cellular reproduction and metabolism have been revealed through autoradiographic studies. Figure 13.9 is an autoradiograph of a cell labeled with H³-cytidine, which is utilized in the synthesis of both DNA and RNA. The photograph actually is taken through a light microscope, looking at the developed film that had been in contact with the cell. The radioactive atoms that produced the black spots (silver grains) over this nucleus were incorpor-

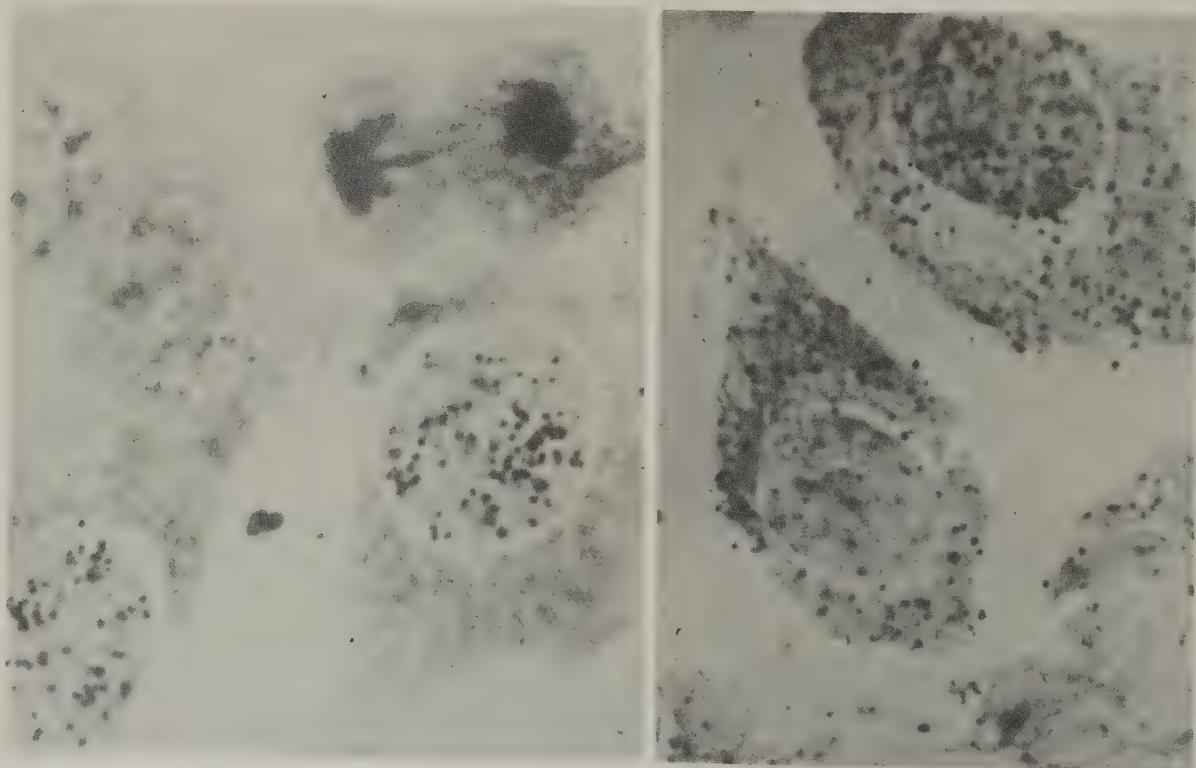


Figure 13.10. Living cancer cells (HeLa strain) attached to a glass surface and growing in a monolayer culture. The ovoid cell in the center is in mitosis (metaphase). Note the condensed chromosomes aligned on the equatorial plate. The bright halo effect surrounding the mitotic cell is characteristic of rounded objects viewed by phase contrast, which is the type of microscopy used here.



ed primarily in RNA molecules. This fact is not apparent from observation of the photograph but is determined from a knowledge of the experimental procedures and the intermediate substances from which the cell originally received the isotope.

TISSUE CULTURE

Cells can often be isolated from tissues and grown in cultures, much as bacteria are grown in the laboratory. Through repeated mitotic divisions, a single cell may produce a population of cells very similar in both appearance and behavior. Such cells grow independently in the culture, much as microorganisms do. Because all of the cells in the culture behave similarly, it is possible to remove samples of cells at intervals and examine them with various techniques to determine what the entire population is doing at each stage.

The same technique can be used with some populations of cells taken from a particular organ or tissue—for example, cells from the root of a plant or from the liver or kidney of an animal. However, the populations of cells from such organs are not as uniform as cloned cells—that is, cells derived by division from a single cell.

Vertebrate animal cells grown in cultures have many uses in addition to the study of cell division. For example, they are useful in the study of the growth and reproduction of viruses. Such cells have been used as hosts for growing viruses for the production of vaccines. Many cancer cells have been grown in culture, and studies of such cells may eventually contribute the necessary links for understanding the nature of cancer.

Isolated cells in culture typically grow best when they can attach to glass or certain plastic surfaces (Figure 13.10). However, experimenters have isolated a few kinds of cells that can grow suspended in the appropriate fluids. By using either attached or free cells, the experimenter can vary the cell's environment in ways that are impossible within the whole organism. Isotopic labels can be supplied or removed. Samples of the population can be harvested for experimental study, leaving a supply of similar or identical cells for future studies or for the production of new cultures. The cells can be frozen by appropriate methods and stored for years at low temperatures; when thawed, they will grow and initiate new cultures.

Over the past few decades, cell and tissue culture studies have complemented the studies of cells taken from living organisms. Each kind of experimentation contributes some information to the growing knowledge of cell biology.

Figure 13.11 (left). Untagged chromosomes of *Bellevalia* in metaphase. Note how large and distinct the eight chromosome pairs are in this specimen.

Figure 13.12 (right). Radioactively tagged chromosomes of *Bellevalia* in metaphase. These chromosomes have duplicated once in the radioactive solution, and both members of each pair are labeled.

In most studies of fixed and stained tissues, about two-thirds of the cells are found to be in interphase even in tissues where rapid division is known to occur. Therefore, early investigators assumed that a cell spends about two-thirds of the cell cycle in interphase. Studies with autoradiographic techniques and time-lapse cinematography have confirmed this picture of the cell cycle. The time required for a complete cell cycle and the amount of time required for each stage in the cycle vary greatly among different kinds of cells but are extremely constant among different individual cells of the same kind. The length of the cell cycle does vary with physiological conditions and temperature. Motion pictures of cell division and experiments dealing with the effects of temperature on cell division have been made possible by techniques of tissue culture (Interleaf 13.2).

The length of the cell cycle varies from less than an hour to more than 20 hours in various kinds of cells. In most cases, interphase occupies 60 to 95 percent of the cell cycle. The other phases of the cycle are accomplished fairly rapidly, usually within 10 to 60 minutes. About two-thirds of this time is devoted to prophase and about one-third to telophase. Metaphase and anaphase occupy small periods of time within the mitotic cycle.

Chromosomal Replication

Synthesis of the major components of the chromosomes occurs during S-phase, although some events essential to chromosomal reproduction may occur in G₂ or in prophase. In addition to DNA replication, the synthesis of the basic proteins (histones) associated with chromosomal DNA occurs during S-phase. RNA and other protein components of the nucleus are synthesized throughout interphase.

Studies in which selective labeling of RNA was used show that nearly all of the RNA synthesized in the nucleus is either transported out to the cytoplasm or broken down in the nucleus within a few hours. The histone molecules are as stable as the DNA with which they are associated. Molecules of DNA and histone remain intact for many cell generations, although additional new molecules are synthesized in each cycle of cell division.

Chromosome replication has been extensively studied through the use of H³-thymidine labeling. This substance is known to be used almost exclusively in DNA synthesis. Root cells of several plants were the first test subjects for H³-thymidine labeling studies. Because the plant *Bellevalia* has only eight large chromosomes in each cell, particularly clear results were obtained from studies of its root cells (Figure 13.11). Bulbs and roots of the plant were immersed in a solution containing H³-thymidine for one to two

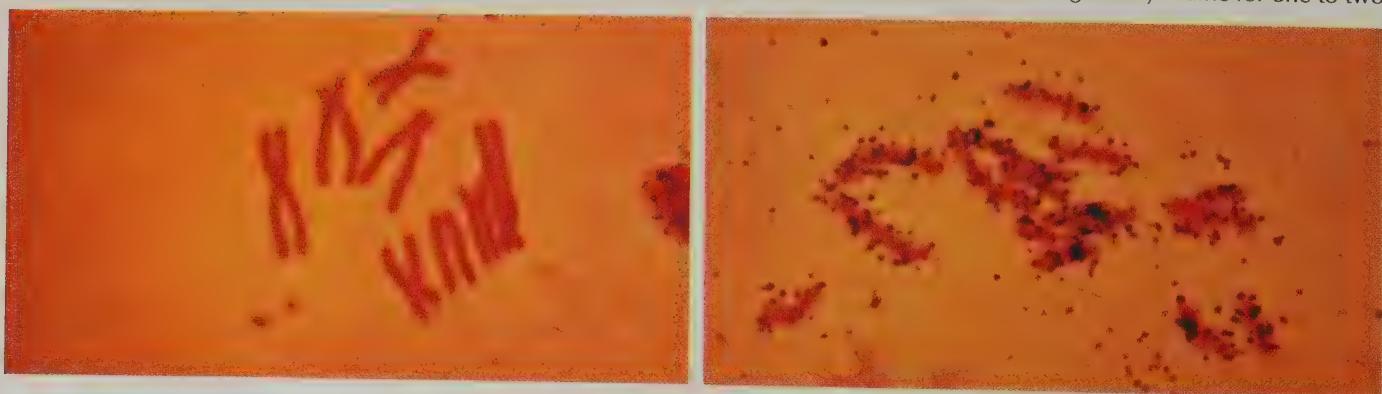


Figure 13.13 (above). Radioactively tagged chromosomes of *Bellevilia* in metaphase that have been allowed to duplicate once in the radioactive solution and once after the cells were removed. Only one member of each chromatid pair is tagged, except where segments have broken and have been exchanged between sister chromatids.

Figure 13.14 (below). Proposed diagrammatic representation of chromosome organization and replication that interprets the autoradiographic results. Solid lines represent nonlabeled units, and those units



hours. Some of the roots then were fixed and squashed on glass slides, while the others were put into a nonradioactive solution to continue growing. The autoradiographs showed that about one-third of the cells had incorporated the labeled thymidine into their DNA. This observation was consistent with the expectation that about one-third of the cells would be in S-phase at any given time.

Autoradiographs of labeled cells reaching metaphase showed that all of the chromosomes were about equally labeled and that both chromatids of each pair had incorporated the H^3 -thymidine into their DNA (Figure 13.12). This observation confirmed the model of DNA replication suggested by Watson and Crick (Chapters 4 and 15). Each chromosome entering S-phase contains a double strand of DNA. During DNA replication, this strand separates and a new complementary strand is built on each of the original strands. Thus, each chromatid would be expected to contain one original unlabeled strand and one labeled strand built during immersion in the H^3 -thymidine solution.

Next, samples were taken from roots that had grown in nonradioactive solutions for 36 hours after immersion in H^3 -thymidine solution. Because the complete cell cycle was known to take about 24 hours, these cells would be expected to have passed through one S-phase in the unlabeled solution. The chromosomes of each labeled cell would have entered that S-phase with a double strand of DNA in which one strand was labeled and the other unlabeled. When the strands separated, each would acquire an unlabeled complementary strand. Thus, cells in metaphase after 36 hours would be expected to have chromosomes in which one chromatid was labeled and its sister chromatid was unlabeled. Some cells did show the expected pattern (Figure 13.13), but there were significant exceptions.

Some chromatids had only a portion of their length labeled. However, when both sister chromatids were examined, enough labeled segments were available in each metaphase chromosome to account for one fully labeled chromatid in each chromosome. The most reasonable way to account for these observations is to assume that the whole unit of DNA in a chromosome does not necessarily remain intact through an entire S-phase. Some breaks and exchanges between sister chromatids must occur (Figure 13.14). If each chromatid is composed of one long DNA molecule or of many shorter molecules joined end to end, the observations are explainable.

The arrangement of DNA in chromatids is still a controversial matter, but it seems clear that very long chains are folded to form nucleoprotein fibrils about 250 Å in diameter. The DNA molecules, which are only about

in dashed lines are labeled. The dots represent grains in the autoradiographs.

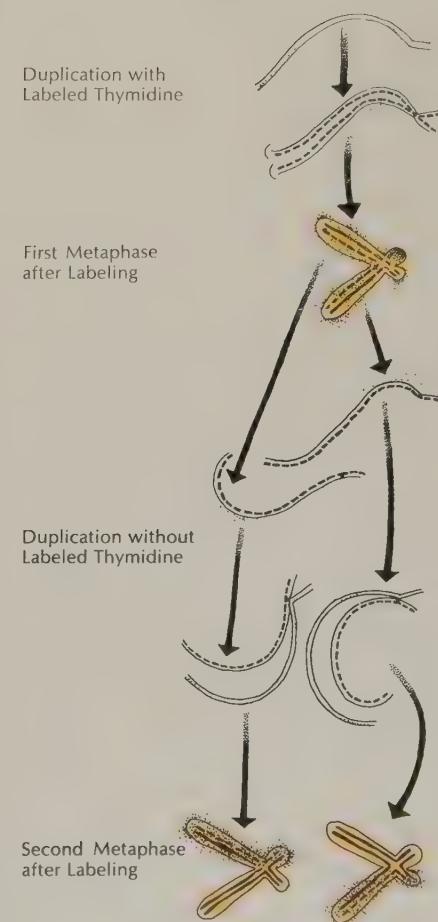
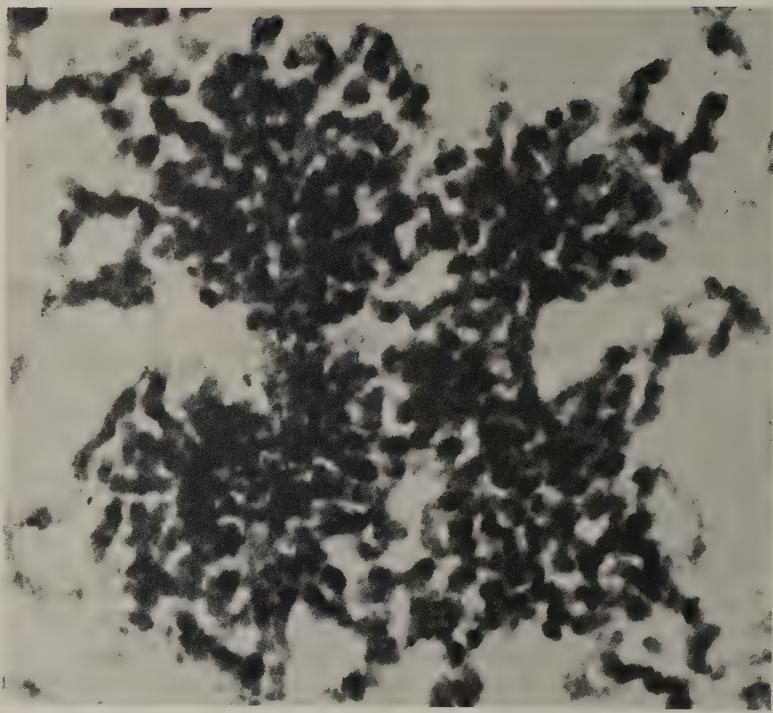


Figure 13.15. Electron micrograph of a thin section of nucleus. The predominant fibers are 250 Å, but finer fibers suggest a possible unwinding of the chromatin.



20 Å in diameter, are folded or coiled in some unknown way in the larger fibrils. The fibrils, in turn, appear to extend as rather loose loops from the central axis of the chromatid (Figure 13.15).

In certain tissues, such as the salivary glands of the larvae of flies and mosquitoes, giant chromosomes are formed. These giant chromosomes are more than 1,000 times the volume of the corresponding normal chromosomes. They are thought to be formed by repeated replications of the DNA molecule, forming perhaps 1,000 copies of the DNA molecule within a single giant chromosome. Because the giant chromosome is about the same length as the corresponding normal, uncoiled interphase chromosome, the giant chromosome is thought to be made up of DNA strands lying side by side with little or no coiling. For this reason, the giant chromosomes are called *polytene* (many-stranded) chromosomes.

Regular patterns of light and dark bands appear on the polytene chromosomes. These patterns have been mapped and have been shown to be consistently the same for a given kind of chromosome from a given species. Mutations that are expressed in various physiological abnormalities in the insects in some cases cause alterations in the band pattern of the polytene chromosomes. Studies of these changes made it possible to map the location of particular genes on the chromosomes of organisms such as the fruitfly (Chapter 14).

Centrioles

All eucaryotic cells except those of higher plants have centrioles, and these structures appear to play a major role in the formation of the mitotic spindle. Two pairs of centrioles exist in the interphase cell, with the centrioles of each pair at right angles to each other. As prophase begins, the pairs of

centrioles separate. Each pair lies at the center of an aster, and the spindle fibers appear to be spun from the centrioles as they separate. Electron micrographs, however, show that the spindle fibers do not actually contact the centrioles. Each centriole is made up of nine groups of microtubules, usually with three microtubules in each group. These microtubules are similar in appearance to the microtubules that make up the spindle fibers. The centrioles also appear to play a role in the formation of cilia and flagella. In the cilia and flagella, patterns of microtubules show a clear relationship to the microtubules of the centriole, but the microtubules of the structure apparently formed by the centriole do not appear to be attached to the centriole.

During telophase, as the two daughter nuclei form, each pair of centrioles replicates to give two pairs of centrioles to each daughter cell. The replication of centrioles thus may be regarded as the first step in preparation for the next cycle of mitosis, occurring even before the previous cycle is completed. The mechanism by which the centrioles replicate is unknown. The role of the centrioles in construction of spindles and asters also is unknown. Although the centrioles appear to play a major role in this process in most eucaryotic cells, the higher plant cells that lack centrioles are able to construct a mitotic apparatus almost identical to that of the cells having centrioles.

Cytokinesis

The final step in cell division, occurring at the end of telophase, is cytokinesis, or cell cleavage. In this process, the nuclei and cytoplasm of the daughter cells are separated by the plasma membrane to form two complete and independent cells. In some cells, such as those of striated muscle tissues, mitosis may occur without cytokinesis, thus forming multinucleate cells.

The mechanism of cytokinesis is markedly different in animal and in plant cells. In plant cells, a structure called the *cell plate* forms along the equatorial plate of the spindle during telophase. This cell plate appears to be composed of membranes from the Golgi complexes, which gradually fuse to form new plasma membranes that separate the two daughter cells. The growth of the new membranes probably proceeds from near the center of the cell outward, until they fuse with the membrane of the parent cell.

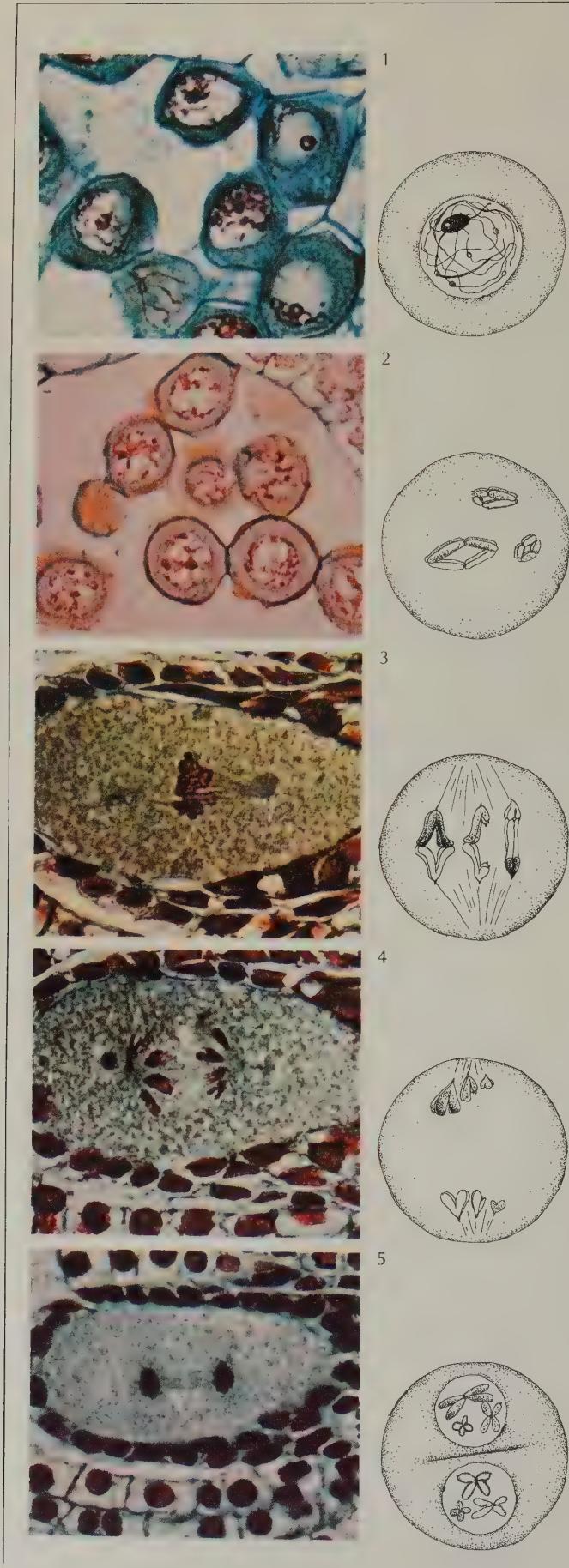
In animal cells, the cell membrane constricts or furrows, gradually closing in until the two daughter cells are separated. The mechanism by which this furrowing is carried out is unknown but probably involves contractile microfilaments. The fact that cell cleavage has been observed in cells from which the mitotic apparatus has been removed rules out the hypothesis that the cell membrane is pulled inward by the spindle or aster fibers. Hydrolysis of ATP is apparently involved in the process of furrowing, and there is some evidence that a contractile protein is involved in the process, obtaining energy for its contraction from ATP.

MEIOSIS

Mitosis maintains the constancy of chromosome number from one cell generation to the next. Mitosis alone, however, does not make sexual reproduction possible, for fusion of sex cells with the full chromosome number would double the number of chromosomes in the resulting cell. The possibility of sexual recombination is provided by the process of meiosis, which produces haploid cells from diploid ones, thereby making subsequent cell fusion feasible (Figure 13.16). The genetic reshuffling made possible by

Figure 13.16. Sequence of meiotic stages in the division of *Lilium michiganense*. (Courtesy Carolina Biological Supply Company)

1. Early prophase. The chromosomes are becoming visible.
2. Late prophase. The thick double threads (chromatids) lie close to the periphery of the nucleus.
3. Metaphase I. The chromosomal pairs are arranging themselves along the equatorial plate.
4. Anaphase I. The individual chromosomes of each pair separate and move toward the poles, possibly pulled by the contracting spindle fibers.
5. Telophase I. The chromosomes have reached the poles and are bunched up tightly. They are separating into the chromatin network of the daughter nuclei, and a new nuclear membrane is forming.

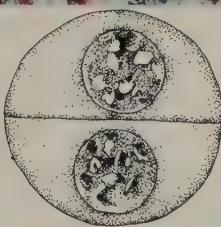




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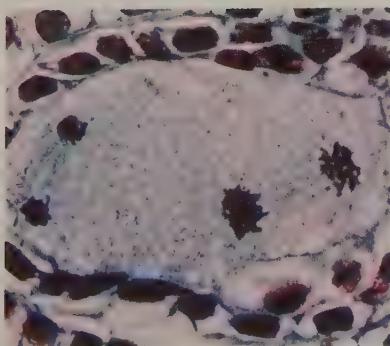
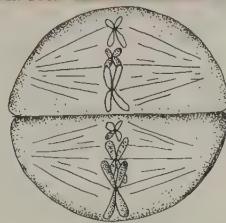
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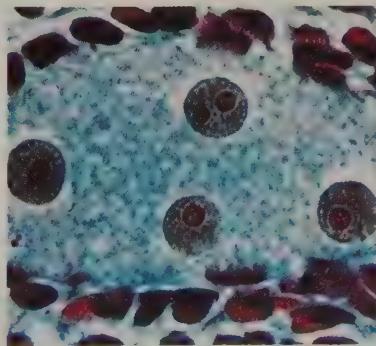
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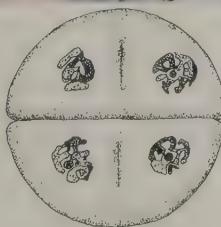
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10



11



6. Interphase. Two nuclei are visible. Note the remnants of the spindle fibers.
7. Prophase II. Chromosomes are condensing, and the spindle is forming as the nuclei prepare for the second division.
8. Metaphase II. Spindle fibers are appearing, and the chromosomes are lining up along the equatorial plate.
9. Anaphase II. The centromeres have separated, and the chromosomes are moving to the opposite poles of the spindle.
10. Telophase II. The chromosomes have separated, and nuclear membranes are forming around the haploid sets of chromosomes. The plasma membranes will separate the daughter cells.
11. Spores. The diploid cell has become four haploid spores.

sexual reproduction almost certainly accounts for the explosive evolution of eucaryotic organisms into the spectacular array of higher animals and plants that now populate the earth.

Sexual reproduction is identified almost exclusively with diploid organisms, occurring only as exceptional processes among haploid organisms. However, sexual reproduction probably first arose as a means of genetic reshuffling among haploid organisms. Its introduction required the development of meiosis as a method of returning the fused cell to its normal haploid condition. Thus, diploidy, which provides enormous adaptive advantages for an organism (Chapter 36), was at first an intervening condition existing briefly after sexual reproduction. The condition was reversed in the evolution of higher organisms, with diploidy becoming the dominant phase and haploidy the intermediate one.

Meiosis in diploid organisms involves two successive cell divisions accompanied or preceded by a single chromosomal replication (Figure 13.17). The first meiotic division (meiosis I) is similar to mitosis in some respects but involves an exceptionally long and complex prophase. In this division, pairs of homologous chromosomes are separated, but each chromosome retains both chromatids. In the second meiotic division (meiosis II), which follows a brief interphase, the chromatids are separated. Mitosis produces two diploid daughter cells from a single diploid parent, and meiosis produces four haploid daughter cells from a single diploid parent.

The movements of chromosomes and other cell structures during meiosis, as seen in the light microscope, were described before 1900, but the molecular events that initiate the process, that lead to pairing and segregation of the chromosomes, and that result in reciprocal changes between homologs are only now beginning to be understood.

Meiosis I

Meiosis I is characterized by a very long and complex prophase. As in other prophases, the first visible change is a condensation of chromatin into long, tangled, threadlike chromosomes less than a micron in diameter. Because prophase I of meiosis is so complex, it has been subdivided into several stages. The first stage is called the *leptotene* stage. The leptotene chromosomes appear longer and thinner than the chromosomes of mitotic prophase and are distinguished by the presence of a series of dark granules, or chromomeres, along the chromosomes. Although studies of DNA synthesis indicate that each chromosome must already contain two double strands of DNA at the beginning of the leptotene stage, the chromosomes are not visibly double as they are in mitosis.

In the *zygotene* stage, each chromosome pairs with its homolog in a very regular fashion. Each chromomere can be seen to match up with the corresponding chromomere on the homolog. During this stage, the chromosomes continue to shorten and thicken. The joining of homologous chromosome pairs is called *synapsis*. Homologous chromosomes always synapse in pairs even in polyploid nuclei, where several copies of each homolog are present. At the close of the zygotene stage, the nucleus appears to have only the haploid number of chromosomes, but each apparent chromosome is actually a pair of homologs closely bound together. These pairs are called *bivalents*.

In the *pachytene* stage, further shortening and thickening of the chromosomes occurs. During this stage, segments are interchanged between pairs

Figure 13.17. Comparative diagram of meiosis and mitosis. Printed by permission from J. D. Watson, Molecular Biology of the Gene, © 1965, J. D. Watson.

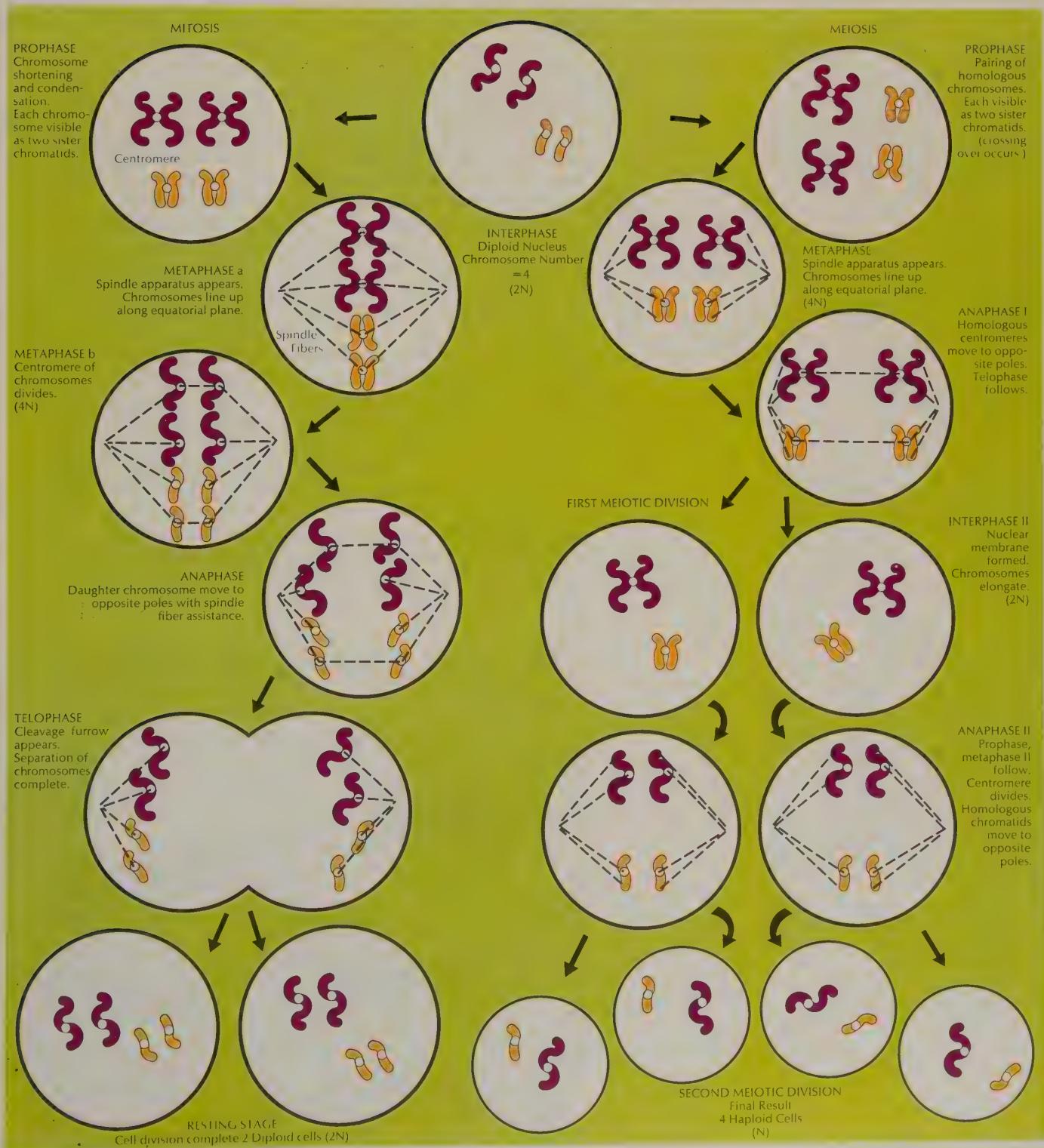


Figure 13.18. Micrograph of a single bivalent. The chromatids cross each other at the points of the two chiasmata.

of homologous chromatids. This process apparently involves breakage of the chromatids at corresponding points, interchange of the two segments, and rejoining of the chromatids.

In the *diplotene* stage, the homologous chromosomes begin to separate, and the pair of chromatids making up each chromosome is visible. The chromosomes do not separate entirely but are joined together at their ends and cross each other at points called *chiasmata* (Figure 13.18). Each chiasma represents the approximate point at which an exchange of chromatid segments between the two chromosomes occurred. One chiasma is formed for each bivalent, and longer bivalents generally form several chiasmata.

In the next stage, *diakinesis*, the chromosomes become maximally shortened and thickened. The chiasmata appear to move toward the ends of the bivalents until each bivalent is held together only by chiasmata at its ends. Diakinesis is the final stage of prophase I, and it is accompanied by disintegration of the nucleolus and nuclear membrane and the formation of a spindle.

Metaphase I is similar to the metaphase of mitosis, with the fully condensed chromosomes becoming aligned on the equatorial plate and spindle fibers attaching to the centromeres. However, in metaphase I of meiosis, the centromeres of each bivalent usually are separated and lie somewhat off the equatorial plate toward the poles.

In *anaphase I*, the chromosomes move toward the poles of the spindle. Each chromosome is made up of a pair of chromatids, joined by the centromere. As the two centromeres of the pairs of bivalents move toward opposite poles, any remaining chiasmata slide apart, and the pairs of homologous chromosomes are separated from each other. Anaphase I ends with a complete haploid set of chromosomes clustered at each of the poles, but these chromosomes are not identical to the chromosomes that existed



at the beginning of prophase I. Each chromosome contains one of its original chromatids and one chromatid that is a mixture of segments from its own original chromatid and from a chromatid of its homolog.

In telophase I, nuclear membranes form around the two sets of chromosomes, the chromosomes uncoil, and a cell membrane is formed between the two nuclei.

After a relatively brief *interphase*, the two haploid cells enter meiosis II. In some species, there is no noticeable interphase, and telophase I may proceed without any formation of nuclear or cell membranes and without uncoiling of the chromosomes. In any case, the chromosomes pass from meiosis I to meiosis II without further chromosomal replication.

Meiosis II

Prophase II is relatively brief, particularly in those species with minimal telophase I and interphase. It is marked by condensation of the chromosomes and formation of the spindle. In *metaphase II*, the chromosomes become aligned on the equatorial plate of the spindle, and the chromosomes replicate and become attached to the spindle fibers. In *anaphase II*, the daughter centromeres separate, and the individual chromatids move to opposite poles. During *telophase II*, the nuclei are formed around the resulting haploid sets of chromatids, which now may be called chromosomes, and plasma membranes separate the daughter cells.

The second meiotic division is similar to mitosis, but begins with a haploid number of chromosomes and ends with the formation of daughter cells that contain a haploid set of chromatids. Each of the haploid cells (gametes) produced by meiosis could fuse with a haploid cell from another individual to form a normal diploid cell. This diploid cell will contain a complete set of chromosomes from each of the parent organisms.

Germ Cells

The process of meiosis occurs only in the germ cells of sexually reproducing plants and animals. In animals, these cells are found in organs called gonads.

In the male animal, the germ cell gives rise by repeated mitotic divisions to a large number of cells called *gonocytes*, which become specialized to *spermatogonia*. Each spermatogonium divides twice mitotically to form four *spermatocytes*. Each of the spermatocytes then undergoes meiosis to form four haploid *spermatids*. These cells are then transformed into the specialized structures called *spermatozoa*, or sperm.

In the female animal, the gonocytes become specialized to form *oogonia*. Each oogonium divides twice mitotically to form four *oocytes*. Each oocyte undergoes meiosis to form four haploid *ootids*. One of the ootids develops into an *ovum*, or egg. The other three become *polar cells*, which eventually disappear. The function of the polar cells is unknown.

In plants, meiosis occurs at various times during the life cycle. In some cases, male and female sex cells similar to the sperm and ova of animals are formed. In other cases, the products of meiosis are asexual spores. In some plants, the haploid stage involves a significant portion of the life cycle of the organism.

The result of meiosis and sexual reproduction is to give each diploid cell a reshuffled combination of genetic information. Each zygote obtains a complete haploid set of chromosomes from each of its parents. Both sets

are duplicated in each of the body cells, which arise by mitotic division of the zygote. When gametes are formed by meiosis, the assortment of the members of each chromosome pair occurs in an independent fashion, so that each gamete is likely to contain a mixture of maternal and paternal chromosomes in its complete haploid set. Thus, in sexually reproducing organisms, each mutation that happens to arise is shuffled and recombined with many other possible mutations to increase astronomically the combinations of different genetic messages that can be found within a population.

Research on Meiosis

Many questions about meiosis remain unanswered today, and research is under way in many laboratories to learn more about this vital process. The pairing of homologous chromosomes during the zygotene stage of meiosis I is an extremely important and little-understood process.

One of the basic problems is that of explaining the mechanism by which the homologous pairs of chromosomes locate each other. Because the homologs contain the same sequences of genes, their DNA nucleotide sequences are similar. It is known that single-stranded DNA molecules of similar nucleotide sequences will locate one another in a solution and become attached to form a double strand. There is no evidence that a similar chemical attraction will occur between double-stranded DNA molecules. Furthermore, the DNA of the chromosomes is thought to be enclosed by protein molecules that protect it from hydrolyzing enzymes.

The process of synapsis, in which the homologs become attached to form a bivalent, is also poorly understood. Recent electron-microscope studies have suggested that, in at least some species, the ends of all chromosomes are attached to the nuclear membrane during prophase. The ends of homologous chromosomes are located close to each other on the nuclear membrane, suggesting that synapsis might begin at the ends of the chromosomes and proceed toward the middle. This suggestion has been confirmed in light-microscope studies of *Tradescantia* and of *Agriotes mancus* (a species of beetle). On the other hand, studies of synapsis in *Drosophila* indicate that synapsis begins at a few apparently random points on the chromosomes. The points of synapsis increase as time elapses until eventually the two homologous chromosomes are joined all along their length.

Electron-microscope studies have shown that a structure called the *synaptinemal complex* exists between homologs in all bivalents. Cycloheximide, a potent inhibitor of protein synthesis, also acts to inhibit synapsis and maintenance of the synaptinemal complex. Apparently, the continuous synthesis of protein is required to maintain synapsis. The chemical structure of the synaptinemal complex is not yet known, but preliminary studies suggest that it contains protein and lipid. Other important studies are under way in an attempt to discover the nature of the mechanism that forms chiasmata and interchanges segments of homologous chromatids.

CELL DIVISION AND HEREDITY

By 1900 the general sequence of events in mitosis and meiosis had been described. Scientists had not discovered that chromosomes exist in pairs of different kinds, but they did know that meiosis results in a halving of the chromosome number. Most biologists, acting on what was known about cell division, assumed that hereditary information is carried in the nucleus.

By observing the regular assortment of chromosomes between daughter cells, several biologists were able to speculate that the chromosomes are involved in transmission of hereditary information.

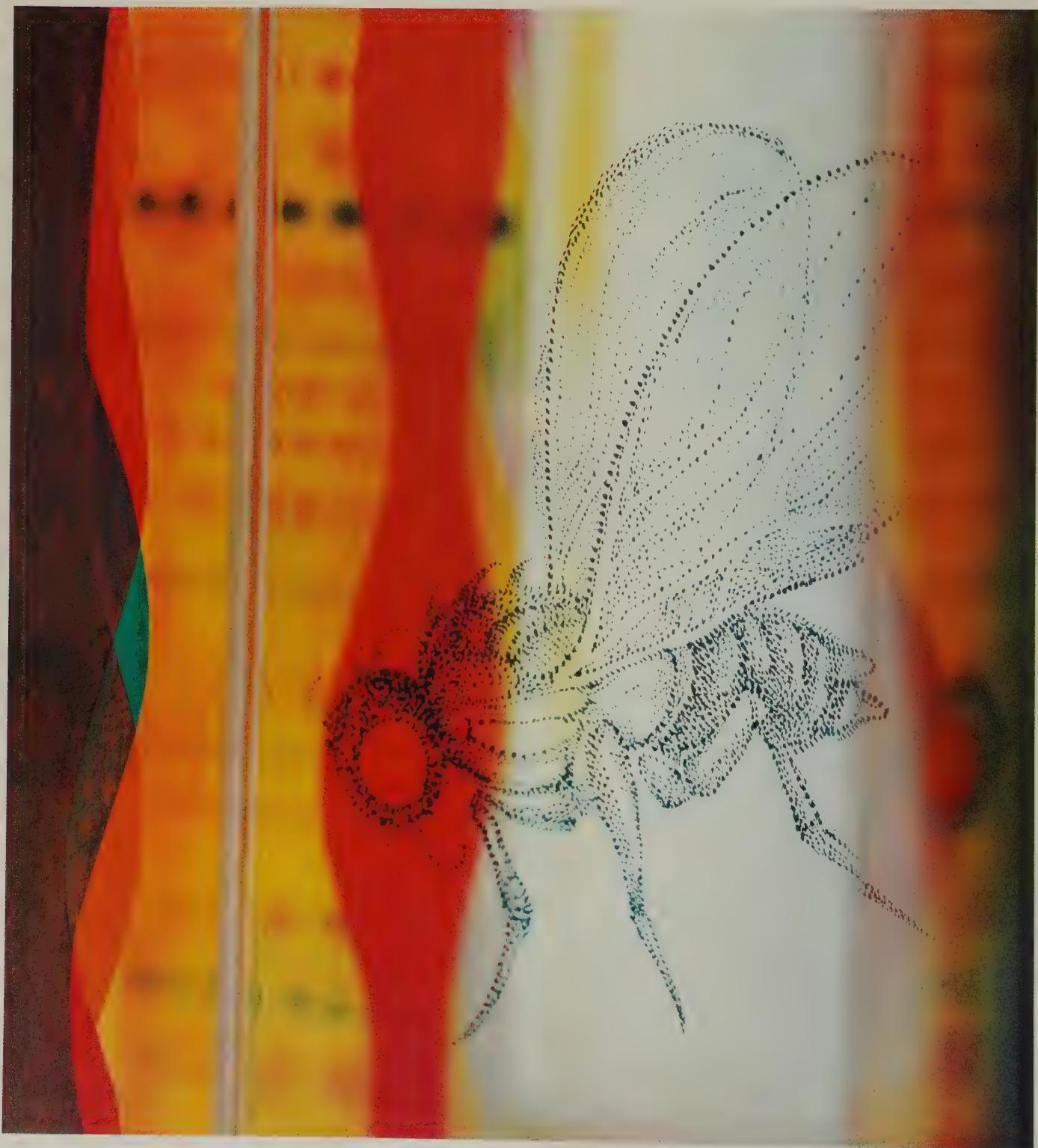
Thus, the stage was set for rapid progress with the rediscovery of Mendel's concept of hereditary factors. Biologists at the turn of the century knew that a zygote is formed by the fusion of sex cells from male and female parents and that the fusion of nuclei plays an important role in this process. They knew that all cells of the mature organism are derived through mitotic divisions of the zygote and that the sex cells are formed by meiotic divisions of certain specialized body cells. These processes provided obvious parallels to the reassortment of factors hypothesized by Mendel. Thus, twentieth-century geneticists moved rapidly into a search for the physical carriers of heredity within the cell nucleus.

FURTHER READING

More detailed information about mitosis and meiosis will be found in books by Brachet and Mirsky (1961), Harris (1963), Hughes (1952), and White (1961). More general discussions are found in articles by Mazia (1953, 1955, 1961).

14

Classical Genetics



The first decade of this century was a period of great activity in biology, and the studies of heredity must have seemed only one minor side-light of progress that included discovery of hormones, proof of the existence of viruses, discovery of human blood types, development of techniques for tissue culture, Pavlov's studies of conditioned reflexes, discovery that enzymes act as catalysts, and clarification of the anatomy and organization of the nervous system. In the physical sciences, subatomic particles were being described, and quantum theory and relativity were upsetting old ideas.

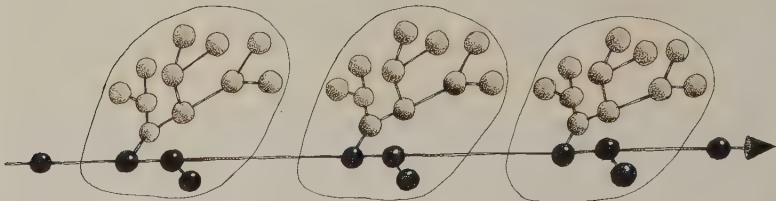
In retrospect, it is possible to pick out certain key observations and theories that seem to mark a steady progress toward understanding of inheritance. Such historical selection always tends to make things much simpler than they appeared at the time.

For the first two decades of this century, students of heredity extended Mendel's principles to explain the many apparent exceptions they had discovered and attempted to discover the physical basis for Mendel's hypothetical hereditary factors. William Bateson played a leading role in this work. He introduced such terms as "homozygote," "heterozygote," and " F_1 and F_2 generations" to clarify discussions of experiments and theories. He coined the word "genetics" to describe the study of heredity. The word "gene" was introduced by the Danish botanist Wilhelm Ludwig Johannsen as a term for the specific unit within the cell that corresponds to one of Mendel's hereditary factors.

Most investigators had assumed that all chromosomes in a cell are essentially equivalent. The mechanisms of mitosis and meiosis were viewed simply as devices that put half of the chromosomes into each daughter cell. The American cytologist T. H. Montgomery, Jr. showed in 1901 that the cell contains pairs of distinctive kinds of chromosomes. Each sperm or egg cell contains a complete haploid set—one of each chromosome kind. Nuclear fusion during fertilization gives the diploid zygote (and each body cell that forms from it) two complete chromosome sets—one of maternal origin and the other paternal. Montgomery assumed that meiosis again separates these sets, giving half of the gametes the maternal set and the other half, the paternal set. He worked with cells from grasshoppers because the chromosomes in many insect cells are unusually visible and few in number. Another American working with grasshoppers suggested that the chromosomes play a role in determining the sex of an individual. C. E. McClung described the existence of two kinds of sperm cells—one with an extra, or accessory, chromosome that is not present in the other kind of sperm cell. McClung concluded that eggs fertilized by sperm having the accessory chromosome develop into males, whereas eggs fertilized by sperm lacking the accessory chromosome develop into females (the reverse of the actual situation, as it later turned out).

Another study of grasshopper cells was made by W. S. Sutton. In the species he studied, Sutton found that the 11 chromosomes in a sperm or egg cell can be distinguished by size and that a fertilized egg cell or body cell contains a pair of each kind of chromosome. In meiosis, one member of each pair goes into each reproductive cell, as Montgomery had observed. At first, Sutton thought—as Montgomery had—that the maternal chromosome set is separated from the paternal set in meiosis. However, Sutton strongly suspected that the chromosomes are the carriers of the hereditary factors. If so, the chromosomes must be assorted independently

Figure 14.1. Germ plasm continuity model. Only the germ cells (black) carrying the hereditary information are sustained through the generations. The body, or somatic, cells (yellow) comprising the individual (encircled) are destined for momentary existence, then death.



among the reproductive cells, as are the hereditary factors in Mendelian theory. Although Sutton was unable to prove that the gametes do receive a mixture of maternal and paternal chromosomes, his microscopic observations convinced him that his suspicions were probably true.

Sutton concluded that the hereditary factors are closely associated with the chromosomes. He pointed out that there are far more hereditary factors than there are chromosomes in any species, so that a number of different factors must be associated with a single chromosome. Thus, a variety of recessive and dominant factors might be expected to remain together on a single chromosome, rather than assorting independently as assumed by Mendel.

By 1905 one of Sutton's professors, Edmund B. Wilson, was able to clarify the nature of sex determination in insects. In some species, the cells of males contain an unpaired accessory chromosome, whereas the cells of females contain a pair of this chromosome. Half of the sperm cells contain the accessory chromosome and half do not. An egg fertilized by a sperm cell containing the accessory chromosome forms a zygote with a pair of this kind of chromosome and develops into a female. An egg fertilized by a sperm cell without the accessory chromosome forms a zygote with an unpaired extra chromosome and develops into a male.

In a second group of insect species, all sperm cells contain the same number of chromosomes, but there are nevertheless two kinds of sperm. The sperm may contain either a large or a small form of one of the chromosomes, which Wilson called the idiochromosome. Every egg cell contains a large idiochromosome. Thus, fusion of egg and sperm can lead either to a zygote with a pair of large idiochromosomes (a female) or to a zygote with one large and one small idiochromosome (a male).

Wilson observed that those species with an accessory chromosome may be regarded as special cases in which the small idiochromosome has disappeared. Later research has confirmed most of Wilson's conclusions and has shown that the same pattern of sex determination exists in most sexually reproducing plants and animals. However, there are many species—for example, butterflies, moths, birds, amphibians, and some fishes—in which the zygote with identical idiochromosomes, now called sex chromosomes, develops into a male and the zygote with dissimilar sex chromosomes or an unpaired sex chromosome develops into a female. In these species, there are two kinds of eggs and only a single kind of sperm.

Wilson's report provided one clear example of chromosomal control of a hereditary character and thus helped to support the theory of chromosomal inheritance. In the meantime, the Mendelists were struggling to explain the results of the ever-increasing number of hybridization experiments being performed. They attempted to fit some of these results to various odd ratios such as 7:1:1:7 or 15:1:1:15. In some cases, they were forced to assume that a single factor can exist in more than two forms, that a single character is controlled by two or more independently assorted factors, or that dominance can be incomplete in certain factors' (Bateson, 1913; Bateson, et al., 1902–1909).

THOMAS HUNT MORGAN AND THE FRUITFLY

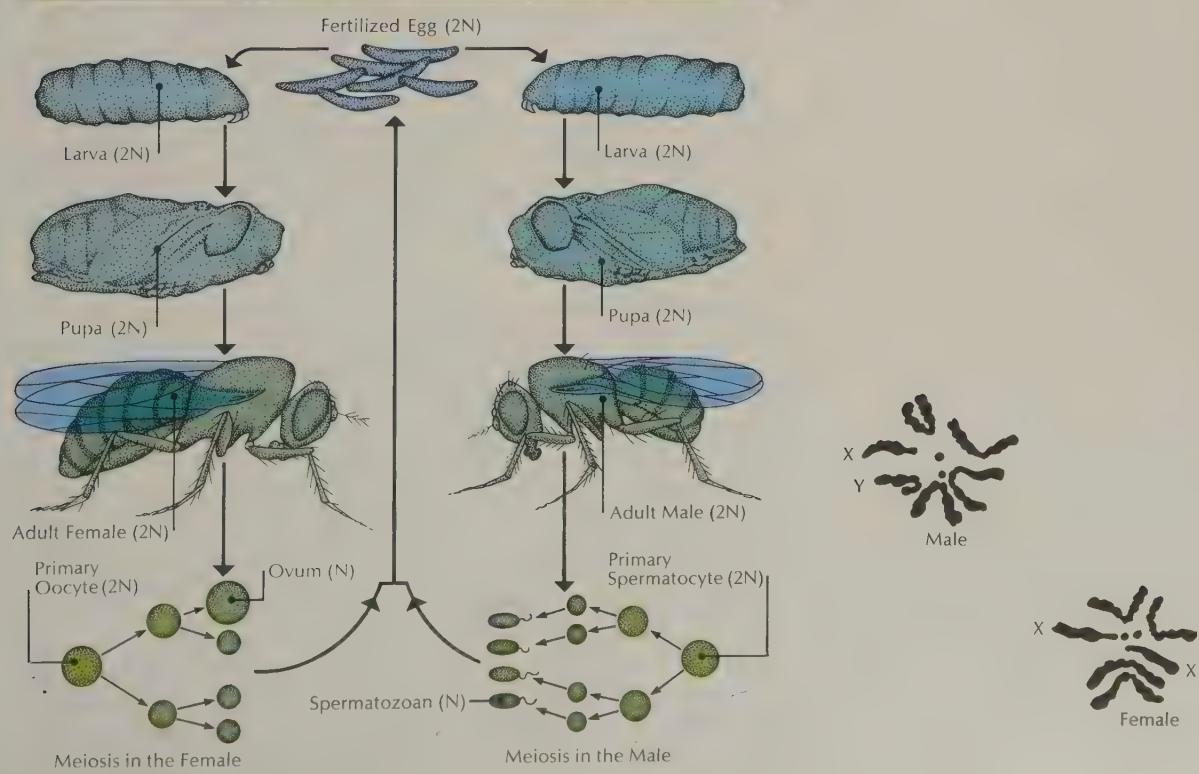
Thomas Hunt Morgan, who had specialized in embryology before becoming interested in genetics, was convinced that sudden changes, or mutations, play a more important role in evolution than do the hypothetical recombinations of hereditary factors described by the Mendelists. Like many



Figure 14.2 (above). The fruitfly *Drosophila melanogaster*.

Figure 14.3 (lower left). Life cycle of *Drosophila*.

Figure 14.4 (lower right). Chromosomes of *Drosophila*. There are four pairs of chromosomes in each cell. Note the differences in the sex determiner chromosomes of males and females.



other researchers exploring heredity and the chromosomes, Morgan turned to insects as experimental subjects. It is easy to maintain large populations of small insects; they reproduce and grow rapidly, and their cells and chromosomes can be viewed easily under the microscope. Morgan had heard of some experiments being done with *Drosophila melanogaster*, a small fruitfly. These little flies thrive on a diet of mashed fruit or yeast, can be kept by the hundreds in half-pint milk bottles, and require only about 12 days to reach maturity—thus providing some 30 generations each year for genetic studies. Furthermore, each *Drosophila* cell has only four chromosome pairs, making this an ideal organism for study in the search for simple relationships between heredity and chromosomes.

Morgan subjected his fruitflies to heat, cold, x-rays, radioactivity, and various chemicals, but he was unable to detect any mutations produced by these treatments. Then, in April 1910, Morgan discovered a single, white-eye male fly in a bottle of normal, red-eye flies. No other white-eye flies had appeared in the dozens of generations through which Morgan had observed this population, so he was sure that the white-eye male represented a mutation.

Morgan mated the white-eye male with wild-type, red-eye females from the same generation. The F_1 generation produced by this cross was entirely red-eye, as Mendelian principles would predict if the white eyes are produced by a recessive gene. Next, Morgan allowed the F_1 generation to interbreed. The resulting F_2 generation contained 3,470 red-eye and 782 white-eye flies. This result is rather far from the 3:1 ratio predicted by Mendel's laws, but the reappearance of the white-eye character showed that Morgan had indeed located a new heritable character.

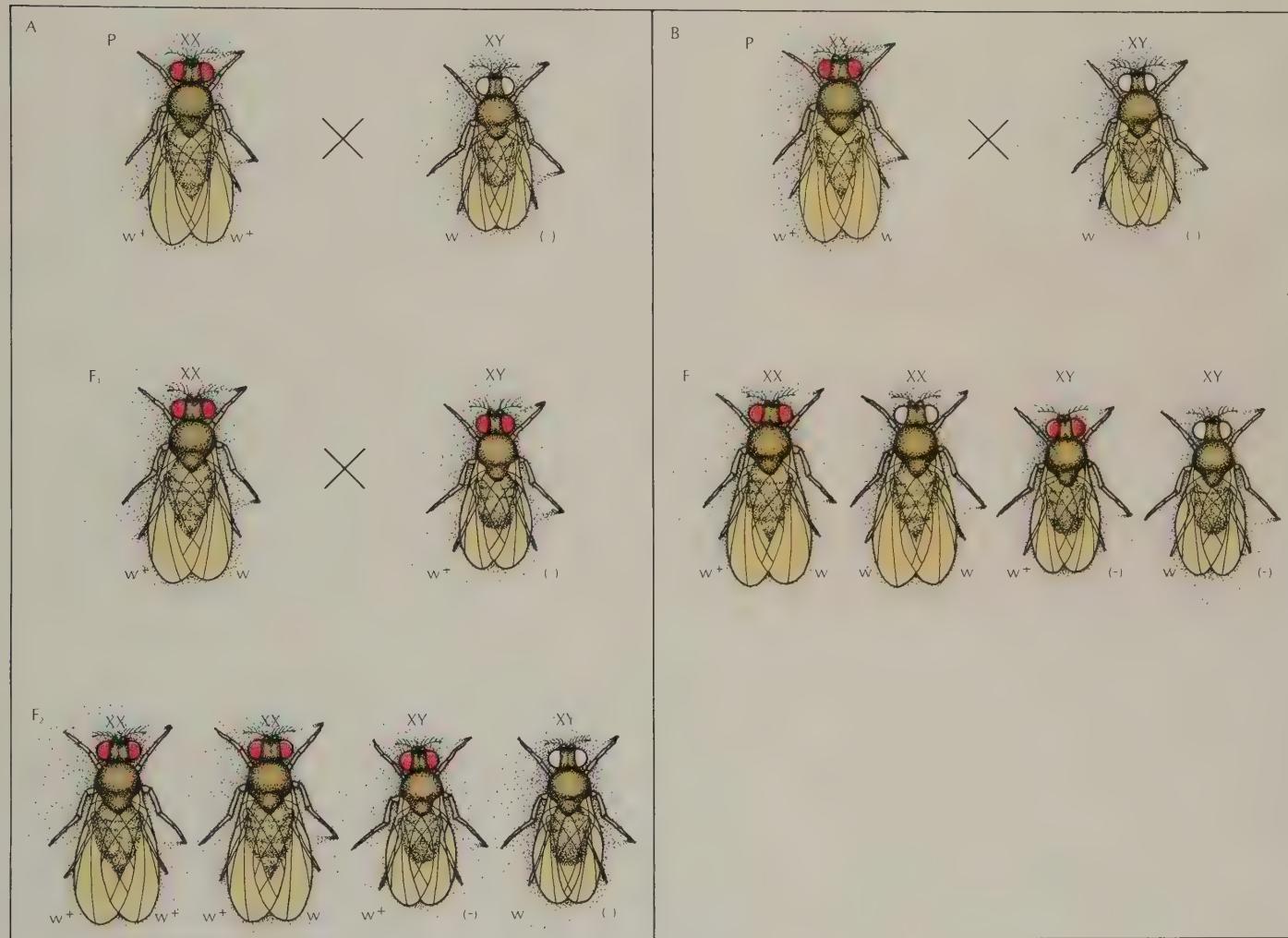
One property of the F_2 generation was incompatible with Mendel's laws. All 782 white-eye F_2 flies were males. The red-eye F_2 flies included 2,459 females and 1,011 males. At first, Morgan thought that the white-eye character might somehow be impossible in females, but when he crossed the original white-eye male with some of his red-eye daughters from the F_1 generation, he obtained 129 red-eye females, 132 red-eye males, 86 white-eye males, and 88 white-eye females. In the results of this cross, white-eye females were as common as white-eye males.

Clearly, the inheritance of the white-eye character is somehow related to the hereditary determination of sex. In his first report on the white-eye mutant, Morgan (1910) used Mendelian principles to explain the results of his crosses by assuming that the eye-color factor and the sex-determining factor are linked together rather than assorting independently. Morgan was well aware of the sex chromosomes in insects and knew that one of *Drosophila*'s four chromosome pairs is responsible for sex determination. In the cells of females, this pair is made up of two rod-shape chromosomes (now called X chromosomes), whereas in the male the pair consists of one rod-shape X chromosome and one J-shape Y chromosome. Later, Morgan showed that the results of his experiments with red-eye and white-eye flies are consistent with the assumption that the eye-color gene is carried only on the X chromosome—that is, eye color is a sex-linked trait.

Because the white-eye factor is recessive, the female will have white eyes only if she carries the white-eye factor on both X chromosomes. In a male, on the other hand, the presence of the white-eye factor on the single X chromosome will lead to development of white eyes. This fact explains why only males of Morgan's original F_2 generation showed white eyes.

Figure 14.5. The inheritance of white-eye color, a sex-linked recessive trait, in *Drosophila*. (A) A wild-type (red-eye) crossed with a white-eye produces an F_1 generation in which all are red-eye, but the females are "carriers" (W^+W). The F_1 flies are crossed among themselves to produce an F_2 , three-quarters of which are red-eye males and females and one-quarter of which are white-eye males. (B) A homozygous recessive white-eye crossed with a wild-type (red-eye) produces an F_1 in which all females are red-eye and all males are white-eye. The F_1 flies are crossed among

themselves to produce an F_2 of one-half red-eye males and females and one-half white-eye males and females.

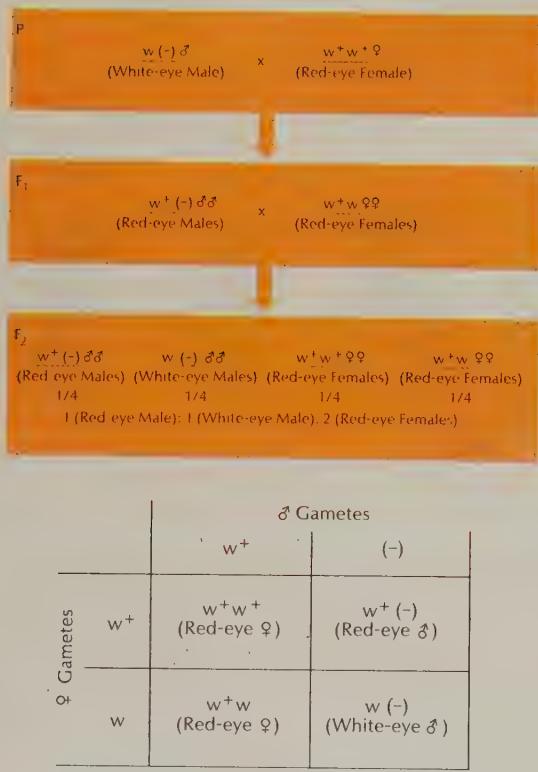


Morgan soon found another mutant fruitfly, this one with miniature wings—a characteristic that also proved to be sex-linked. Before the end of 1910, Morgan had discovered 40 different mutant characters in *Drosophila*, a number of which are sex-linked. Other characters that are not sex-linked proved to be linked to one another. For example, if a fly with purple eyes and a black body is crossed with a wild-type fly (red eyes and "gray" body—actually yellowish gray with dark bands), the characters of purple eyes and black bodies tend to appear together in offspring generations, rather than assorting independently.

Every bit of shelf space in Morgan's laboratory was crowded with bottles of flies, each bottle with a label describing the genetic background of the flies inside. Morgan and his students devoted themselves to the laborious task of anesthetizing each bottle of flies with ether and carefully examining each fly under the microscope to classify its characters. Some of the mutant characters involved subtle alterations of the shape of minute bristles on the fly body or slight changes in the shade of red in the eyes. It was not unusual for the ether to begin to wear off, and the weary researcher in the midst of a

Figure 14.6. Morgan's crosses of the white-eye mutant. The results of the original cross are shown in the 2 top figures, and back cross results are shown below.

Interleaf 14.1 DIAGRAMS OF MORGAN'S CROSSES



♂ Gametes:

	w	$(-)$
w^+	w^+w (Red-eye ♀)	$w^+(-)$ (Red-eye ♂)
w	ww (White-eye ♀)	$w\ (-)$ (White-eye ♂)

Morgan's crosses with his original white-eye mutant fruitfly can be represented with checkerboard diagrams. The symbols for male (δ) and female (φ) used by biologists are derived from the astrological symbols for Mars (a shield and spear) and Venus (a looking glass). The symbols $\delta\ \delta$ and $\varphi\ \varphi$ represent the plurals, "males" and "females." Figure 14.6 outlines the crosses that Morgan performed.

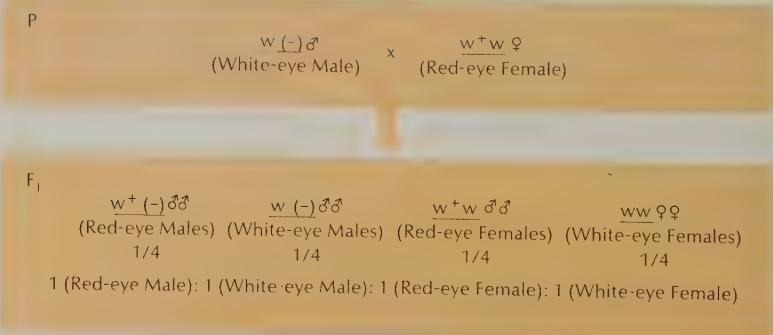
In the original cross of the white-eye male with wild-type females, the females produce only w^+ gametes, whereas the male produces both w and $(-)$ gametes. Thus, the F_1 generation contains equal numbers of $w^+(-)$ and w^+w genotypes, or equal numbers of red-eye males and red-eye females. (Actually, in his F_1 generation, Morgan obtained 1,237 red-eye males and females and 3 white-eye males. He assumed that these three males represented the appearance of new mutations, and he did not include them in his statistics.)

When the members of the F_1 generation are interbred, each sex produces two kinds of gametes. (The possible combinations are shown in Figure 14.6a.) The predicted composition of the F_2 generation is 25 percent red-eye males, 25 percent white-eye males, and 50 percent red-eye females. Actually, Morgan obtained about 24 percent red-eye males, 18 percent white-eye males, and 58 percent red-eye females.

Morgan also crossed the white-eye male with heterozygous red-eye females from the F_1 generation of the previous cross. Again, each sex produces two kinds of gametes, but this time equal numbers of four different phenotypes are predicted (Figure 14.6b). Morgan actually obtained 30 percent red-eye females, 30 percent red-eye males, 20 percent white-eye females, and 20 percent white-eye males.

A cross such as this one—in which an individual from the F_1 generation is crossed with its parent or with an individual of a genotype identical to the parent—is called a *back cross*. Another useful cross is the *test cross*, in which a heterozygote is crossed with an individual homozygous for the recessive alleles of the gene or genes being studied. Morgan performed a number of back crosses and test crosses to be sure that the white gene does behave in the predicted fashion in every case.

Morgan's experimental results are much further from the predicted ratios than were Mendel's results. These results are not surprising in view of the relatively small numbers of flies in Morgan's populations. In fact, even with his large populations of pea seeds, Mendel's results show less random deviation from the predicted ratios than would be expected. In the crosses cited above, white-eye flies consistently appear in smaller numbers than predicted. This result might be merely chance, but if it is consistently found in a great many experiments, it will have to be explained. Perhaps another gene is involved, or perhaps the white-eye flies are more likely than red-eye flies to die before the zygote develops into an adult. A great many test crosses and the counting of a very large number of flies may be necessary to resolve such uncertainties.



count of hundreds of flies would find his research population beginning to take wing and disappear into the corridors.

By 1915 Morgan and his students had identified nearly 100 different mutant characters in *D. melanogaster*. More than 20 of these characters are sex-linked and are controlled by factors carried on the X chromosome. The remaining characters fall into three groups, with the characters of each group tending to remain linked together. The four linkage groups correspond nicely with the four chromosome pairs of *Drosophila*, and one of the groups contains only a few characters, as might be expected from the fact that one of *Drosophila*'s chromosome types is little more than a small dot.

Further evidence that genes are carried by the chromosomes was soon to come from Morgan's laboratory. His important work with *Drosophila* led many other geneticists to begin experimenting with this insect, and it became the most common organism for genetic research. In fact, because this inconspicuous little fly is of minor importance to man as a pest or otherwise, someone once remarked that God must have created *Drosophila* just for Morgan.

LINKAGE AND CROSSING OVER

Geneticists working with *Drosophila* developed a standard set of symbols to represent the various genes. This symbolism has been used with some other organisms, but it is not universally adopted. The various forms of the same gene are now called *alleles*. For example, the eye-color gene first discovered by Morgan and carried on the X chromosome has a recessive mutant allele (*w*), which tends to produce white eyes, and a dominant wild-type allele (*w⁺*), which tends to produce red eyes. In genetics, the symbol + always indicates a wild-type allele. The letter used for a particular gene is an abbreviation of the name given to the mutant character (in this case, white). A capital letter is used if the mutant allele is dominant; a lower-case letter is used if the wild-type allele is dominant. Thus, *B* represents a mutant allele for a condition called bar-eye, which is dominant to its wild-type allele, *B⁺*.

The white gene is carried only on the X chromosome, so the white-eye phenotype is produced by a *ww* genotype in females or by a *w(−)* genotype in males. The symbol (−) is used to represent the Y chromosome, which carries no allele for this gene. The red-eye phenotype is produced by a *w⁺(−)* genotype in males, but the genotype of a red-eye female may be either the wild-type *w⁺w⁺* or the hybrid *w⁺w*.

The bar-eye character is also sex-linked. In the pure bar-eye character, the form of the eye is a narrow red bar (Figure 14.7). An intermediate form called wide-bar-eye is observed in some females produced in cross-breeding experiments. Males may be either wild-type or bar-eye in phenotype, corresponding to the genotypes *B^{+(−)}* or *B(−)*. Three genotypes are possible in the female: *B^{+(−)}*B^{+(−), *B^{+(−)}*B, or *BB*. Females with the heterozygous *B^{+(−)}*B genotype show the intermediate wide-bar-eye phenotype—an example of incomplete dominance. The presence of the recessive, mutant allele does have some effect on the phenotype of the heterozygote.}

In his early work with *Drosophila* mutants, Morgan found some mutant characters that appear only as a result of the combined effects of two recessives. For example, certain wing defects do not occur if the individual has a dominant wild-type allele for either of two different genes. Thus, Morgan found himself postulating multiple genes as he had once criticized the

Figure 14.7a (above). Variations in eye structure and color in the fruitfly *Drosophila melanogaster*.

Figure 14.7b (below). Inheritance of the sex-linked recessive trait bar-eye in the fruitfly. In a male that has the recessive gene and a female that is homozygous recessive for the trait, the eye form is a narrow bar. A female heterozygous for the trait has a wide-bar phenotype—an example of incomplete dominance.

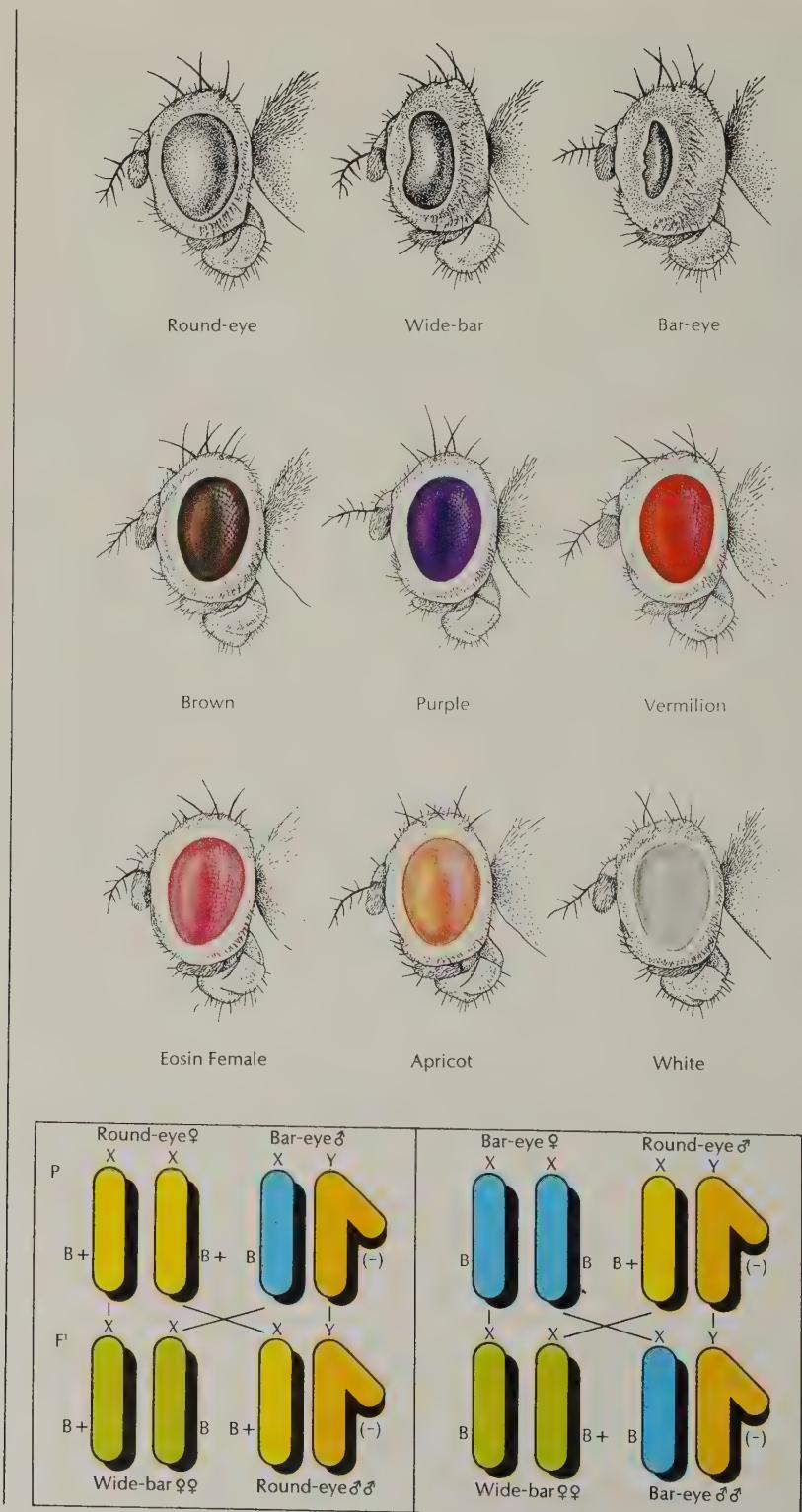


Figure 14.8. Sturtevant's cross utilizing two sex-linked recessive genes. Sturtevant crossed a long-wing, vermilion-eye female (m^+v/m^+v) with a miniature-wing, red-eye male ($(-)/mv^+$). These F_1 offspring were interbred to produce an F_2 generation. The gametes and predicted genotypes are shown in the Punnett square.

Mendelists for doing, but Morgan's postulates were based upon following hundreds or thousands of individuals through many generations of various test crosses. Incomplete dominance can be explained by a relatively minor modification of Mendel's original laws, merely recognizing that in some cases the heterozygous phenotype may be intermediate between the homozygous phenotypes. Gene linkage can be explained by adopting Sutton's hypothesis that the genes are linked together on chromosomes and thus cannot assort independently.

Among the early mutant genes Morgan discovered in *Drosophila* are those for miniature wings (m) and vermilion eye color (v). Both are sex-linked genes carried by the X chromosome, and therefore the two genes may be expected to be linked to each other. In both cases, the mutant allele is recessive to the wild-type allele. In an early experiment, one of Morgan's students, A. H. Sturtevant, crossed a long-wing, vermilion-eye female with a miniature-wing, red-eye male. The female was homozygous for both genes, so her genotype may be represented as m^+v/m^+v . In cases of linked genes, the slant is used to separate the alleles found on the two chromosomes of the pair, making it easier to keep track of the linked alleles in later assortment. The genotype of the male was $(-)(-)/mv^+$.

All of the gametes produced by the female must be of the genotype m^+v . The male will produce equal numbers of gametes containing an X chromosome with genotype mv^+ and of gametes containing a Y chromosome with genotype $(-)(-)$. Therefore, the offspring, or F_1 , generation, of this cross should consist of equal numbers of the two genotypes, mv^+/m^+v and $(-)(-)/m^+v$. These genotypes correspond to the two phenotypes: long-wing, red-eye females and long-wing, vermilion-eye males. As expected, Sturtevant obtained an F_1 generation with approximately equal numbers of these two phenotypes.

An F_2 generation was then produced by interbreeding members of the F_1 generation. Because the alleles appearing on the same chromosome should remain linked together, the F_1 females should be able to produce only two

P	$\frac{(-)(-)}{mv^+} \delta$ (Miniature Red Male)	X	$\frac{m^+v}{m^+v} \varphi$ (Long Vermilion Female)	
F_1	$\frac{(-)(-)}{m^+v} \delta\delta$ (Long Vermilion Males)	X	$\frac{mv^+}{m^+v} \varphi\varphi$ (Long Red Females)	
F_2	$\frac{(-)(-)}{mv^+} \delta\delta$ (Miniature Red Males) 1/4	$\frac{(-)(-)}{m^+v} \delta\delta$ (Long Red Males) 1/4	$\frac{m^+v}{mv^+} \varphi\varphi$ (Long Red Females) 1/4	$\frac{m^+v}{m^+v} \varphi\varphi$ (Long Vermilion Females) 1/4

		δ Gametes	
		$(-)(-)$	m^+v
♀ Gametes	mv^+	$(-)(-)$ mv^+ (Miniature-wing Red-eye ♂)	m^+v mv^+ (Long-wing Red-eye ♀)
	m^+v	$(-)(-)$ m^+v (Long-wing Vermilion-eye ♂)	m^+v m^+v (Long-wing Vermilion-eye ♀)

Figure 14.9. Schematic model of chromosome crossover. (1) Two homologous double-stranded chromosomes, one bearing the linked alleles A and B and the other bearing the linked alleles a and b are joined in synapsis. (2) Corresponding breaks occur in one chromatid of each pair and the fragments are exchanged. (3) After crossing over, one chromatid of the first chromosome bears alleles A and b and one chromatid of the second chromosome bears alleles a and B.

kinds of gametes: mv^+ and m^+v . The male gametes should also be of two kinds: $(-) (-)$ and m^+v . Recombination of these gametes yields four genotypes, each of which corresponds to a different phenotype. Thus, the expected F_2 generation would contain equal numbers of long-wing, red-eye females; long-wing, vermilion-eye males; long-wing, vermilion-eye females; and miniature-wing, red-eye males. Table 14.1 compares the predicted and observed phenotypes of the F_2 generation.

Table 14.1
Observed and Predicted Phenotypes of F_2 Generation

PHENOTYPE	Observed		Predicted Percentage
	Number	Percentage	
Long-wing, red-eye males	8	1.7%	0.0%
Long-wing, red-eye females	138	29.4	25.0
Long-wing, vermilion-eye males	117	24.9	25.0
Long-wing, vermilion-eye females	110	23.4	25.0
Miniature-wing, red-eye males	97	20.5	25.0
Miniature-wing, red-eye females	0	0.0	0.0
Miniature-wing, vermilion-eye males	1	0.2	0.0
Miniature-wing, vermilion-eye females	0	0.0	0.0

Source: A. H. Sturtevant, "The linear arrangement of six sex-linked factors in *Drosophila*, as shown by their mode of association," *Journal of Experimental Zoology* 14 (1913): 43–59.

There are two unexpected results. First, although three of the four expected phenotypes are present in approximately the expected proportions, there are fewer miniature-wing, red-eye males than expected. Morgan and Sturtevant found that miniature-wing phenotypes always occur in much smaller numbers than predicted in any cross. They concluded that this phenotype has a low viability. That is, zygotes that would develop into miniature-wing adults tend to die before reaching maturity and thus are not counted by the researcher.

The second unexpected result is the presence in the F_2 generation of small numbers of two phenotypes that were not expected to appear. Morgan suggested that the appearance of such phenotypes might be due to an exchange, or *crossing over*, of alleles between the homologous chromosomes in the female during the meiotic division that forms the gametes. Such crossing over cannot occur in the male because there is no second X chromosome with which alleles can be exchanged. (It was later discovered that crossing over never occurs on any of the chromosomes of the male *Drosophila*, but this species is unusual in this respect.)

Crossing over, or exchange of alleles, would make it possible for some female gametes to have the genotypes mv and m^+v . Combination of these female gametes with the two kinds of male gametes could produce the additional genotypes (and phenotypes): $(-) (-)/mv$ (miniature-wing, vermilion-eye male), $(-) (-)/m^+v^+$ (long-wing, red-eye male), m^+v/mv (long-wing, vermilion-eye female), and m^+v/m^+v^+ (long-wing, red-eye female). The new female genotypes produce phenotypes indistinguishable from those expected by linked assortment, but the two new male phenotypes are those that Sturtevant actually observed. The very small number of miniature-wing, vermilion-eye males is consistent with the assumed low viability of the miniature-wing phenotype. Among the F_2 males, there are 214 of the expected phenotypes and 9 of the cross-over phenotypes. There-

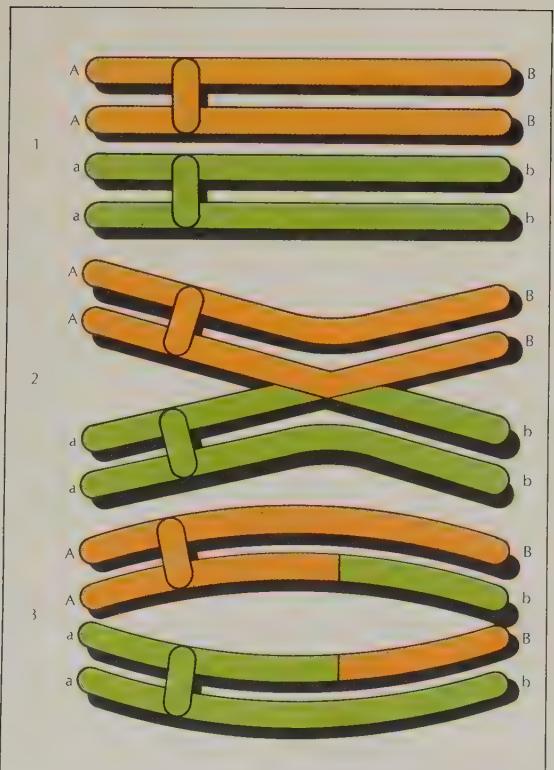


Figure 14.10. Map of the salivary gland chromosomes of *Drosophila melanogaster*.

fore, crossing over occurred during formation of 9/223, or 4 percent of the gametes. The frequency of crossing over in the females cannot be determined from this cross.

Morgan immediately began to search for a physical explanation of the phenomenon of crossing over and found a clue in the observations of the Belgian cytologist F. A. Janssens, who described the process of chiasmata formation at the beginning of meiosis. When homologous pairs of chromosomes come together in synapsis, it appears that some material is interchanged between chromatids of the two chromosomes. Janssens suggested that the chromatids break at corresponding places and interchange equal segments. Thus, when the chromosomes are pulled apart in anaphase, each of the separating chromatids contains some segments derived from the other chromosome of the pair.

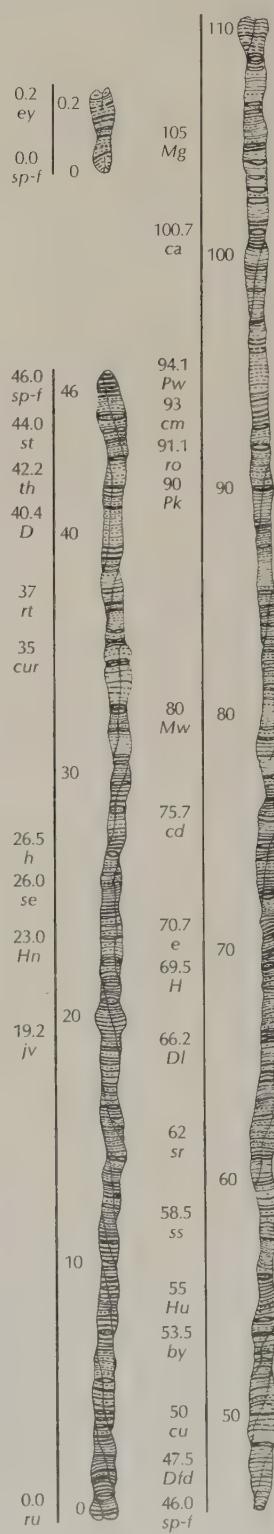
Morgan and his colleagues quickly realized that the physical crossing over described by Janssens provides exactly the mechanism needed to account for the crossing over of genes indicated by their experiments with *Drosophila*. Chiasmata do not form between the X and Y chromosomes during meiosis in the male. Furthermore, the chromatids always seem to exchange exactly corresponding segments. These observations are consistent with the genetic observations that crossing over does not occur with sex-linked genes in the male and that genes are exchanged but never lost during crossing over.

Morgan recognized another important implication of this mechanism for crossing over. He had found that the percentage of crossing over remains roughly constant for any given pair of genes but varies greatly among different pairs of genes. Some pairs of genes are completely linked so that crossing over is never observed between them. With other pairs, the percentage of crossing over reaches about 50 percent. Morgan suggested that the genes fall at particular locations along the chromosomes. If two genes happen to be located close together, they will rarely be separated during chiasmata formation; if crossing over occurs, they will cross over together, and the crossing over will not be detected by breeding experiments. On the other hand, if the two genes are located near opposite ends of the chromosome, crossing over would be expected almost every time that chiasmata are formed. The fact that cross-over frequency seldom rises above 50 percent is probably due to multiple crossing over, which could not always be detected in breeding experiments. Morgan pointed out that cross-over frequencies should provide a means of mapping the relative locations of genes on the chromosomes.

By observing an unusual chromosomal abnormality in *Drosophila*, Curt Stern (1931) was able to provide definite proof of the physical reality of crossing over. Chromosomal fragments attached to the ends of the X chromosome made it possible to show microscopically when segments of the X chromosomes were interchanged. In every case, a physical interchange of the chromosome segments was accompanied by genetic crossing over (determined through breeding experiments) between genes located near the ends of the chromosome.

CHROMOSOME MAPPING

Morgan and Sturtevant soon were able to show the relative positions of a number of genes on the X chromosome of *D. melanogaster*. Within a few years, they prepared *chromosome maps* (Interleaf 14.2) for each of the four



Interleaf 14.2

CHROMOSOME MAPS

Sturtevant (1913) prepared the first chromosome map of *Drosophila melanogaster*, using Morgan's suggestion that the cross-over frequency provides an index of the distance between any two genes. He used six sex-linked genes studied in the early work in Morgan's laboratory. Two of the genes were found to be at the same location and have subsequently been found to represent different mutant alleles of the same gene. In the following discussion, modern terminology is used for the genes, but Sturtevant's original experimental results are used as data.

The genes used in this mapping are *w* (white eyes), *v* (vermilion eyes), *m* (miniature wings), *r* (rudimentary wings), and *y* (yellow body color). Sturtevant determined the cross-over frequencies for various pairings of these genes (Table 14.2).

Table 14.2
Cross-Over Frequencies for Sex-Linked Gene Pairs

GENES CONCERNED	Cross-Over Frequency
<i>w</i> and <i>v</i>	29.7%
<i>w</i> and <i>m</i>	33.7
<i>w</i> and <i>r</i>	45.2
<i>w</i> and <i>y</i>	1.0
<i>v</i> and <i>m</i>	3.0
<i>v</i> and <i>r</i>	26.9
<i>v</i> and <i>y</i>	32.2
<i>m</i> and <i>y</i>	35.5
<i>r</i> and <i>y</i>	37.6

Source: Adapted from A. H. Sturtevant, "The linear arrangements of six sex-linked factors in *Drosophila*, as shown by their mode of association," *Journal of Experimental Zoology* 14 (1913): 43–59.

Sturtevant realized that distance between genes might not be the only factor affecting cross-over frequency. For example, some parts of the gene might break more easily than others, thus giving a higher cross-over frequency for that part of the gene. However, the maps were prepared to represent only the "statistical distance" between genes based on the assumption that distance is proportional to cross-over frequency. Later work did show that various parts of the gene are more susceptible to crossing over, and therefore the cross-over maps are now known to represent somewhat distorted pictures of the physical locations of the genes on the chromosomes. However, independent techniques confirm the sequence of genes determined by cross-over studies.

As a unit of distance, Sturtevant chose a length of the chromosome such that, on the average, 1 crossing over will occur in that length for every 100 gametes formed. In other words, cross-over frequency expressed as a percentage is used as an index of distance. For example, the distance between genes *w* and *v* is 29.7 units, between genes *w* and *m* is 33.7 units, and so on.

On a line representing the X chromosome, *w* may be placed at an arbitrary position, with *v* 29.7 units away from it. The distance between *w* and *m* is 33.7 units, and the distance between *v* and *m* is only 3.0 units. The only position for *m* that is compatible with these distances is 3.0 units beyond *v*. (A position on the other side of *w* would be much too far from *v*.) The distances do not quite add up—the distance from *w* to *m* (33.7 units) should be equal to the sum of the distance from *w* to *v* (29.7 units) and the distance from *v* to *m* (3.0 units). However, the discrepancy is quite small and could be due to inaccuracies in determining the cross-over frequencies.

Next, *r* can be added to the map. It is 45.2 units from *w* and only 26.9 units from *v*, so it must be located beyond *v* and *m*. (Sturtevant had not yet measured the cross-

Figure 14.11a. Maps of Sturtevant's experiment involving *w*, *v*, *m*, *r*, and *y* genes.

over frequency between *m* and *r* when he prepared this first map.) This time the discrepancy is more serious; the sum of cross-over frequencies between *w* and *v* and between *v* and *r* is 56.6 percent, whereas the cross-over frequency between *w* and *r* is only 45.2 percent.

Finally, *y* can be placed on the map. It is only 1.0 unit from *w*, but which side should it go on? The data are inconsistent, because *y* is farther from *v* and *m* than is *w*, but it is closer to *r* than is *w*. Sturtevant chose to trust the figures obtained for the shorter distances and to put *y* on the map to the left of *w*. Using *y* as the arbitrary beginning of the chromosome and using the shortest distance measurements available to place the chromosome distances between genes, Sturtevant obtained his first simple map of the X chromosome.

He found that cross-over frequencies over long distances on the map are always smaller than the value predicted by adding the cross-over frequencies for intermediate distances. He explained this discrepancy by pointing out that "double crossovers" within the long distance would leave the genes being studied on their original chromosomes and thus would not be detected in the breeding experiments. Therefore, any cross-over frequency measured by the outcome of crossing experiments will be lower than the true frequency of crossing over. The discrepancy will be largest for the longest distances. For this reason, Sturtevant used measurements between adjacent genes to construct his map (Figure 14.11a).

Crossing experiments involving three or more genes can be used to check the hypothesis of double crossovers. Figure 14.11b summarizes an experiment of this

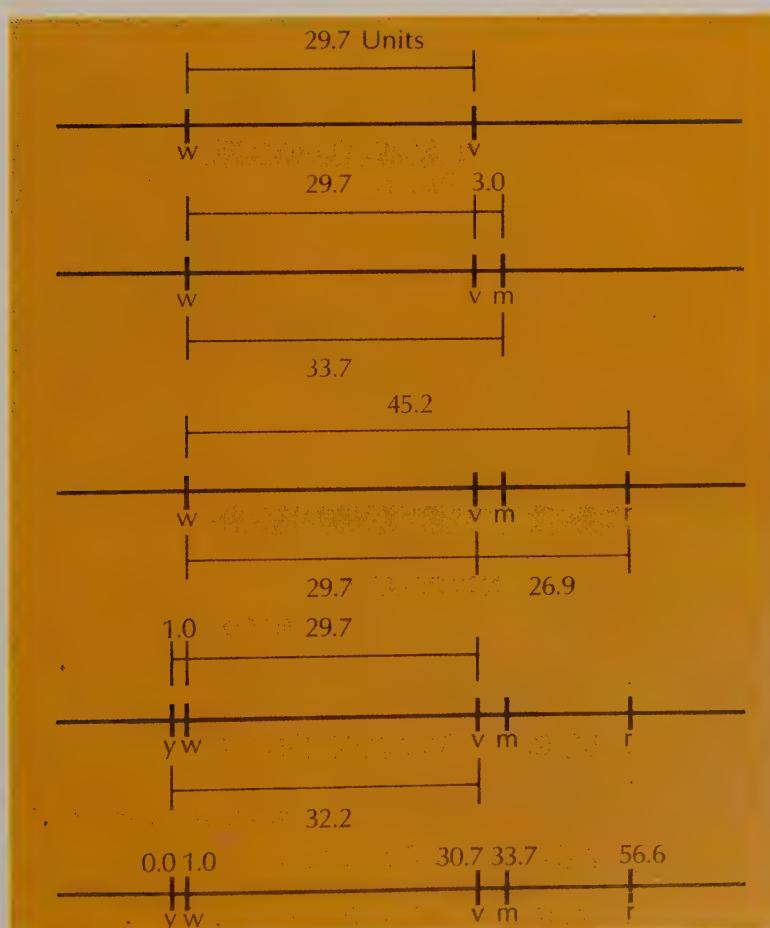


Figure 14.11b. Summary of an experiment used to check the hypothesis of double crossovers.

P $\frac{\text{sn m fu}}{\text{sn+m+fu+}}$ ♀ X $\frac{\text{sn m fu}}{\text{sn+m+fu+}}$ ♂

Noncross-over Gametes	Male	$\frac{\text{sn m fu}}{\text{sn+m+fu+}}$
	Female	$\frac{\text{sn m fu}}{\text{sn+m+fu+}}$
Cross-over Gametes (female)	$\frac{\text{sn m fu}^+}{\text{sn+m+fu+}}$	$\frac{\text{sn m }^+ \text{ fu}^+}{\text{sn+m+fu+}}$; $\frac{\text{sn+m fu}}{\text{sn+m+fu+}}$
	$\frac{\text{sn m fu}}{\text{sn+m+fu+}}$	$\frac{\text{sn m fu}^+}{\text{sn+m+fu+}}$; $\frac{\text{sn+m fu}}{\text{sn+m+fu+}}$
	$\frac{\text{sn m fu}}{\text{sn+m+fu+}}$	$\frac{\text{sn m }^+ \text{ fu}^+}{\text{sn+m+fu+}}$; $\frac{\text{sn+m fu}}{\text{sn+m+fu+}}$

F₁

		Phenotype	Number	Percentage
Noncross-over	$\frac{\text{sn m fu}}{\text{sn+m+fu+}}$ ♀; $\frac{\text{sn m fu}}{\text{sn+m+fu+}}$ ♂	Singed-bristle Miniature-wing Fused-wing	3,661	66.3
	$\frac{\text{sn m fu}}{\text{sn+m+fu+}}$ ♀; $\frac{\text{sn+m+fu}^+}{\text{sn+m+fu+}}$ ♂	Wild-type	3,672	
<u>sn/m</u> Cross-over	$\frac{\text{sn m }^+ \text{ fu}^+}{\text{sn m fu}}$ ♀; $\frac{\text{sn m }^+ \text{ fu}^+}{\text{sn m fu}}$ ♂	Singed-bristle Normal-wing	665	12.1
	$\frac{\text{sn+m fu}}{\text{sn m fu}}$ ♀; $\frac{\text{sn+m fu}}{\text{sn m fu}}$ ♂	Normal-bristle Miniature-wing Fused-wing	676	
<u>m/fu</u> Cross-over	$\frac{\text{sn m fu}^+}{\text{sn m fu}}$ ♀; $\frac{\text{sn m fu}^+}{\text{sn m fu}}$ ♂	Singed-bristle Miniature-wing	1,041	18.5
	$\frac{\text{sn+m fu}^+}{\text{sn m fu}}$ ♀; $\frac{\text{sn+m fu}^+}{\text{sn m fu}}$ ♂	Normal-bristle Fused-wing	1,003	
Double Cross-over	$\frac{\text{sn m }^+ \text{ fu}^+}{\text{sn m fu}}$ ♀; $\frac{\text{sn m }^+ \text{ fu}^+}{\text{sn m fu}}$ ♂	Singed-bristle Fused-wing	165	3.1
	$\frac{\text{sn m }^+ \text{ fu}^+}{\text{sn m fu}}$ ♀; $\frac{\text{sn+m fu}^+}{\text{sn m fu}}$ ♂	Normal-bristle Miniature-wing	173	

kind. Three genes located on the X chromosome are studied. The *miniature* gene (small wings) has already been discussed. The other two genes are *singed* (curled and twisted bristles) and *fused* (certain wing veins joined together). A female heterozygous for all three characters is crossed with a male showing the recessive mutant traits.

In addition to the four kinds of gametes that can be produced by linked reassortment, there are six kinds of female gametes that can be produced by crossing over. A hooked line has been used to represent the Y chromosome to emphasize the fact that crossing over cannot occur between X and Y chromosomes. In this kind of test cross, both males and females can be used in estimating cross-over frequency. Both the Y chromosome and the male's completely recessive X chromosome allow the alleles on the chromosome from the female to be detected in the phenotype.

Double crossing over did occur in 3.1 percent of the gametes. Because these cases represent crossing over both between *sn* and *m* and between *m* and *fu*, these cases should be counted in determining cross-over frequencies for both regions. In other words, the map distance between *sn* and *m* is 15.2 units ($12.1 + 3.1$), and the map distance between *m* and *fu* is 21.6 units ($18.5 + 3.1$). In a two-gene crossing experiment using only *sn* and *fu*, the double cross-overs would have been indistinguishable from the noncross-overs. Such an experiment would have indicated a distance of 30.6 units between *sn* and *fu* instead of the 36.8 units obtained in this experiment. It is possible that some double cross-overs have occurred in the regions between genes and not been detected or that some of the gametes recorded as single cross-overs actually represent triple cross-overs. Therefore, cross-over frequencies must always be regarded as minimum values for the true map distance being determined.

Even if the sequence of the three genes had not been determined earlier from two-gene crossings, it could be deduced from the results of this experiment by assuming that the smallest frequency phenotypes in the F₁ generation represent double cross-overs. Because the *m* alleles are exchanged in that group, *m* must lie between the other two genes.

chromosomes of this species, showing the relative locations of each of the 85 mutant genes then known (Morgan, et al., 1915).

The consistent results of the mapping convinced Morgan and most other biologists that the genes are indeed located in linear order along the chromosomes. They found no evidence of branching or parallel chains of genes on a single chromosome. Every known gene could be assigned a consistent location on one of the chromosomes. Occasionally, two genes were found to have the same apparent location. In some cases, further experiments with larger numbers of flies revealed a very low frequency of crossing over, so that the two genes could be assigned locations very near to each other. In other cases, no crossing over could be found. For example, at a particular point near one end of the X chromosome, several mutant genes for different eye colors have been assigned the same location. It appears that all of these are different mutant alleles of the same gene, for all are recessive to the wild-type character.

Another of Morgan's graduate students, Calvin B. Bridges, discovered something that put the chromosomal theory of heredity almost beyond doubt. Bridges (1916) noticed that a vermilion-eye female occasionally turns up among the offspring of a cross between vermilion-eye females and red-eye males. Because the allele for vermilion eyes is recessive, a vermilion-eye female must have two of these alleles. Yet, in this cross, the male cannot contribute a mutant allele. Bridges guessed that these two alleles might occur if the X chromosomes of the mother fly failed to separate during meiosis, thus giving the egg a pair of X chromosomes, each carrying the mutant allele for vermilion eyes. If this egg were fertilized by a sperm carrying a Y chromosome, the resulting zygote would have the abnormal genotype $vv(-)$. When Bridges examined the cells of the unexpected vermilion-eye females, he found exactly what he had predicted: two X chromosomes and a Y chromosome. This experiment provided dramatic and convincing support for the theory that genes are carried on the chromosomes.

Thus, Morgan — who had begun his career in genetics as an opponent of the Mendelists — became one of the outstanding proponents of a modified, chromosomal version of Mendelian genetics. He and his colleagues became the recognized leaders of genetic research and theory.

CHROMOSOMAL ABNORMALITIES AND MUTATIONS

The extra X chromosome in some vermilion-eye females was not the only chromosomal abnormality that Bridges discovered. If some eggs received an extra X chromosome during meiosis, it might be equally possible for both X chromosomes to go into the polar body, leaving the egg with no sex chromosome. Fertilization of such an egg by a sperm with an X chromosome would produce a zygote with a single unpaired X chromosome. Bridges found this condition in certain male flies that produce immotile sperm and thus do not reproduce. Apparently, the Y chromosome plays some role in the normal development of sperm. Fertilization of the egg without a sex chromosome by a sperm with a Y chromosome would produce a zygote with an unpaired Y chromosome. Bridges was unable to locate any flies with this condition, so he concluded that such zygotes die at a very early stage of development. Apparently, this condition is a lethal chromosomal abnormality. A fourth possibility would involve fertilization of an egg with two X chromosomes by a sperm with a third X chromosome.

Figure 14.12a (upper left). The normal male karyotype. This photomicrograph of a chromosome smear preparation was taken from a white blood cell of a normal male. The 23 chromosome sets have been repositioned in rank order to facilitate examination. Note the size disparity between the X and Y chromosomes.

Figure 14.12b (upper right). The normal female karyotype. This photomicrograph of a chromosome smear preparation was taken from a white blood cell of a normal female. These cytological preparations are made during the metaphase stage of the cells' mitotic division. Note the X chromosomes.

Figure 14.13 (lower left). Photomicrograph of the chromosomes found in the white blood cell of a human female with Turner's syndrome. Note the single X chromosome. Phenotypic expression of this chromosome abnormality results in retarded sexual development and sterility.

Figure 14.14 (lower right). Photomicrograph of the chromosomes found in the white blood cells of a Down's syndrome male. Note the additional chromosome to set number 21. This chromosome abnormality is phenotypically expressed as a form of Mongolian idiocy.

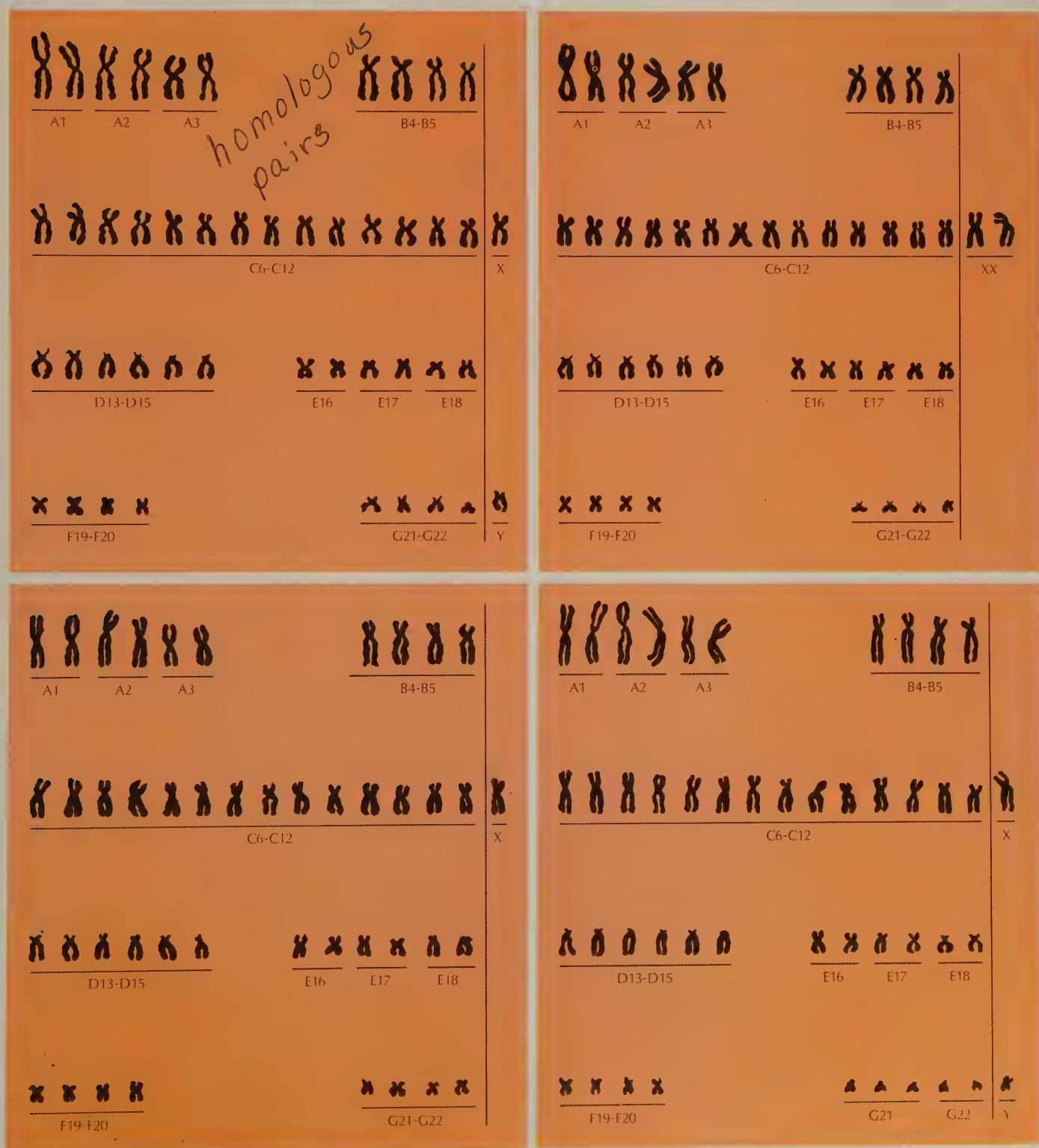


Figure 14.15. Photomicrograph of the chromosomes found in the white blood cell of a human male with Klinefelter's syndrome. Note the XXY genotype, which phenotypically results in severe mental retardation and may result in either a lack of male secondary sex characteristics or in the development of some female secondary sex characteristics.



Bridges found cells containing three X chromosomes in certain female flies that die before reaching maturity.

Such variations in chromosomal number have subsequently been shown to cause many hereditary abnormalities. The absence of one chromosome is now called *monosomy*. An example of monosomy in humans is Turner's syndrome, which results from the lack of one of the X chromosomes and is expressed phenotypically in retarded sexual development. The presence of an extra chromosome is called *trisomy*. The presence of two X chromosomes in a human male causes a condition called Klinefelter's syndrome. Such a male is severely retarded mentally and either fails to develop normal secondary sexual characteristics of a male or develops some female characteristics such as enlarged breasts or broad hips. The XXY human male is invariably sterile. Down's syndrome—caused by the presence of three copies of one of the other human chromosomes—results in a form of Mongolian idiocy.

Most cases of trisomy in humans result in highly abnormal embryonic or fetal development and are lethal. The general term for the absence or duplication of part of the normal diploid chromosome set is *aneuploidy*. In most cases of aneuploidy in animals, the zygote fails to develop into an adult. In cases where adulthood is reached, the aneuploid individual is usually unable to reproduce. Thus, aneuploidy is seldom transmitted from one generation to the next. In some plants, a number of varieties with extra copies of particular chromosomes do exist, and the aneuploid condition may be passed along to offspring.

The presence of one or more complete extra sets of chromosomes is a condition called *polyploidy*. Cells with three complete sets of chromosomes are called triploid, those with four sets are called tetraploid, and so on. Because polyploidy interferes with normal synapsis during meiosis, polyploids are characterized by extremely low fertility.

Polyploidy in plants often results in exaggeration of certain characteristics of the flowers or of the fruit—for example, the size and number of petals or the fleshiness and sugar content of the fruit. Because such characteristics may be prized by farmers or gardeners, many domesticated plants are

Figure 14.16. The frequency of sex-linked lethal recessive mutations in *Drosophila* is a function of the x-ray dosage. The relationship between dosage and lethal mutations is a linear one.

polyploid mutants. Many of these mutants (particularly triploids) do not reproduce effectively by sexual means but must be reproduced through grafting or slipping techniques. Those polyploid species that can reproduce sexually (such as many crop plants that are tetraploid) pass the polyploidy along to their descendants. Polyploids, however, are usually not viable in animals.

Some hybrid plant species are formed by a combination of complete chromosome sets from the two parental species. For example, suppose that one species has the diploid set of chromosomes AABBCC and a fairly similar species has a different set XXYYZZ. In most cases, fusion of an ABC gamete from one species with an XYZ gamete from the other will produce a sterile hybrid with chromosomes ABCXYZ. Because the chromosomes do not pair properly at synapsis, the gametes of the hybrid will have incomplete chromosome sets and will be inviable. If each species becomes polyploid and thus produces diploid gametes, the fusion of an AABBCC gamete with an XXYYZZ gamete can produce a zygote with a complete diploid set of six chromosomes: AABBCCXXYYZZ. This hybrid can produce gametes with full chromosome sets because all of its chromosomes have homologous pairs. Furthermore, the hybrid will produce relatively infertile triploid zygotes if it crosses with one of the parental species. Thus, the hybrid becomes reproductively isolated and is apt to evolve rapidly into a species of quite different characteristics from the parental species. It appears that this process has been of great importance in the evolution of new plant species.

Other chromosomal abnormalities result from the breakage and rejoining of chromosomes. When a chromosome breaks, the broken ends act as if they are sticky and tend to rejoin. In most cases, the rejoining results in restoration of the original chromosomal configuration, but other things can happen. Segments may be duplicated or deleted, a part of the chromosome may be inverted, or a part of one chromosome may be joined onto another. Many of these changes are lethal to the cell in which they occur or result in severe abnormalities of zygote development. If the alteration does not prevent mitosis or meiosis, the altered chromosome may be replicated and passed on to future generations. For example, the bar-eye mutation of *Drosophila* is now known to involve duplication of a short segment of the X chromosome.

The genetic research in Morgan's laboratory was hampered by the extreme rarity with which new mutations appear naturally. Hundreds of thousands of flies were examined in order to find a few hundred mutant genes. Yet another of Morgan's students, Herman J. Muller, hit upon a new idea. He reasoned that most mutations involve only a single gene and that he needed to attack the chromosome with some agent that would affect only a tiny part of its length. High-energy radiation seemed to be the only thing that could accomplish the necessary microscopic damage. Muller (1927) subjected a group of *Drosophila* to a dose of x-rays so strong that some of the flies became sterile. He then crossed the remaining fertile flies with wild-type flies. When the offspring matured, Muller found more mutants than he could have hoped for. Comparison with a control group of untreated flies showed that the radiation had increased the mutation rate by 15,000 percent.

A large number of the new mutant genes proved to be lethal. Some were "dominant lethals," detected by the decreased reproduction rate of the treated flies. Others were "recessive lethals," which allowed the heterozygous

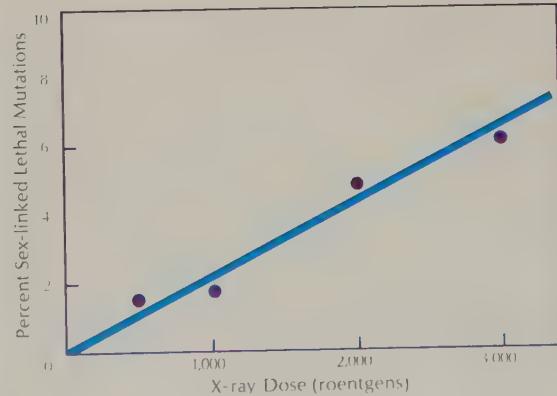


Figure 14.17. Photomicrograph depicting the giant salivary cell chromosomes of *Drosophila*. Note the distinct banding—dark bands containing DNA and light bands containing RNA. Note the two distinct chromosome puffs. These puffed-out regions may be areas of gene activity where RNA transcription is taking place.

gous first-generation flies to mature but proved fatal to their offspring that were homozygous for the mutant allele. Many of the mutant characters already known were produced among the radiated flies, as well as a number of new mutant characters of similar nature—splotched wings, sex-combless, and so on. Under the microscope, many cases of chromosome breakage, inversion, and exchange could be seen. Chromosome mapping of some of the mutants confirmed the alteration of gene locations in a number of cases.

Other investigators soon showed that x-rays are equally effective in producing mutations in other organisms. With this new research tool to produce large numbers of mutations, genetic research could proceed much more rapidly. The nature of mutations could be studied far more easily. And perhaps of most importance, the fact that radiation produces mutations provided an explanation of the source of naturally occurring mutations. Cosmic rays, ultraviolet light, and natural radioactivity provide a constant source of low-level radiation that strikes all organisms. This natural radiation accounts for the natural appearance of mutations at a low rate in all organisms. These mutations provide the variability of characters that can be reassorted through Mendelian mechanisms to provide a variety of phenotypes within a population. This variety makes possible evolution through natural selection.

GIANT CHROMOSOMES

Cytologists had known since 1881 that the chromosomes in the salivary glands of *Drosophila* larvae are about 100 to 200 times as long as the chromosomes of other cells and have a banded structure. This fact did not come to the attention of geneticists, however, until T. S. Painter (1933) made a careful study of these cells using staining to make the banded patterns more visible. Painter showed that the pattern of banding is very regular for homologous chromosomes within individuals or between individuals of the same species. Using the banding pattern, he identified even small cases of inversion, duplication, deletion, exchange of chromosomal fragments, and so on. By comparing characteristics of organisms and the banding patterns of their salivary gland chromosomes, Painter identified the particular bands that correspond to particular genes. Thus, he prepared chromosome maps by a method entirely independent from the crossing over method used by Morgan's group. Comparison of Painter's physical maps with Morgan's sta-



tistical maps revealed exactly the same sequence of genes, although the spacing between genes was different. Apparently, the frequency of crossing over does not depend solely upon distance between genes; some parts of the chromosome are more liable to cross over than are others. However, Painter's work dramatically confirmed the validity of the indirect inferences about gene order made by Morgan and Sturtevant.

Use of the giant chromosomes—which are produced by several consecutive duplications of the chromosomes to produce a total of more than 1,000 chromosomes lying side by side—made possible much more detailed chromosome maps (Bridges and Brehme, 1944). Studies of such detailed modifications of the chromosome revealed facts that forced a revision of prevailing ideas about genes. Most geneticists had assumed that genes are independent units that are merely carried by the chromosomes. If so, the effects of a gene should be the same regardless of its location. However, studies with the giant chromosomes showed that the same two genes may have different effects if they are on the same chromosome instead of on two different chromosomes, or if their sequence is inverted. Such *position effects* indicate that the genes are more closely related to one another on the chromosomes than had earlier been thought (Dobzhansky, 1936; Lewis, 1950).

GENES AND PHENOTYPIC CHARACTERS

As Morgan's group discovered and mapped genes in *Drosophila*, they found that almost every character of the adult organism is affected by a number of different genes on different chromosomes. For example, the color and shape of the eye are controlled by more than a dozen different genes on each of the three large chromosomes and by a few other genes on the small fourth chromosome. In addition, some genes have several different recessive alleles, each leading to development of a slightly different phenotypic character. To complicate matters further, there are many cases of incomplete dominance.

While Morgan's group and many other geneticists concentrated upon locating the genes and working out their relationships to various characters in the adult organism, other geneticists began to seek an understanding of the way in which the gene controls the development of these characters. How can a change in a minute segment of a chromosome affect a character such as eye color or wing shape in an adult *Drosophila*?

A brilliant suggestion was made early in the history of genetics by a British biochemist, Archibald Garrod (1909). A number of human diseases had long been known to be most common among the descendants of persons who also had the disease—that is, to be hereditary. Many of these diseases were known to be associated with the presence of unusual substances or the absence of normal substances in the urine or in the affected parts of the body. Combining ideas from the latest discoveries in genetics and in biochemistry, Garrod suggested that these diseases are "inborn errors of metabolism." An inherited, defective gene leads to the body's failure to produce a needed enzyme. Because of the absence of this enzyme, some portion of the normal metabolic processes goes astray. Chemicals that should have been broken down accumulate, or other necessary chemicals are not manufactured. Garrod showed that the same explanation can be applied to many of the mutant characters in *Drosophila* and other organisms. Furthermore, some human diseases show sex linkage similar to that

Figure 14.18 (above). Dr. George Wells Beadle, Nobel Prize-winning geneticist noted for his work on *Neurospora* leading to the one gene—one enzyme hypothesis.

Figure 14.19 (below). Photograph of the red bread mold *Neurospora crassa*.



observed in *Drosophila*. Wherever enough data were available to study the appearance of these diseases in successive generations, the ratios of phenotypes found proved quite similar to the results of experiments with recessive mutant genes in *Drosophila* and other organisms.

Some geneticists attempted to discover the relationships between genes and enzymes. Most of these attempts were relatively unsuccessful because the genetics and development of organisms are so complex that it is almost impossible to find simple relationships.

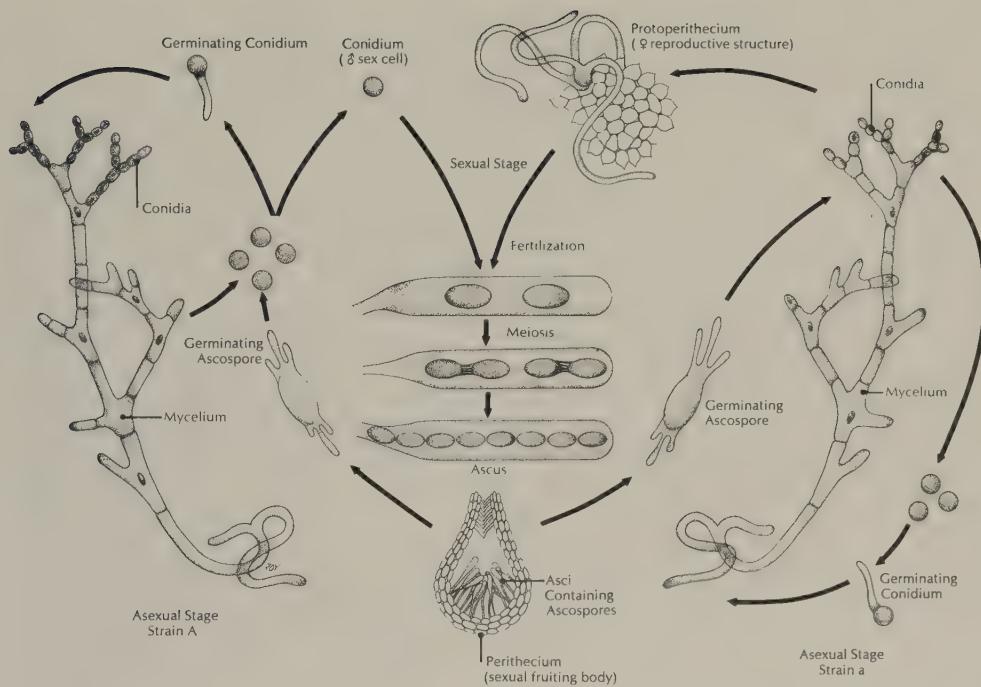
Again, the key to further discoveries was the use of a new organism particularly suited to the problems being investigated, and again the advance was the work of one of Morgan's colleagues. George Wells Beadle worked with Sturtevant on crossing experiments with *Drosophila* and corn, but Beadle felt that this sort of work was a dead end—there were many details to be resolved but little hope of any major new discoveries.

Beadle later joined forces with Edward L. Tatum, a biochemist at Stanford University. They decided to abandon research with *Drosophila*, an organism whose genetic properties had been thoroughly mapped but whose biochemistry was complex and poorly understood. Instead, they needed an organism with simple biochemical properties. In 1940 they began to work with a red bread mold, *Neurospora crassa*. The haploid cells and relatively short life cycle (ten days between sexual generations) of this mold make it suitable for genetic research. Its ability to reproduce asexually and rapidly enables a researcher to create a sizable sample of any genotype in quantities suitable for biochemical analysis. *Neurospora* thrives in a simple culture medium containing mineral salts, sugar, and the vitamin biotin.

When *Neurospora* reproduces sexually, haploid cells from two individuals fuse to form a zygote. Meiotic division of the zygote produces four haploid cells, each of which then divides by mitosis. The eight spore cells that result are neatly lined up in a spore sac. Because of the orderly process of cell division within a confining structure, the sequence of the spores in the sac can be directly correlated with the separation of chromosomes in the various divisions of the zygote. With a microscope, a trained laboratory assistant can isolate one spore sac, remove the eight spores in sequence, and place each into a tube of culture medium. Rapid asexual reproduction



Figure 14.20. The life cycle of *Neurospora crassa*.



leads to a sizable population derived from a single chromosome set. Thus, the complex crossing experiments needed to analyze the genetic composition of *Drosophila* can be avoided for the most part.

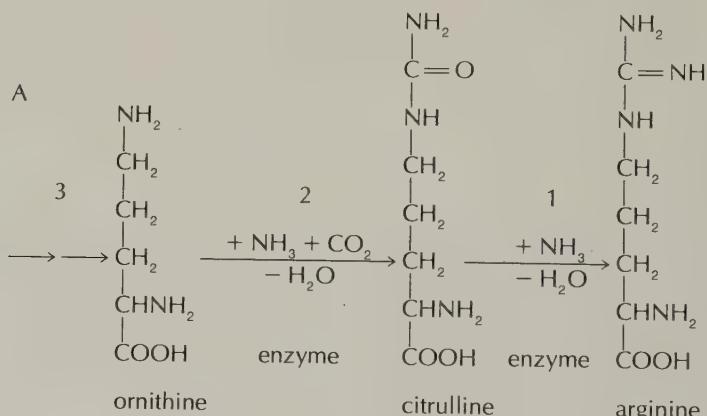
Neurospora synthesizes all the substances it needs from the mineral salts, sugar, and biotin in the minimal culture medium. Beadle and Tatum reasoned that this complex metabolic machinery must involve a great many enzymes. If the presence of a mutant gene results in the absence of a particular enzyme (as they strongly suspected from their earlier work with *Drosophila*), the mutant mold should fail to synthesize some needed substance. It will then fail to survive on the minimal medium. By finding out what substance it needs for survival, they should be able to correlate a particular mutant gene with the failure of a particular synthetic step in metabolism. Biochemical studies could then reveal the enzyme needed for that particular synthesis. Thus, Beadle and Tatum hoped to establish a one-to-one correlation between the genes of *Neurospora* and the enzymes that it normally manufactures.

They began by exposing cultures of *Neurospora* to x-rays in hopes of producing mutations. From the irradiated *Neurospora*, they obtained 2,000 spores, each of which was planted in a tube containing a complete medium made of yeast and malt extracts in addition to mineral salts and sugar. This complete medium contains most of the substances that the mold normally synthesizes for itself, so that even mutant strains will thrive in it. After sizable populations had been established in each tube by asexual reproduction, a small sample of each culture was transferred to a tube containing the minimal medium. Three of the samples failed to grow on the minimal medium. For each of these strains, samples were tested on a sequence of media, each consisting of the minimal media plus one vitamin,

Figure 14.21 (A) A portion of the pathway of arginine biosynthesis in *Neurospora*. An essential enzyme is required in each step of the process in order to catalyze the reactions culminating in arginine synthesis. (B) Growth requirements of three groups of arginine-requiring mutants in *Neurospora*.

amino acid, or other organic chemical present in the complete medium. In this way, Beadle and Tatum (1941) discovered exactly what substance the mutant strains require for growth. One proved unable to synthesize pyridoxine (vitamin B_6), another, thiamine (vitamin B_1), and the third, para-aminobenzoic acid. The thiamine molecule is composed of two parts, a pyrimidine half and a thiazole half. The second mutant strain was not only able to grow with thiamine added to the minimal medium but also was able to grow with only thiazole added. Apparently, it could synthesize the pyrimidine part of the thiamine molecule but not thiazole.

In later studies, Beadle and Tatum worked out various pathways of synthesis in the normal organism by study of mutant strains. For example, one mutant strain grew only if the amino acid arginine was added to its minimal medium. Another mutant strain grew on either arginine or citrulline. A third strain accepted arginine, citrulline, or ornithine. Presumably, arginine is normally synthesized in *Neurospora* by the reaction pathway: ornithine \rightarrow citrulline \rightarrow arginine. The ornithine is synthesized from simpler precursors. Each step in the pathway is catalyzed by a specific enzyme.



B

Arginine-requiring mutant	growth on				reaction blocked
	minimal	ornithine	citrulline	arginine	
1	—	—	—	+	1
2	—	—	+	+	2
3	—	+	+	+	3

The first mutant strain lacked the enzyme that converts citrulline to arginine. Neither ornithine nor citrulline did any good for this strain; it needed arginine to survive. The second strain lacked the enzyme that converts ornithine to citrulline. If supplied with citrulline, it could synthesize arginine. The third strain lacked an enzyme for an earlier step in the pathway and thus was able to convert either ornithine or citrulline into arginine.

In every case, Beadle and Tatum found that a particular mutant strain was deficient in only a single step in a reaction pathway and therefore pre-

sumably in a single enzyme. They concluded that each enzyme is produced under the direction of a single gene. This postulate has come to be known as the *one gene–one enzyme hypothesis*. They demonstrated that each synthetic deficiency is in fact inherited as a single gene in crosses of the mutant strain with normal strains (Beadle, 1945a, 1946).

Analogous experiments with many different organisms have subsequently revealed mutations that affect single steps in many different biosynthetic pathways. The *one gene–one enzyme hypothesis* was modified slightly when it was later discovered that many enzymes are composed of two or more separate polypeptide chains, each of which is produced under the direction of a separate gene. This discovery led to the restatement of the postulate as the *one gene–one polypeptide hypothesis*, and it now has been extensively confirmed (Wagner and Mitchell, 1964).

The work of Beadle and Tatum established *Neurospora* as a genetic research subject second in popularity only to *Drosophila*. Even more important was the dramatic evidence that biochemical studies could carry understanding of heredity a great deal further. From the 1940s onward, genetics and biochemistry became ever more closely related fields. Now that the general mechanism by which the gene directs cell metabolism through enzymes was understood, the next step was clearly an understanding of the mechanism by which the gene controls synthesis of enzymes. What is the mechanism of gene action? Solution of this problem was the work of molecular geneticists in the last two decades.

FURTHER READING

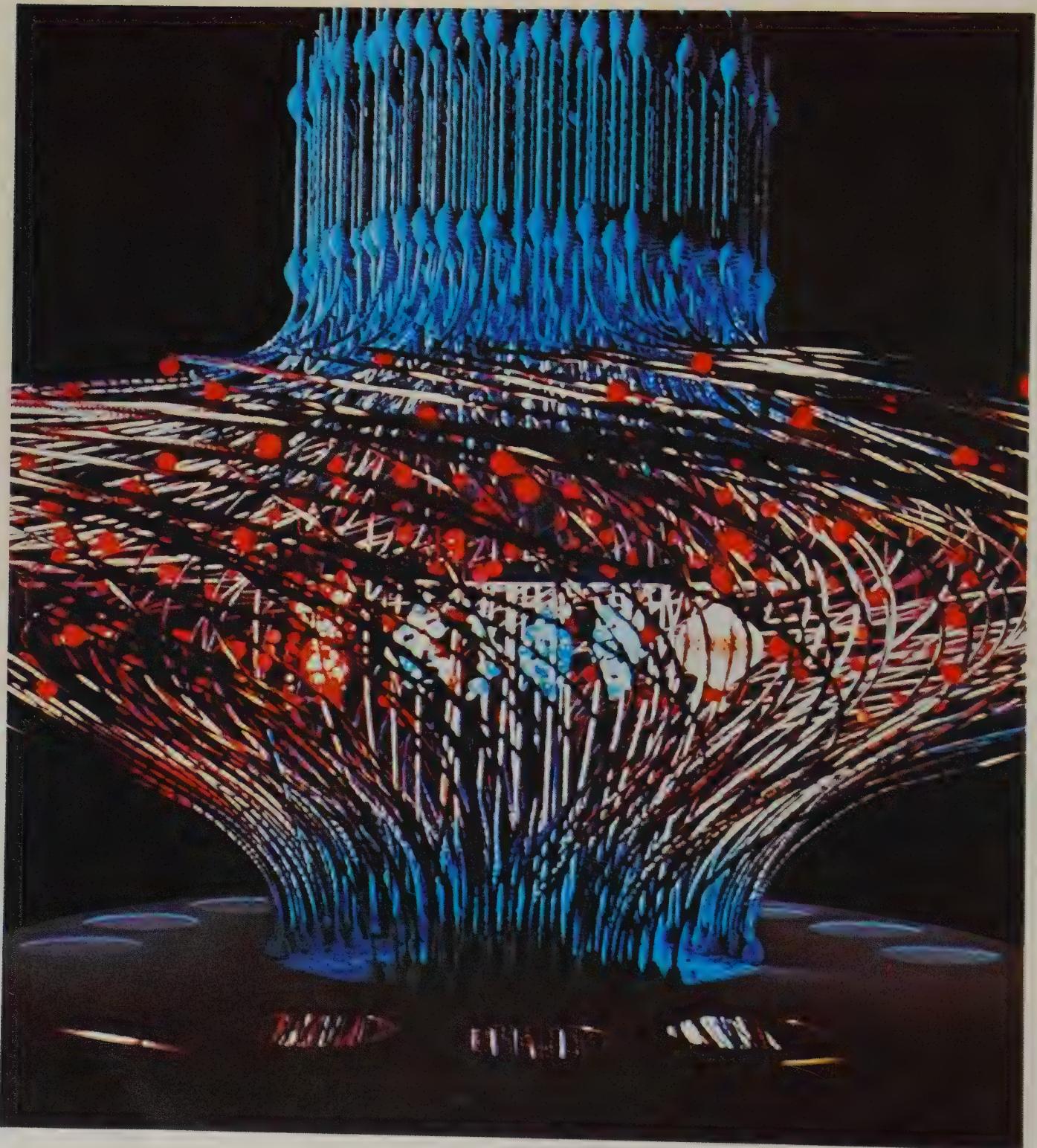
Many of the important theoretical and research reports mentioned in this chapter and the next are reprinted in the collection edited by Peters (1959). Historical accounts and further discussion of the material in this chapter and the next are given by Beadle and Beadle (1966), Muller (1951), and Sullivan (1967). Burdette (1962) describes many of the experimental techniques commonly used in genetics research.

A full understanding of Mendelian and classical genetics principles can best be developed by working through a number of examples. For more detailed information and exercises, see introductory genetics texts such as those by King (1965) and Srb, Owen, and Edgar (1965).

For further information about *Drosophila* see Bridges and Brehme (1944), Demerec (1950), Demerec and Kaufman (1961), Herskowitz (1952, 1958, 1964), Morgan (1926), Morgan and Bridges (1916), Muller (1939), Sang (1956), and Strickberger (1962). Further information about *Neurospora* is given by Beadle (1945b), Bonner (1946), Horowitz (1950), and Lindegren (1932, 1933).

15

Molecular Genetics



The greatest achievement in biology in this century has been the development of the field of molecular genetics. Ingenious and imaginative experimentation—combined with powerful tools of chemical analysis—has produced a remarkable series of discoveries leading to a precise description of the chemical nature of the gene and a deep understanding of gene function and gene mutation. The central principle by which genes replicate and provide genetic information to the cells of an organism is now understood in detailed molecular terms. These discoveries are now being extended by an enormous and intensive research program on a worldwide scale. The effort is directed toward a full understanding of the biochemical basis of structure and function of all living systems. The potential value of this knowledge is beyond estimation.

Already the accomplishments of molecular geneticists have led to new triumphs of medical technique. The possibility of a prevention or cure for cancer and hereditary disease gives new public significance to experiments and theories, and it is not unusual to find accounts of esoteric research results in the newspapers or the newsmagazines. Some biologists have begun to worry about the wisdom of further progress toward the ability to manipulate human genes. With the example of atomic power always in mind, one wonders whether the human race is ready to use such power in a beneficial fashion. For the time being, the techniques of genetic engineering remain a futuristic speculation, and most geneticists are convinced that the almost limitless beneficial results of further knowledge of genetic processes outweigh the danger of its misuse.

NUCLEIC ACIDS AND CHROMOSOMES

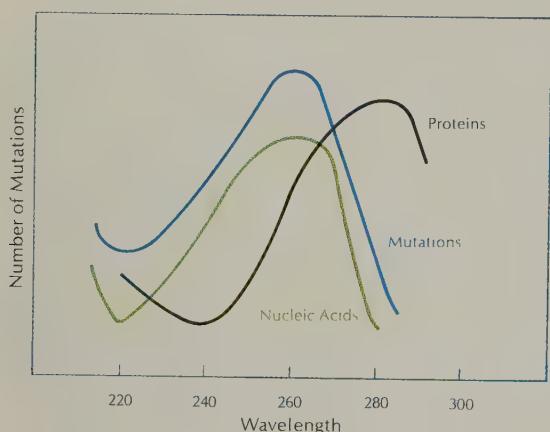
Nucleic acids were discovered at almost the same time that Mendel was experimenting with pea plants. By 1881 it was established that chromatin either is composed of or is closely associated with nucleic acids, but its importance went unnoticed.

At the turn of the century, a number of discoveries forced a reassessment of the role of nucleic acids. Careful study of chromosomes showed that chromatin varies in amount during different parts of the cell cycle and even seems to disappear entirely from many cells during interphase. Because chromatin and nucleic acids were believed to be the same substance, biochemists considered the disappearance of chromatin observed in the staining of many cells as evidence that nucleic acids are broken down during certain parts of the cell cycle. An obvious requirement of the genetic material is that it must be stable, and nucleic acids were not. At the same time, proteins were shown to be polymers that acted as enzymes controlling most of the chemical machinery of the cell. In addition, they were thought to be stable. Biochemists came to view proteins as the primary chemicals of life. Obviously, they reasoned, the persistent and extremely complex proteins—not the impermanent nucleic acids—must be the genes. The relatively simple molecules of nucleic acids, consisting of only four bases, merely provided structural support for the chromosomes; the associated proteins, with their great variety of structures and chemical properties, were the carriers of heredity information.

Suggestive Evidence

During the first half of the twentieth century, much evidence suggested that the nucleic acids, not the proteins, were the genetic material. Ultraviolet

Figure 15.1. Absorption spectrum of DNA and protein in the ultraviolet range. Note that the number of mutations at any wavelength follows the pattern of the DNA absorption spectrum and not that of the protein.



light was known to cause mutations in cells, presumably because its absorption by the cell produced chemical modifications of the genetic material. Therefore, the wavelengths of ultraviolet light that were absorbed most strongly would be expected to produce the most mutations. Because the absorption spectra (that is, the amount of absorption versus different wavelengths) of nucleic acids and proteins are significantly different, a spectrum plotting the number of mutants produced versus wavelength might indicate the identity of the genetic material. The results coincided with the spectrum of nucleic acids but not with that of proteins. In the wavelengths where nucleic acids strongly absorbed ultraviolet light, there were many mutations; where absorption was small, there were few mutations.

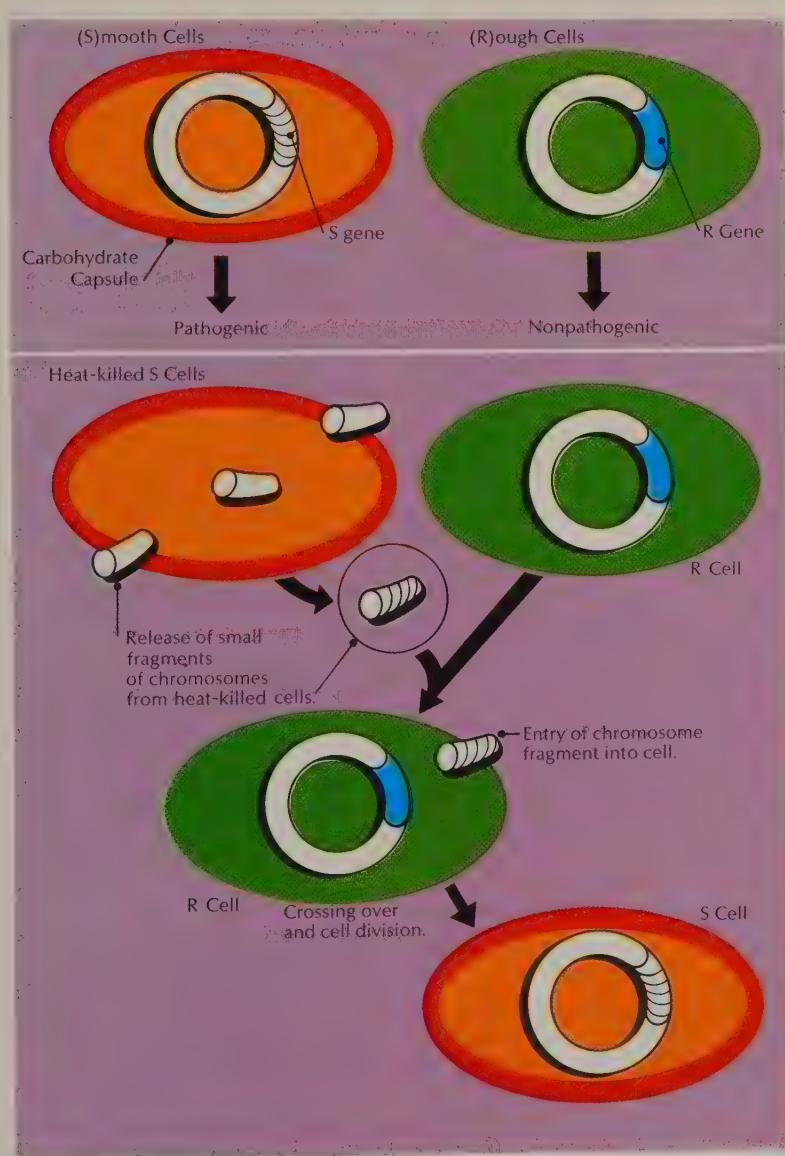
In any given species of animal, the amount of DNA is the same in all cells except sperm or egg cells. Even during the phases of the cell cycle when the chromatin seems to disappear, the DNA content remains constant. The sperm and egg cells contain just half as much DNA as do the other body cells, as would be expected if the offspring received one-half of their genetic material from each parent. Other studies of this nature revealed a much greater variation in the amount of protein or other chemicals present from cell to cell. Many similar experiments on a variety of organisms and cell types gave consistent results: although the amount of DNA in each cell may vary from one type of organism to another, each cell of a particular organism has the same amount of DNA. In short, DNA is present in constant quantity proportional to the number of chromosomes present, just as would be expected for the hereditary material, whereas proteins and other substances fail to meet this criterion of constancy.

Another suggestion of DNA's suitability as a genetic molecule came from studies of its stability. The breakdown of genetic molecules would represent loss of genetic information with no means of rebuilding the destroyed genes. Therefore, the macromolecule that stores genetic information must be highly stable. The stability of DNA was confirmed by use of radioactive labels. In most experiments, DNA is labeled by supplying H^3 -thymidine or C^{14} -thymidine during interphase, when DNA is being synthesized. These experiments reveal that radioactivity, once incorporated into the DNA, is not freed from the DNA molecule so long as the cell remains alive. DNA, once made, does not undergo breakdown and resynthesis in the living cell but shows the high degree of stability expected of the genetic material. On the other hand, similar experiments reveal that all other types of macromolecules within the cell—proteins, carbohydrates, and RNA—undergo breakdown and resynthesis with considerable reshuffling of atoms.

The scientific discovery that established the groundwork for the subsequent era of molecular genetics was made in the 1920s by Fred Griffith. Griffith was studying the bacterium *Diplococcus pneumoniae* (also called *pneumococcus*), an organism that causes pneumonia. He was trying to develop a method for immunization against the disease, but he failed to achieve his objective. Instead, he obtained puzzling experimental results that could not be explained by the knowledge of the time.

Griffith worked with two strains of bacteria. On a nutritive plate, one strain formed colonies that had a smooth surface; the other strain formed colonies with a rough surface. The smooth (S) strain was highly virulent (infectious and damaging to the host organism), whereas the rough (R)

Figure 15.2. Diagram of Griffith's experiment. The genetic composition of the bacteria *Diplococcus pneumoniae* is transformed by the addition of heat-killed cells of a different strain. This experiment set the stage for the proof that DNA is the genetic material of the cell. Printed by permission from J. D. Watson, Molecular Biology of the Gene, © 1965, J. D. Watson



strain was avirulent (noninfectious or harmless to the host). Griffith injected S bacteria into mice and found, as he expected, that the bacteria multiplied rapidly and the mice developed pneumonia and died within a few days. The injection of R bacteria into mice led to no signs of illness, and the bacteria disappeared from the host animals within a short time. The explanation for these results was well known. The S bacterium has a polysaccharide cell wall, or capsule, that inhibits the attack of leucocytes (white blood cells). No such capsule protects the R bacterium, and the leucocytes readily ingest and destroy it.

The ability of a bacterium to produce a capsule is a hereditary trait. When S bacteria undergo binary fission, the offspring are also S bacteria.

Similarly, the offspring of R bacteria possess the avirulent R traits. It is not the capsule of the virulent strain that damages the host organism. Large populations of R bacteria would be equally damaging, but the body's defense mechanisms eliminate defenseless R invaders before they can establish large populations.

In a series of routine experiments designed to confirm these theories about the pneumonia bacteria, Griffith prepared a vaccine consisting of S bacteria that had been killed by heating to 60°C. Although the capsules still surrounded these dead bacteria, their presence in the bloodstream produced no ill effects in the mice. Next, Griffith prepared a vaccine containing a mixture of live R bacteria and dead S bacteria, a mixture that also should have been harmless to the mice. To his surprise, mice injected with this vaccine developed pneumonia and died. Microscopic examination showed that the sick mice contained large populations of living bacteria with polysaccharide capsules. When isolated on nutrient plates, these bacteria continued to produce offspring with virulent S traits. Somehow the presence of dead S bacteria in the mouse transformed the live R bacteria into S bacteria—a transformation that involved permanent change of a hereditary trait. How could the presence of dead bacteria transform the genetic information of live bacteria? The only explanation that Griffith could suggest was that live R bacteria ingested the dead S bacteria and somehow were transformed by this diet.

Griffith's experiments were later duplicated outside the living cell. A mixture of dead virulent bacteria and living avirulent bacteria in a suitable medium led to the genetic transformation of the living bacteria. Once again, this transformation occurred only in the presence of dead virulent cells. Later experiments showed that a fluid extracted from the dead virulent bacteria can carry out the genetic transformation of the avirulent strain. This material came to be known as the *transforming factor*. It was Griffith's work that set the stage for the proof that DNA is the genetic material.

The Proof

In 1944 O. T. Avery, C. M. MacLeod, and M. McCarty succeeded in purifying the transforming factor. This material proved to be DNA, as evidenced by its susceptibility to DNase, an enzyme that specifically destroys DNA molecules; an extract treated with DNase will not cause genetic transformation. Other substances such as proteins proved ineffective in transforming avirulent bacteria.

The work of Avery, MacLeod, and McCarty represented the first direct proof that a molecule of DNA can carry genetic information.

Further confirmation of the genetic role of DNA soon came from studies of the viruses that infect the bacterium *Escherichia coli*. These viruses were known to consist only of protein and DNA, but it had been assumed that the protein plays the active role in the virus' takeover of a bacterial cell for its own reproduction. However, early electron micrographs using shadowing techniques showed that the phage attach to the bacterial cell wall and become flat, empty shells, suggesting that only part of the phage enters the cell.

The assumption that phage DNA is the active agent in takeover of the bacterial cell was confirmed in an experiment performed by Alfred Hershey and Martha Chase (1952). They grew phage-infected bacteria in media containing either radioactive sulfur (sulfur-35) or radioactive phosphorus

The following series of electron micrographs and autoradiographs describes the progressive sequence of the "central dogma," which can be schematically shown as:

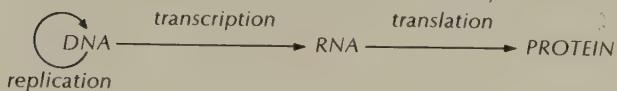


Figure 1 is an autoradiograph of DNA replicating in *E. coli*. Figure 2 shows a tracer experiment indicating

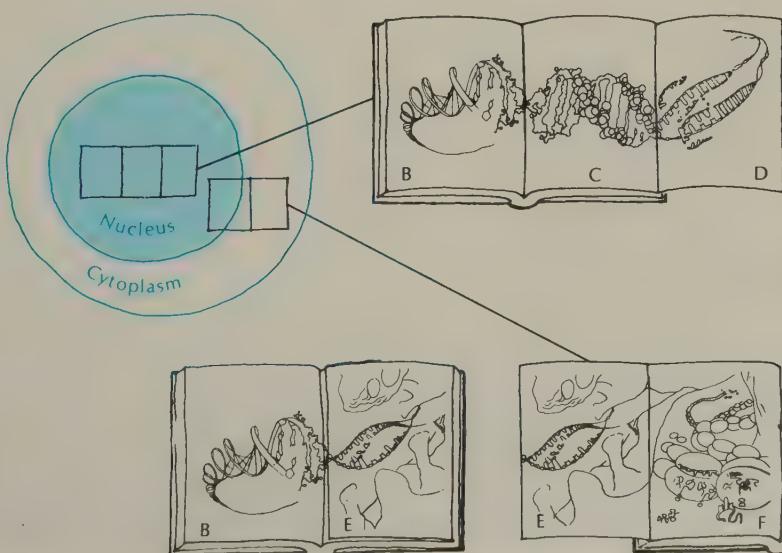
the role of RNA as an intermediate in protein synthesis. Radioactive uridine (found only in RNA) is first seen in the nucleus and later in the cytoplasm. Figure 3 is an electron micrograph of a polyribosome with a thin strand of linking mRNA—the level of translation. The final autoradiograph (Figure 4), completing the sequence, shows the production of protein with tritium-labeled leucine (an amino acid) in guinea pig pancreatic cells.

The process of life is intimately connected to molecules. Nucleic acid and proteins are two of the main containers of that life. The progressive panels of this interleaf reveal the mechanisms by which information is transmitted from the nuclear DNA out to the cytoplasm, where proteins are formed. Panels B, C, D, and part of E show the events that take place in the nucleus, whereas panels E and F show those occurring in the cytoplasm.

The "central dogma" is the key to understanding this molecular basis of life. Panels B and C depict DNA in the various conventions that biochemists use to define molecules. DNA is progressively shown by its chemical formula, stick-ball form, softened stick-ball form, space-filling—or effective size—model (based on electron-cloud size), and finally in symbolic form in panel D. Panel D shows the two strands separating and self-replication occurring. It is this self-replication mechanism that ensures that genetic information is preserved and transmitted from one generation to the next.

In order to present a clear view of events, the perspective of these illustrations has been distorted somewhat and the individual strands separated so that the three-dimensional quality of the molecule can be observed. Enzyme-mediated reactions throughout this interleaf are depicted symbolically by yellow-orange "clouds" and the hydrogen bonding between the bases by yellow shading. The student will find that the rendition of this complex process has been portrayed with accuracy, and in exploring its intricacy, he will be able to note strand polarity, synthesis direction, hydrogen bonding, base pairing, and so on.

When panels B and E are brought together, the formation of messenger RNA on DNA templates, or transcription, is shown. The final panels E and F are interpretations of the frozen reality of electron micrographs. The panels show an illustrative view of a protein being formed on the mRNA template—the process of translation. Ribosomal subunits are shown on the endoplasmic reticulum (ER) linking up with the mRNA. They form one long polyribosome. Transfer RNAs, with the individual amino acid that each one carries for a specific codon on the mRNA, then participate in the protein building. As the polyribosome recedes into the ER, the final product, a complicated protein, is released. A certain degree of liberty has been taken in sacrificing scale proportions so that the student can begin to appreciate the more dynamic and architectural realities of the inner space of the cell world.



Interleaf 151 THE "CENTRAL DOGMA"

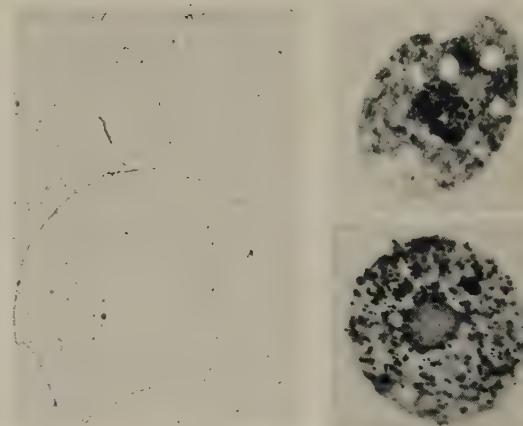


Figure 1

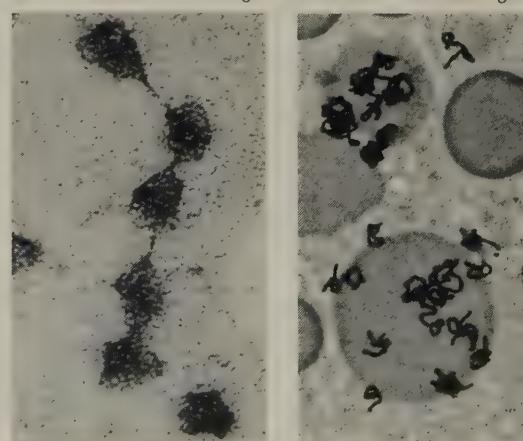


Figure 2

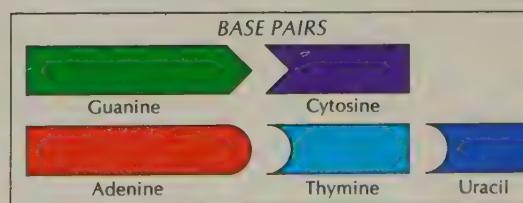
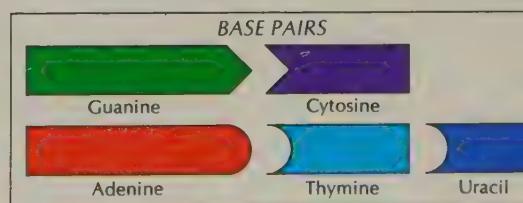
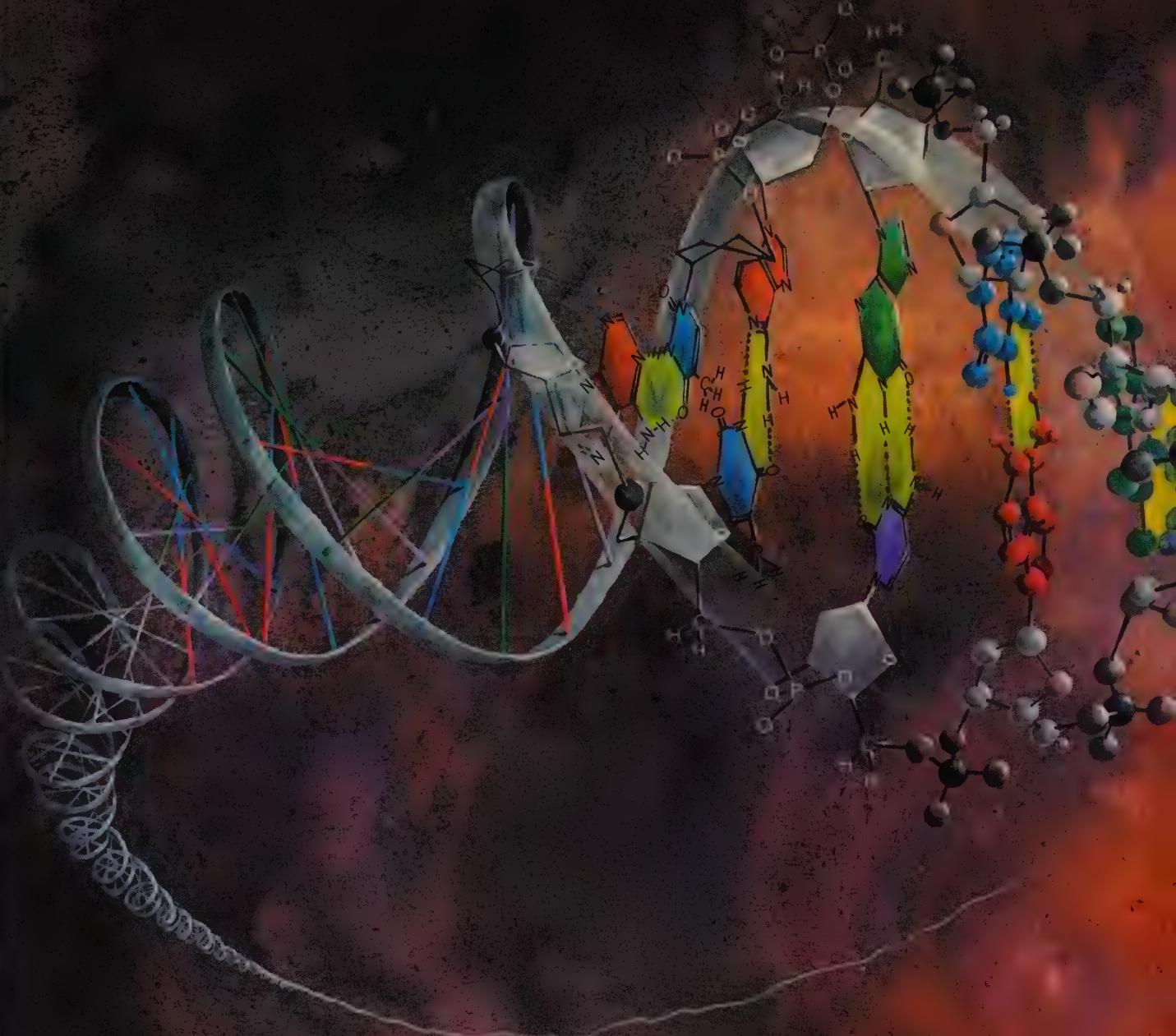
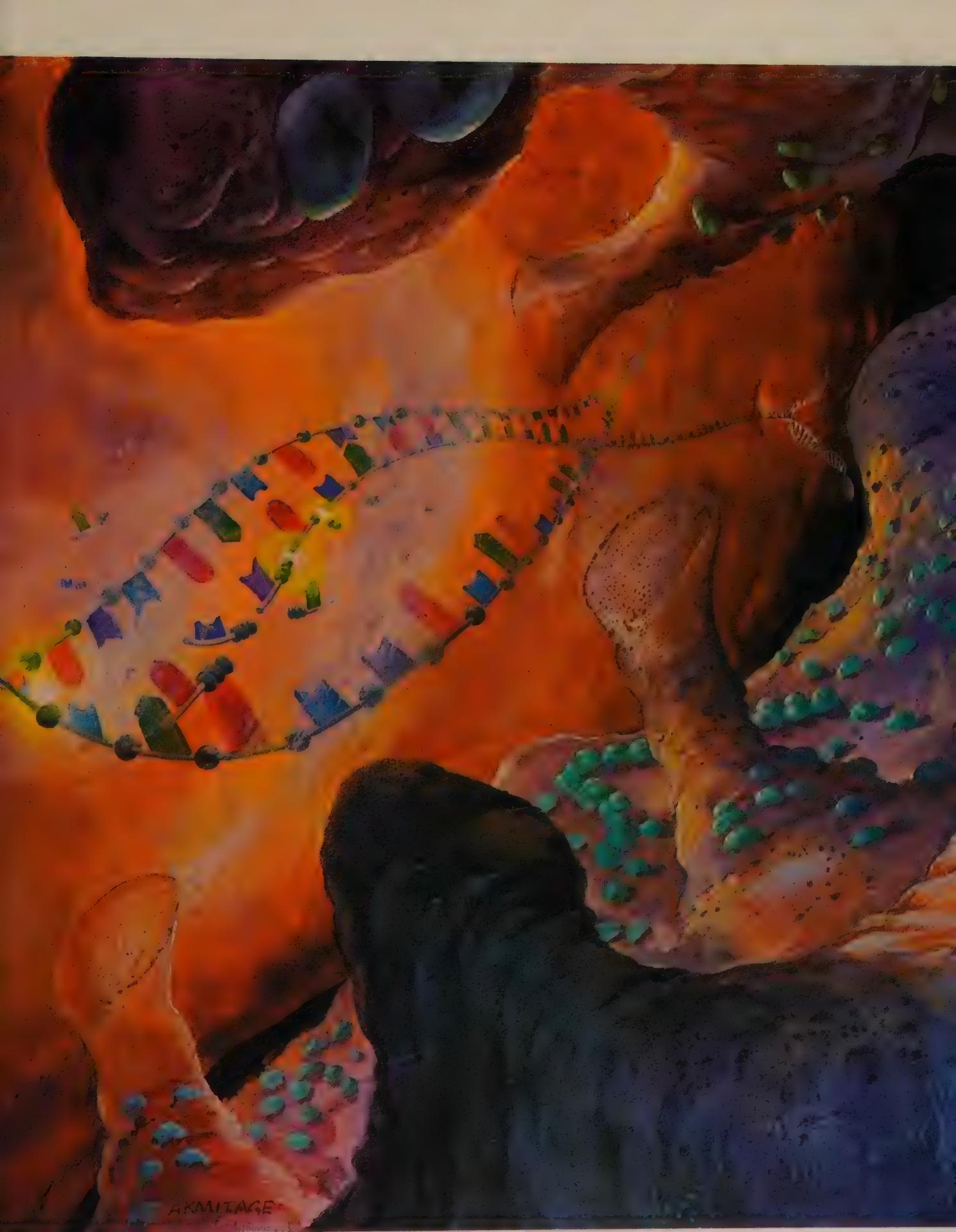


Figure 3

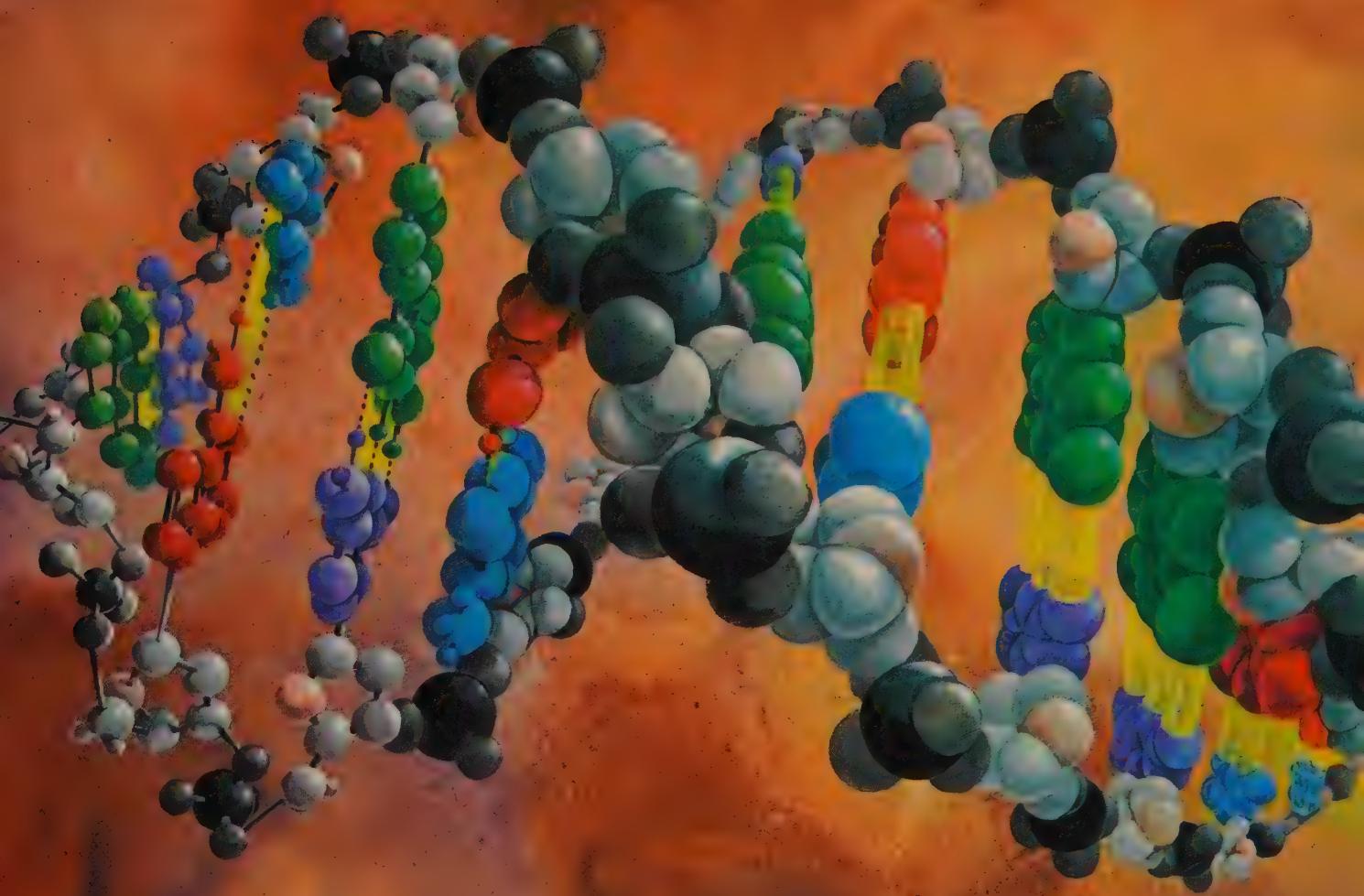




B



AKMILAGE



C

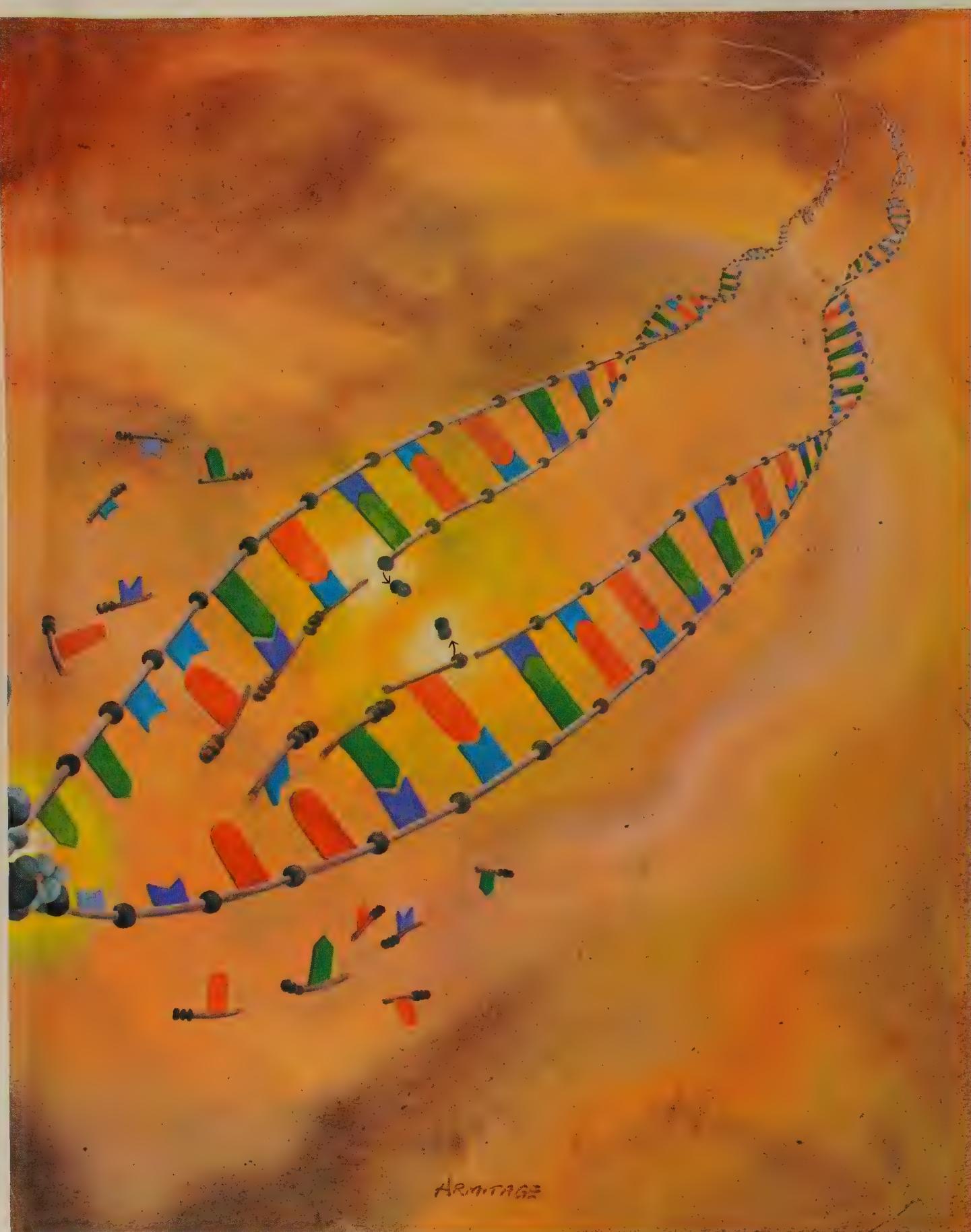
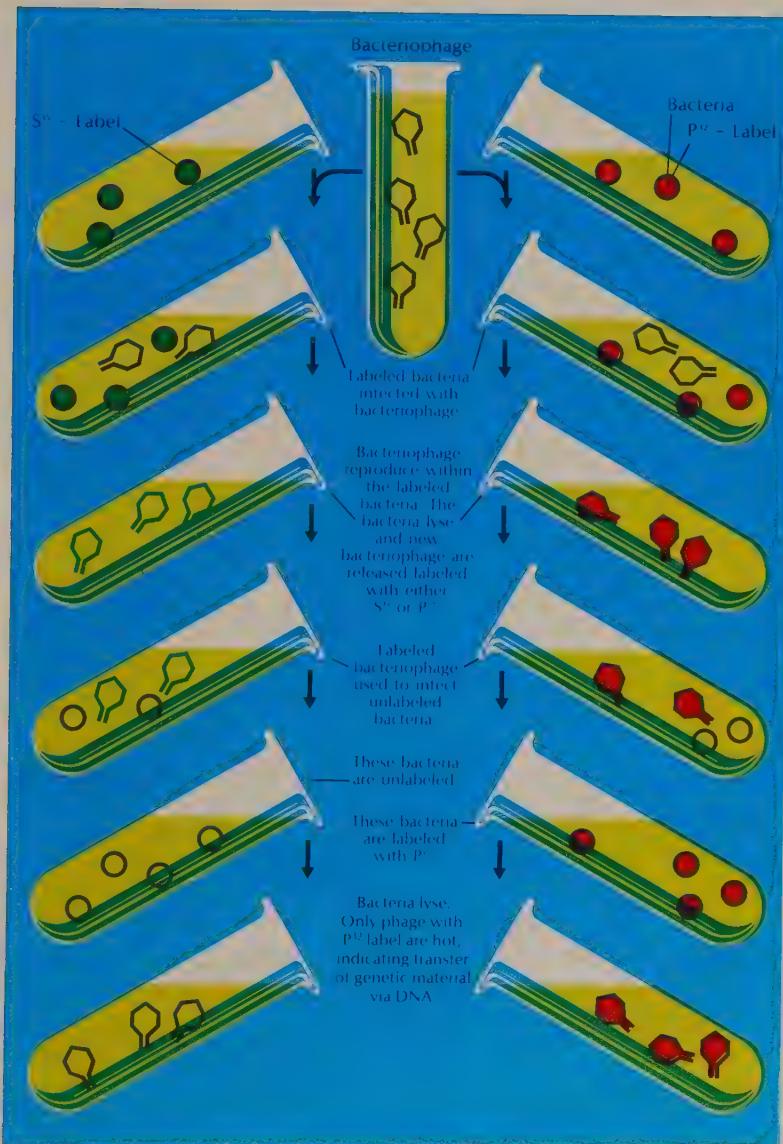


Figure 15.3. Diagram of the Hershey-Chase experiment with radioactive labeled bacteriophage. The radioactive nucleic acid enters the bacterial cells, and its entry alone (without protein) is sufficient for faithful reproduction of more viruses.



(phosphorus-32). Sulfur atoms were incorporated into proteins but not into DNA. Phosphorus atoms were built into nucleic acids but not into proteins. Thus, phage that were produced within bacteria in the S^{35} medium incorporated the radioactive atoms into their proteins, whereas phage grown in the P^{32} medium incorporated radioactive atoms into their DNA (Figure 15.3).

When phage grown in the S^{35} medium are used to infect nonradioactive bacteria, almost no radioactivity is found inside the bacterial cells after infection, showing that little if any of the phage protein enters the cells. When the bacteria are infected with phage containing P^{32} , most of the radioactivity is found inside the bacterial cell after infection, showing

that most of the phage DNA does enter the cell. This experiment revealed that only the phage DNA is needed in order for the phage to take over the bacterial cell and reproduce itself. The DNA must carry all of the genetic instructions needed to put together large numbers of complete phages inside the bacterial cell.

Other viruses, such as the tobacco mosaic virus (TMV), were known to contain only RNA and proteins. Heinz Fraenkel-Conrat showed that he could cause mutations in TMV by chemical modification of the RNA but not of the protein. Thus, he proved that RNA is the carrier of genetic information for TMV. Fraenkel-Conrat later succeeded in infecting tobacco leaves with pure RNA extracted from TMV. Thus, RNA could also serve as genetic material.

From observations such as those described above and many others, it has been established beyond any doubt that DNA is the genetic substance in all cells, as well as in some viruses. Genes must be composed of DNA.

THE STRUCTURE OF DNA

The powerful evidence—obtained in the 1940s and early 1950s—that DNA served the genetic role stimulated an intense effort to determine the chemical structure of the DNA molecule. It had long been known that DNA is composed of the four deoxynucleotides—thymidylic acid, adenylic acid, cytidylic acid, and guanylic acid. The molecular structures of these four nucleotides were known, but the structure and size of the polymer remained to be determined.

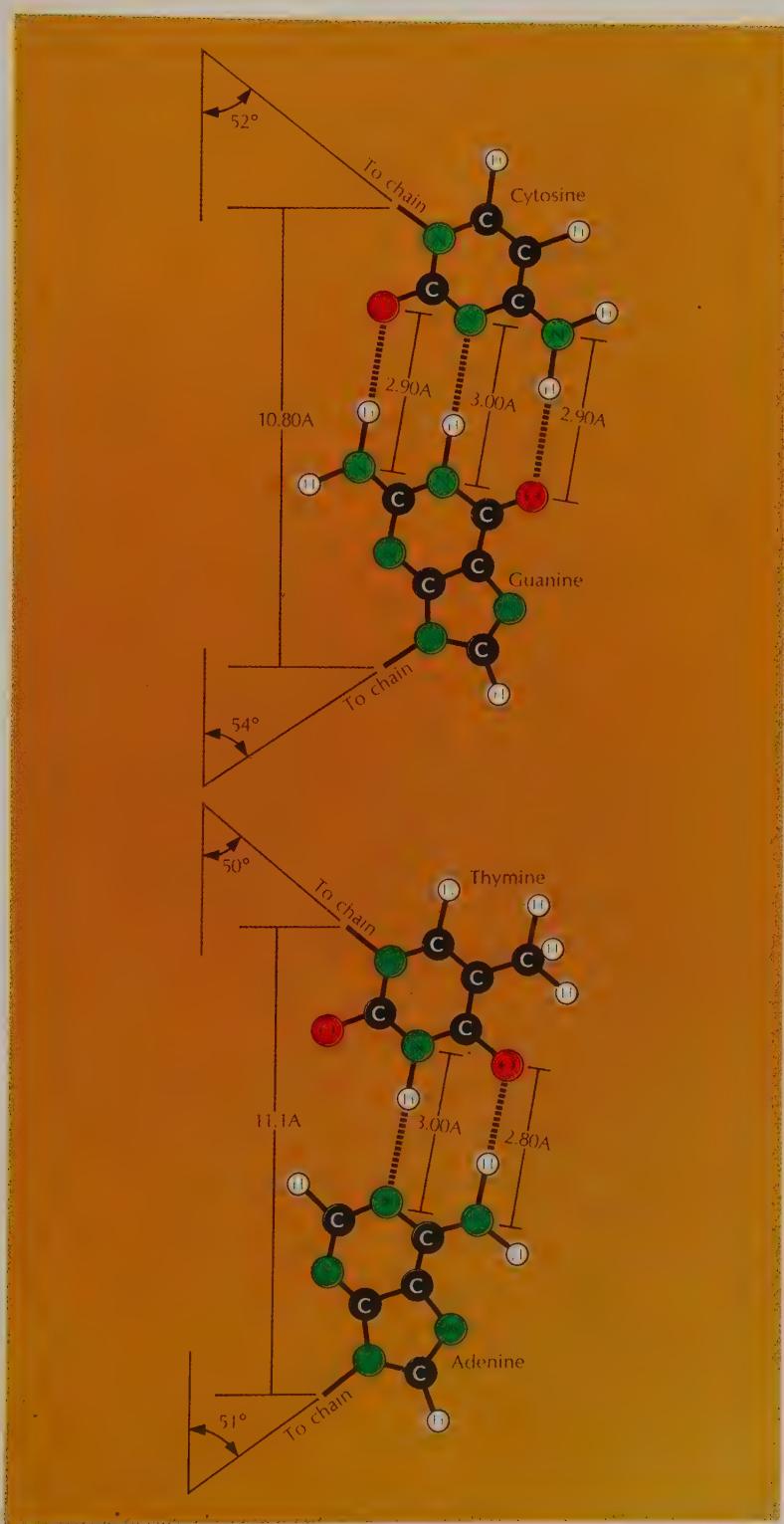
Erwin Chargaff analyzed the relative amounts of the four bases in DNA from various kinds of organisms. Using the technique of paper chromatography for separation of the bases, Chargaff found considerable variation in the base ratios of DNA from different species, but his results showed remarkable constancy in the base ratios of DNA from different cells of a single species. The DNA of each kind of organism has its own specific ratio of different nucleotides, a ratio that might be the result of a specific sequence of nucleotides that could serve as a means of encoding genetic information. Chargaff noted that in all the DNA samples he studied the ratios of adenine to thymine, of guanine to cytosine, and therefore of total purines to total pyrimidines, “were not far from one.”

William Astbury had made an attempt at x-ray diffraction study of DNA in 1938. From the diffraction patterns, he concluded that the DNA molecule is a long chain of repeating units, with the flat planes of the organic bases oriented at right angles to the long axis of the molecule, rather like “a pile of pennies.” Pauling and Corey later concluded that DNA has a helical structure, and the British biochemist J. M. Gulland argued from chemical considerations that DNA is probably a double chain in which hydrogen bonds between bases hold the two chains together. Another group headed by Maurice Wilkins had begun a crystallographic analysis of DNA structure in 1950.

Enter Watson and Crick

When James D. Watson arrived at Cambridge University to work in the laboratories of Francis H. C. Crick, he was convinced that the structure of DNA would be found through x-ray crystallography. Crick shared his enthusiasm, and both set about to solve the riddle of the DNA structure. Together they brilliantly synthesized the results of many workers into a

Figure 15.4. The base pairs of DNA. Adenine is bonded to thymine by two hydrogen bonds, and guanine is bonded to cytosine by three hydrogen bonds.



coherent model consistent with the known data on DNA. Most importantly, their model could account for Chargaff's data as well as offer a plausible mechanism for DNA replication.

Watson and Crick decided that there was good evidence that DNA was a two-chain, helical molecule with a constant width of 20 Å. The twin phosphate chains ran up the outside, and the base pairs in the center were held together by hydrogen bonds. However, the manner in which the bases were arranged was still a mystery.

Because purines are much larger than pyrimidines, pairing of like bases along the chains would cause the width of the helix to vary considerably from one base pair to the next. After Watson had built unsuccessful models using purine-purine or pyrimidine-pyrimidine base pairing, a friend pointed out to him that he was using the forms of the bases commonly shown in textbook diagrams, and these were almost certainly the wrong stereochemical isomers for the base groups in DNA. It was this clue that enabled Watson to discover the true nature of the base pairing. With this structure in mind, he cut scale models of the base groups out of cardboard and began trying to fit them together in various ways. Suddenly, he realized that an adenine-thymine pair held together by two hydrogen bonds is identical in size to a guanine-cytosine pair joined by two or three hydrogen bonds. Such pairing would explain Chargaff's base ratios showing equal amounts of purines and pyrimidines, and it would also give the double helix a very regular structure, meaning it would have constant width. Furthermore, it would provide an intriguing means of DNA replication. The molecule could split and each strand could then build upon itself a complementary strand to restore the original molecule. As Watson and Crick remarked in their publication on DNA structure: "It has not escaped our notice that the specific pairing we have postulated immediately suggests a possible copying mechanism for the genetic material." Soon afterward, the model structure was found to be fully consistent with the x-ray data of Wilkins. Almost immediately, the Watson-Crick model of DNA structure was acclaimed by biologists and geneticists as one of the major biological discoveries of the century.

Replication of DNA

The most exciting feature of the Watson-Crick model of DNA structure is the obvious possibility for such a structure to act as a self-replicating molecule. The mechanism of DNA replication causes at least a partial separation of the strands of the duplex at the replicating point, so that the hydrogen-bonded bases are exposed. The free nucleotides then "recognize" the complementary base via hydrogen bonding, and the sequence of bases in the parental strand orders them in the daughter strand. Once again, the important point is base pairing. These monomers are then polymerized to form the daughter strand. Because the two initial strands are themselves complementary, the result is two double-stranded molecules. In this way, DNA molecules replicate without loss of information. It is thus useful to think of parental strands as templates for the synthesis of daughters.

The process described above is called *semiconservative replication* because each of the daughter molecules contains one of the two strands of the parent DNA molecule. Proof that DNA replication is semiconservative was soon provided by Matthew Meselson and Franklin W. Stahl in an experiment with *Escherichia coli* (Figure 15.5). Bacteria were grown for many

Figure 15.5. Diagram of the Meselson-Stahl experiment, a demonstration of complementary strand separation during DNA replication by the use of the cesium chloride (CsCl) density gradient technique. Printed by permission from J. D. Watson, Molecular Biology of the Gene, © 1965, J. D. Watson

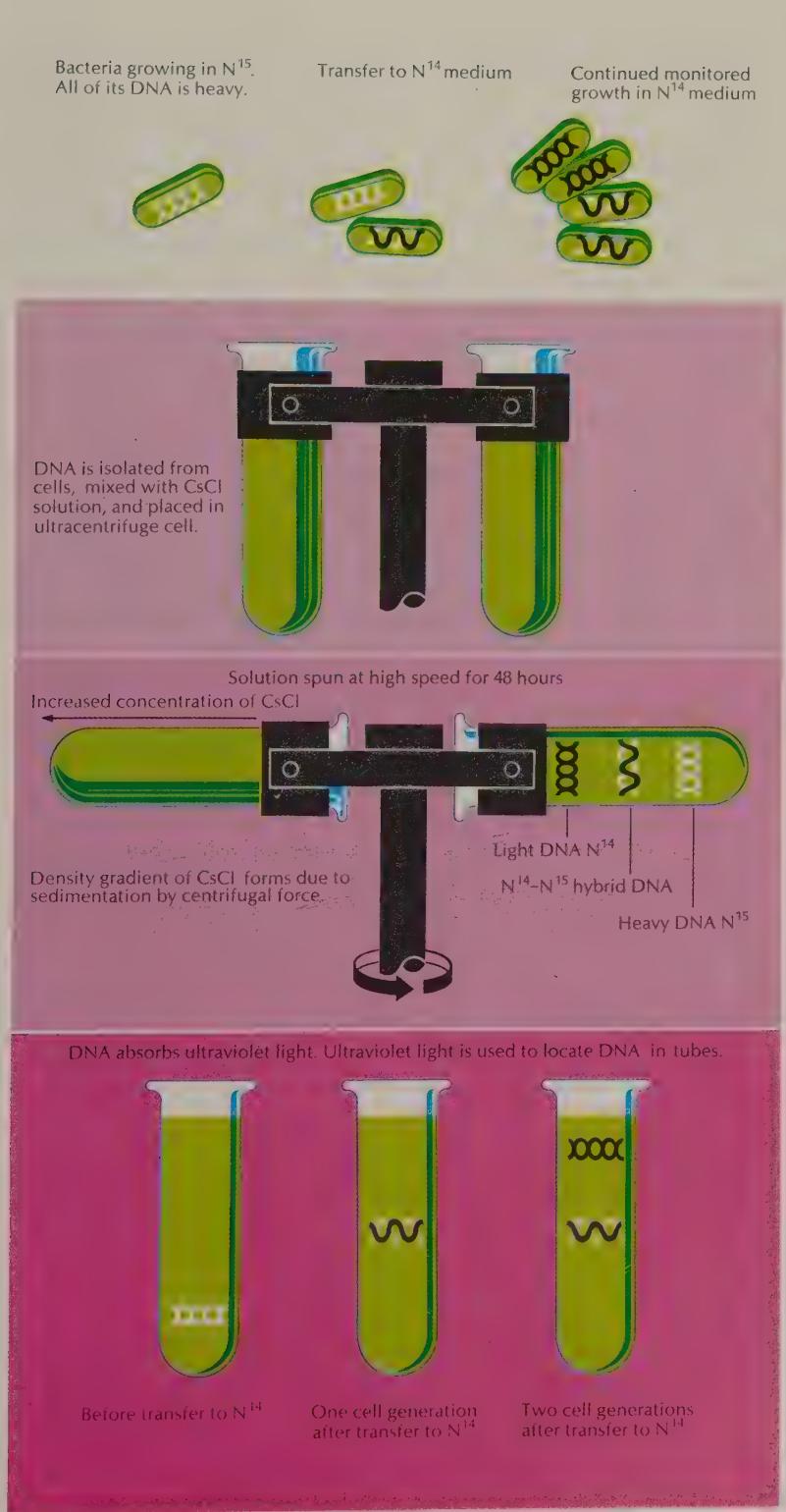


Figure 15.6. Autoradiograph of the replicating *E. coli* K12 Hfr chromosome. The DNA of the chromosome was labeled with H^3 -thymidine for two generations of DNA replication and extracted with lysozyme. The same structure is diagrammed at the upper right. It is divided into three sections (A, B, and C) that arise at the two forks (X and Y). X shows the growing point where replication is occurring, and Y the finishing point. The chromosome is about two-thirds replicated.



generations on a nutrient medium containing the heavy, nonradioactive isotope nitrogen-15. During the DNA synthesis that precedes binary fission, nucleotides containing N^{15} were incorporated into the bacterial DNA. After several cycles of cell division, most of the normal N^{14} in the DNA had been replaced by the heavier N^{15} , making the molecules about 1 percent heavier and denser than normal.

Very small differences in the density of macromolecules can be detected by a technique called density-gradient centrifugation. With this process, Meselson and Stahl (1958) separated DNA containing N^{15} in one or both chains from DNA containing N^{14} in both chains. When DNA from the bacteria grown in the N^{15} medium (heavy DNA) was mixed with DNA from bacteria grown in a normal N^{14} medium (normal DNA) and the resulting mixture centrifuged, the two kinds of DNA formed distinct layers. The bacteria with heavy DNA were then transferred into a normal medium.

After one cycle of cell division, each DNA double strand, or duplex, will have replicated, forming two daughter duplexes. According to the semi-conservative replication scheme, each of the daughter duplexes should contain one of the original heavy chains and one light chain constructed from the precursors available in the normal N^{14} medium. As predicted, DNA extracted from these bacteria after a single division cycle in the normal medium formed an intermediate layer—halfway between the positions of the heavy and the light DNA duplex layers in the first test.

Cells allowed to undergo two replications in the N^{14} nutrient should produce a population of cells whose DNA would consist of an equal mixture of light ($\text{N}^{14}-\text{N}^{14}$) duplexes and intermediate ($\text{N}^{14}-\text{N}^{15}$) duplexes. After three replications of the DNA, 75 percent should be of light intensity and 25 percent of intermediate density. These predictions also were confirmed in this experiment.

The work by Meselson and Stahl showed clearly that each DNA chain remains intact during replication but that the double strand separates and each strand forms a new complementary strand as predicted by the hypothesis of semiconservative replication. Experiments of similar nature have been subsequently performed with eucaryotic cells undergoing mitosis. These experiments also confirmed the hypothesis of semiconservative replication. Other possible replication mechanisms have been proposed, but no evidence has been obtained to support these alternative schemes. It thus appears that DNA replication in all cells occurs through a semiconservative mechanism.

The general requirements for DNA synthesis are now known. A DNA chain must be present to act as a template, nucleotide precursors must be available, and DNA polymerase must be present to catalyze the formation of bonds between nucleotides as they become aligned on the template. There are important details of DNA replication, however, that are not yet clear. Autoradiographs (Figure 15.6) are among the strong evidence that synthesis of complementary strands occurs at the same time as the separation of the two strands of the parent molecule—that is, the chains do not completely separate before synthesis of the daughter strands begins.

One point not yet understood is the process that causes the two chains of the duplex to begin separating from one another so that replication can occur. Presumably, some molecule—possibly a protein—is able to rupture the hydrogen bonds that hold the two chains together. Once the separation

has been initiated at some site on the DNA molecule, the processes of DNA replication may be sufficient to cause the continuing unzipping of the two chains.

Replication is not an easy task. For example, an *E. coli* is able to undergo binary fission about 20 minutes after it has been formed as a daughter cell of a similar fission. The separation of the two strands of the molecule requires them to untwist because the strands are intertwined in the helical coil. To untwist the 360,000 turns of the *E. coli* DNA within a few minutes would require a rate of rotation that is almost incredible. There must be 3.6 million nucleotides of just the right kinds available for each of the strands to build its complementary strand. Within another few minutes, these 7.2 million precursor molecules must be moved to the proper locations, fitted into place, and joined together to form the new double strands. Self-replication of DNA must involve an astounding speed and precision of molecular movements.

Another major problem yet to be solved is the control of initiation of DNA replication. Apparently, some mechanism exists to control the onset of the S-phase when DNA is synthesized. This process is of extreme importance because the health and proper development of an organism depend heavily upon proper control of cell regulation. In cancer, for example, a defect in this regulatory process causes cells to reproduce far too rapidly.

THE ROLE OF RNA

The one gene—one polypeptide hypothesis implies that the genetic information implicit in the sequence of base pairs in the DNA molecule must somehow serve to direct the assembly of a polypeptide chain with a particular sequence of amino acids. Even before the Watson-Crick model was proposed, it had become clear that RNA plays a key role in the process of protein synthesis.

RNA is the intermediate responsible for the transfer and decoding of the genetic information. Here, too, base pairing provides the mechanism. In virtually every case, RNA molecules are synthesized on a DNA template exactly as DNA synthesis is guided by the parental template. However, the monomers are ribonucleotides and the enzymology is somewhat different. Figure 15.4 illustrates synthesis of RNA on a DNA template. It is important to note that the base-pairing rules are exactly the same and that the RNA is complementary to the DNA template. The process of DNA-directed RNA synthesis is known as *transcription*, because the information contained in the DNA sequence is copied into RNA using the same alphabet (remember that uracil pairs exactly as thymine). Again, it must be emphasized that the rules of secondary structure, namely base pairing, govern the expression of genetic information.

The synthesis of RNA requires that the hydrogen bonds joining the two DNA chains in the double helix be temporarily disrupted to allow one chain to act as a template for RNA synthesis. After the newly synthesized RNA molecule is dissociated from the DNA, the DNA duplex might re-form by regenerating the hydrogen bonds, or the process of RNA synthesis might be repeated many times. In fact, it would be possible for many RNA molecules to be synthesized at the same time if a new RNA chain began to form as each partially completed chain began to peel away. In 1969 O. L. Miller, Jr. and Barbara R. Beatty at Oak Ridge National Laboratory succeeded in

Figure 15.7 (left). High-resolution electron micrograph of nucleolar genes from an amphibian oocyte. These genes code for ribosomal RNA. Each cluster of fibrils represents the simultaneous transcription by about 100 polymerase molecules. Each fibril is an rRNA precursor molecule. The triangular shape of each cluster results from each fibril being in a different state of completion. Those near the apex of the triangle are just commencing synthesis. ($\times 25,000$)



Figure 15.8a (middle). Autoradiograph of a cell fed on H³-uridine for 5 minutes and then killed. All the RNA is clustered in the nucleus—the site of RNA synthesis.

Figure 15.8b (right). Autoradiograph of a cell fed as above, but the radioactive uridine has been replaced by normal uridine. The RNA has moved from the nucleus into the cytoplasm.



isolating DNA from the nucleoli of amphibian oocytes and preparing high-resolution electron micrographs that show exactly the predicted pattern of RNA synthesis (Figure 15.7).

RNA synthesis can occur in normal cells only in the presence of DNA. Removal of the nucleus from a cell by means of microsurgery results in the immediate cessation of all RNA synthesis in that cell. Furthermore, if a cell is given a radioactive precursor of RNA (such as H³-uridine), the first RNA molecules to become radioactive are always found in the nucleus (Prescott, 1961).

Figure 15.8a shows an autoradiograph of a cell fed on H³-uridine for five minutes and then immediately killed to halt synthesis. All of the radioactive RNA is restricted to the nucleus, showing that the nucleus is the site of RNA synthesis. Because there is a great deal of RNA in the cytoplasm of any cell, RNA must move from the nucleus to the cytoplasm. This movement can be confirmed by an extension of the experiment. A cell is fed on H³-uridine for five minutes as before; then, the radioactive uridine in the nutrient medium is replaced by nonradioactive (normal) uridine, thereby ending the incorporation of radioactivity into RNA. The cell is allowed to live for 60 additional minutes in the nonradioactive medium, synthesizing nonradioactive RNA. When the cell is killed and analyzed by autoradiography, it is evident that a considerable amount of radioactive RNA has moved from the nucleus into the cytoplasm (Figure 15.8b). These experiments were particularly significant in light of earlier observations that suggested a role of RNA in protein synthesis, because proteins are synthesized in the cytoplasm, not in the nucleus.

TRANSLATION

Two processes fundamental to genetic expression have been sketched out: replication of DNA and transcription of DNA into RNA sequences. A third process also employs the rules of base pairing to achieve *translation* of the nucleic acid code (sequence of nucleotides) into the amino acid code (sequence of amino acid residues in a protein). The information coded in DNA base sequences is transcribed into a complementary RNA molecule. This information must then be translated into the information required to produce the primary structure of proteins.

All cells have three major types of RNA molecules. The smallest are transfer RNA (tRNA) molecules, with molecular weights of 2.5 to 3.0×10^4 . The fact that tRNA molecules are homogenous in size and structure

is important for the role they play in protein synthesis. All tRNA molecules have bases that are chemically modified after transcription by specific enzymes. The reason for these modifications is unknown.

Transfer RNA performs the essential step in translating information from nucleic acid into protein; it is the “adapter” between amino acid and its coded counterpart transcribed from DNA. It reads the coded information of the RNA molecule and inserts the corresponding amino acid into a growing polypeptide chain. It must be specifically recognized by an enzyme, which equips the tRNA with the proper amino acid. It must also interact properly with the ribosome, the subcellular organelle in which protein synthesis occurs. Every cell contains at least one tRNA for each amino acid. Often tRNA also regulates transcription of the message itself. Specialized tRNA does not always serve as an amino acid donor but may be responsible for beginning or terminating synthesis of a polypeptide.

Within the ribosomes are *ribosomal RNA* molecules (rRNA). Their functions are not clearly understood, but it is likely that by specifically binding various protein molecules, they are important in organizing a functional ribosome. In addition, interactions between rRNA and messenger RNA or tRNA could be important in the mechanism of protein synthesis. These rRNA and tRNA molecules are generally *stable* gene products—they are not usually degraded after synthesis. Together, these types of RNA form 95 to 98 percent of the total cellular RNA.

The remaining class of RNA molecules is composed of messenger RNA (mRNA). These molecules are transcribed from DNA sequences—“structural” genes—whose information ultimately codes a protein primary structure. Messenger RNA molecules have molecular weights from 100,000 to several million. Only a small percentage of the total cellular RNA is mRNA, but this molecule acts catalytically. A single RNA molecule may be translated many times.

All RNA molecules (except certain viral RNA) are transcribed from a DNA template using the base-pairing rules. The enzymes that catalyze transcription are *RNA polymerases*. In animal cells, there are distinct enzymes, one involved in synthesis of rRNA and another for mRNA. It is possible to isolate the enzyme and use it in a chemically defined system to transcribe isolated or synthetic DNA sequences.

The Code

The information required to specify protein primary structure is contained in the sequence of nucleotides in an mRNA molecule. There are about 20 different amino acids in proteins, but the RNA contains only 4 different types of subunits. If 1 nucleotide residue specified an amino acid, then only 4 amino acids could be coded, using the nucleic acid alphabet. If 2 bases at a time were used, then 16 amino acids could be specified. This number still is not enough, so it is most likely that at least 3 bases are required to specify 1 amino acid; then 64 different combinations of 3 bases are possible, and this number is more than enough to specify the 20 amino acids found in proteins.

It is now known that the specification of nucleic acid to protein is in fact a *triplet code*. In the linear sequence of residues in an RNA molecule, three bases at a time are required to specify one amino acid residue in a protein chain. Each set of three bases is called a *codon*; each codon specifies an amino acid. There are 64 possible codons and only 20 amino acids, so it is

clear that some amino acids may be specified by more than one codon (Table 15.1). All 64 possible codons are used in the genetic code. Two triplets (UAA and UAG) appear to act as periods in translation, giving the signal for termination of the polypeptide chain being formed.

Table 15.1
Codons* in the Genetic Code

CODON	Message	Codon	Message	Codon	Message	Codon	Message
UUU	phenylalanine	CUU	leucine	AUU	isoleucine	GUU	valine
UUC	phenylalanine	CUC	leucine	AUC	isoleucine	GUC	valine
UUA	leucine	CUA	leucine	AUA	isoleucine	GUA	valine
UUG	leucine	CUG	leucine	AUG	methionine	GUG	valine
UCU	serine	CCU	proline	ACU	threonine	GCU	alanine
UCC	serine	CCC	proline	ACC	threonine	GCC	alanine
UCA	serine	CCA	proline	ACA	threonine	GCA	alanine
UCG	serine	CCG	proline	ACG	threonine	GCG	alanine
UAU	tyrosine	CAU	histidine	AAU	asparagine	GAU	aspartic acid
UAC	tyrosine	CAC	histidine	AAC	asparagine	GAC	aspartic acid
UAA	STOP	CAA	glutamine	AAA	lysine	GAA	glutamic acid
UAG	STOP	CAG	glutamine	AAG	lysine	GAG	glutamic acid
UGU	cysteine	CGU	arginine	AGU	serine	GGU	glycine
UGC	cysteine	CGC	arginine	AGC	serine	GGC	glycine
UGA	tryptophan	CGA	arginine	AGA	arginine	GGG	glycine
UGG	tryptophan	CGG	arginine	AGG	arginine	GGG	glycine

*Codons are sequences of nucleotides in the RNA. Each is represented by a letter symbolizing its base: U = uracil, C = cytosine, A = adenine, and G = guanine. Each codon causes the addition of a particular amino acid to the protein chain, except UAA and UAG, which indicate the end of a protein chain.

An RNA molecule may be regarded as a string of codons. For each amino acid there exist tRNA molecules that are specific for that amino acid. That is, the tRNA molecules make the connection between codons and amino acids. This connection is achieved by a sequence of three residues in the tRNA primary structure that are complementary to the codon in the mRNA. This part of the tRNA molecule is called the *anticodon*. The codon and the anticodon pair through hydrogen bonds by essentially the same rules of base pairing, and it is by this mechanism that the nucleotide code is translated into the amino acid code. Again, base pairing is the essential mechanism by which genetic information is transmitted.

According to the codon meanings in Table 15.1, mRNA with the base sequence AUGUUUCUCGCGGG . . . will code for a polypeptide with the amino acid sequence methionine-phenylalanine-leucine-alanine-glycine . . . This translation is based on a nonoverlapping code. Although it was expected that the code would be nonoverlapping, it was necessary to confirm this assumption. The mRNA message above could conceivably be read in a series of overlapping triplets (Figure 15.9), yielding the code sequence AUG-UGU-GUU-UUU-UUC-UCU . . . and the amino acid sequence methionine-cysteine-valine-phenylalanine-phenylalanine-

Figure 15.9 (above). Comparison of the peptide products resulting from the same DNA base sequence being read as triplets in either an overlapping or nonoverlapping fashion. In the overlapping sequence, the reading frame is advanced one case at a time; in the nonoverlapping, three bases at a time. A different sequence of peptides results with an overlapping code than with a nonoverlapping code. The triplet code is known to be nonoverlapping.

Methionine	Phenylalanine	Leucine	Alanine	Glycine
A U G	U U U	C U C	G C G	G G G
A U G				Methionine
	U G U			Cysteine
	G U U			Valine
	U U U			Phenylalanine
	U U C			Phenylalanine

serine . . . Such a reading of the code would occur if the ribosome advanced only one nucleotide each time it added an amino acid to the polypeptide chain. The genetic code was proven to be nonoverlapping by means of rather intricate genetic studies of the bacteriophage T-4.

An important and unexpected property of the genetic code is its universality. The codons represent the same amino acids in all organisms from viruses to multicellular plants and animals. The universality of the genetic code thus implies a common ancestor for all biological systems now in existence.

BIOSYNTHESIS OF PROTEINS

The central element in the process of translation is the mRNA produced by transcription from DNA. The DNA in a chromosome is one continuous molecule (in some cases, at least a few centimeters in length) containing many genes in series. Presumably, a series of nucleotides in the DNA indicates the beginning and end of a gene. Such identification would be essential for the individualized control of the expression of single genes. In bacteria—and perhaps in eucaryotes—certain genes with related functions are transcribed together as a unit with a single control point at one end of the gene series.

Protein synthesis involves the charging of the various tRNA molecules with the proper amino acid sequence. After the mRNA attaches to the ribosomes, the charged tRNA molecules interact by base pairing with the mRNA and donate their amino acids one by one to the growing polypeptide. The tRNA molecules are matched by a series of amino acid activating enzymes that catalyze the addition of a given amino acid to the stem of the appropriate tRNA. For example, the tRNA with the GAA anticodon is recognized by one particular activating enzyme that joins leucine to that tRNA. Each kind of tRNA becomes charged with the appropriate amino acid.

In the next step of translation, tRNA molecules, with their attached amino acids, are brought together with the mRNA molecule (Figure 15.10). The

Figure 15.10 (below). ATP activation of an amino acid (AA), which is then transferred to the appropriate end of its specific tRNA. The enzyme amino-acyl synthetase catalyzes the activation of free amino acids by ATP to form an AMP-amino acid intermediate. This intermediate then reacts with a tRNA molecule to form an amino-acid “charged” tRNA molecule, releasing AMP in the process.

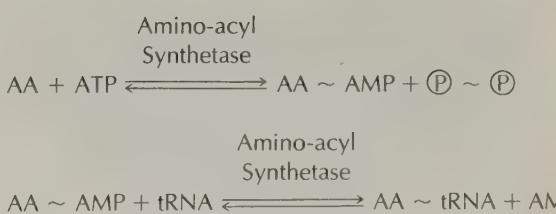


Figure 15.11a (left). Ribosomal subunits. A ribosome is made up of a large and a small subunit that each contain RNA molecules and proteins.

Figure 15.11b (right). The *E. coli* ribosome structure. This structure is generally known as the 70S ribosome ($S = \text{Svedbergs}$)—70S is a measure (sedimentation constant) of the speed of ribosomal sedimentation in a centrifuge. The sedimentation constant of the smaller and larger ribosomal subunits are designated 30S and

50S, and 16S and 23S refer to the same constants of the smaller and larger ribosomal RNA molecules. Bacterial ribosomes are closest in size to the *E. coli*—with 30S and 50S subunits—whereas in larger organisms, the ribosomes are larger (80S) with 40S and 60S subunits.
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ribosome plays an important role in this process. Each ribosome is composed of a small and a large subunit, each subunit containing an RNA molecule and a number of proteins. The function of ribosomal RNA (rRNA) and ribosomal proteins is not yet fully understood. The first codon of the mRNA binds to the small subunit of the ribosome. This codon is then matched by the corresponding anticodon of the appropriate tRNA. This tRNA molecule, with its attached amino acid, becomes bound to the large subunit of the ribosome in such a way that the codon and the anticodon are hydrogen bonded. The second codon on the mRNA now directs the binding of a second tRNA (tRNA_2) by codon-anticodon matching to a second position on the ribosome. The two amino acids on the two bound tRNAs correspond to the first two codons of mRNA.

Because the tRNA molecules are essentially the same size and have the same parallel orientation on the ribosome, the respective amino acids are in proximity to each other. An enzyme on the large subunit is able to link the two amino acids together by a peptide bond. Simultaneously with bond

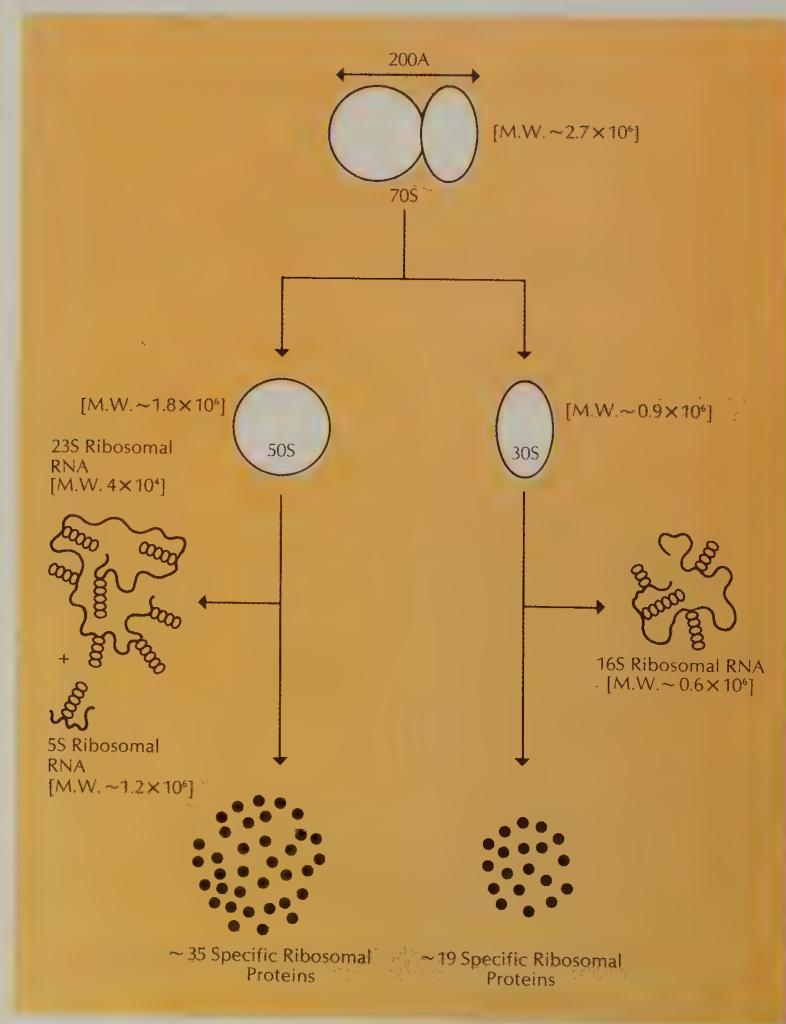


Figure 15.12a (above). Diagram of a polyribosome during protein synthesis, with an mRNA moving from the right to the left. Printed by permission from J. D. Watson, Molecular Biology of the Gene, © 1970, J. D. Watson

Figure 15.12b (below). Electron micrographs of polyribosomes. The thin strand connecting the ribosomes is the mRNA.

formation, the first amino acid is split from its tRNA, thus freeing the tRNA and leaving the dipeptide bound to tRNA₂. Next, the peptide-tRNA complex is shifted to the position originally occupied by tRNA₁. It is still hydrogen bonded to the second mRNA codon, so that, in effect, the message has moved along the ribosome a distance of one codon. This move frees the binding site on the ribosome previously occupied by tRNA₂. That position may now be occupied by a new charged tRNA₃, which corresponds to the third codon of the mRNA. This tRNA is then in a position for its corresponding amino acid to join the growing peptide chain. This process continues polymerizing amino acids until a termination signal is reached, and the completed protein is released from the ribosome and from its tRNA.

There may be a special signal to indicate the beginning as well as the end of a code message in mRNA. Studies of bacterial cells suggest that each code message begins with one of the two codons AUG or GUG. In the starting position on the message, either of these codons directs the placement of a special form of methionine tRNA. In some protein molecules, the

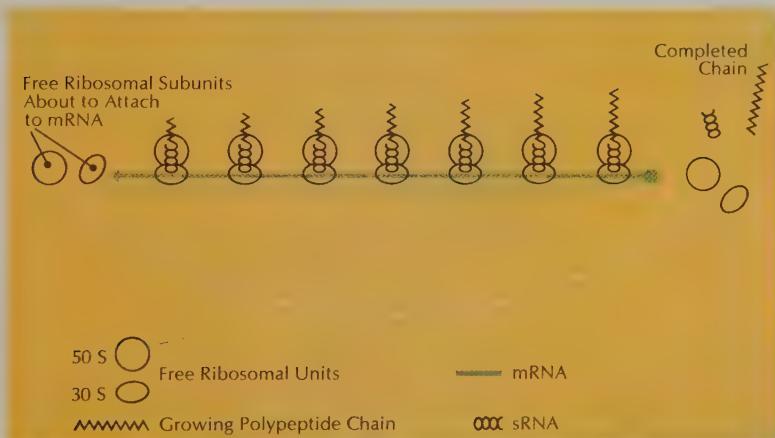
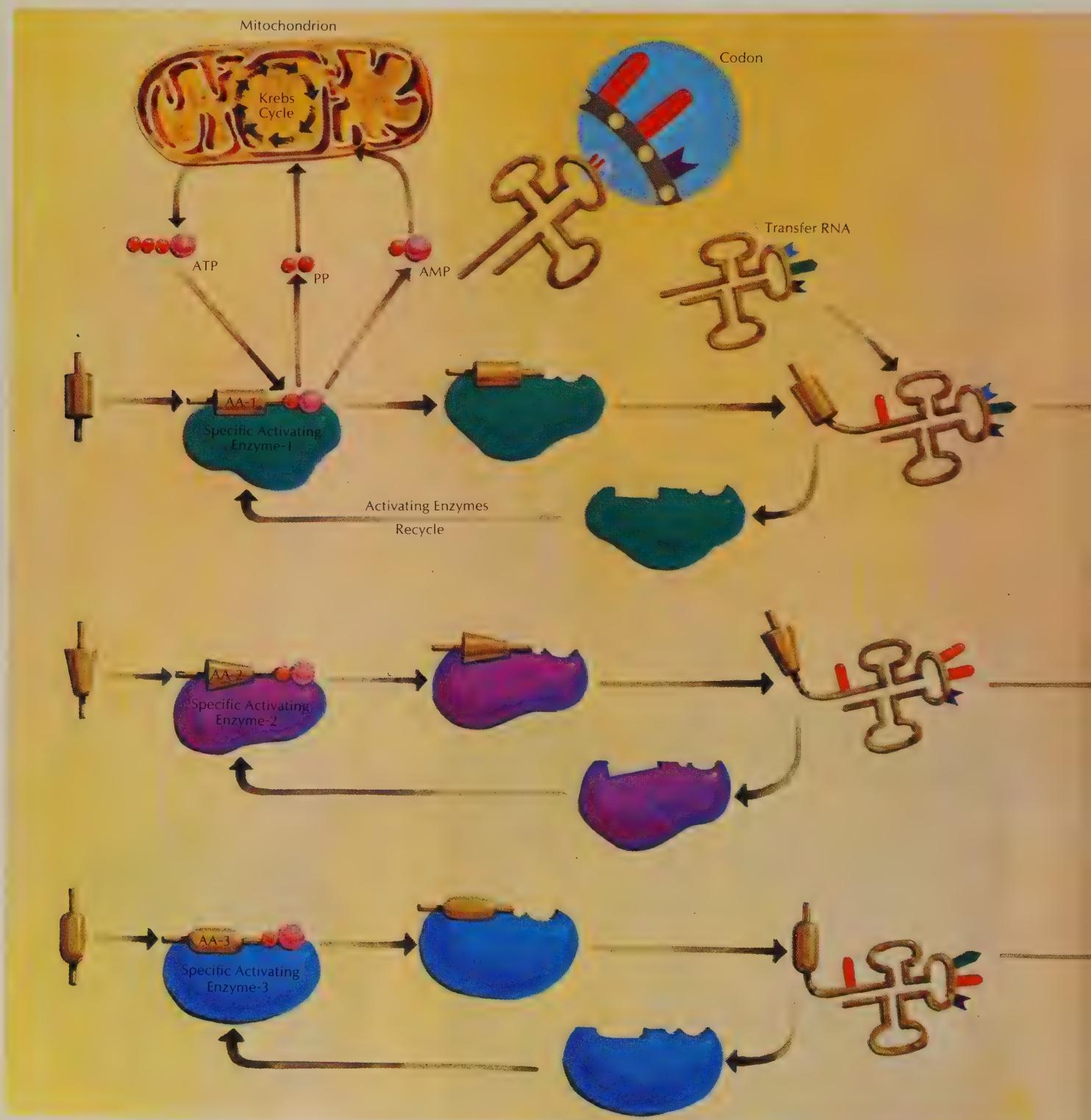
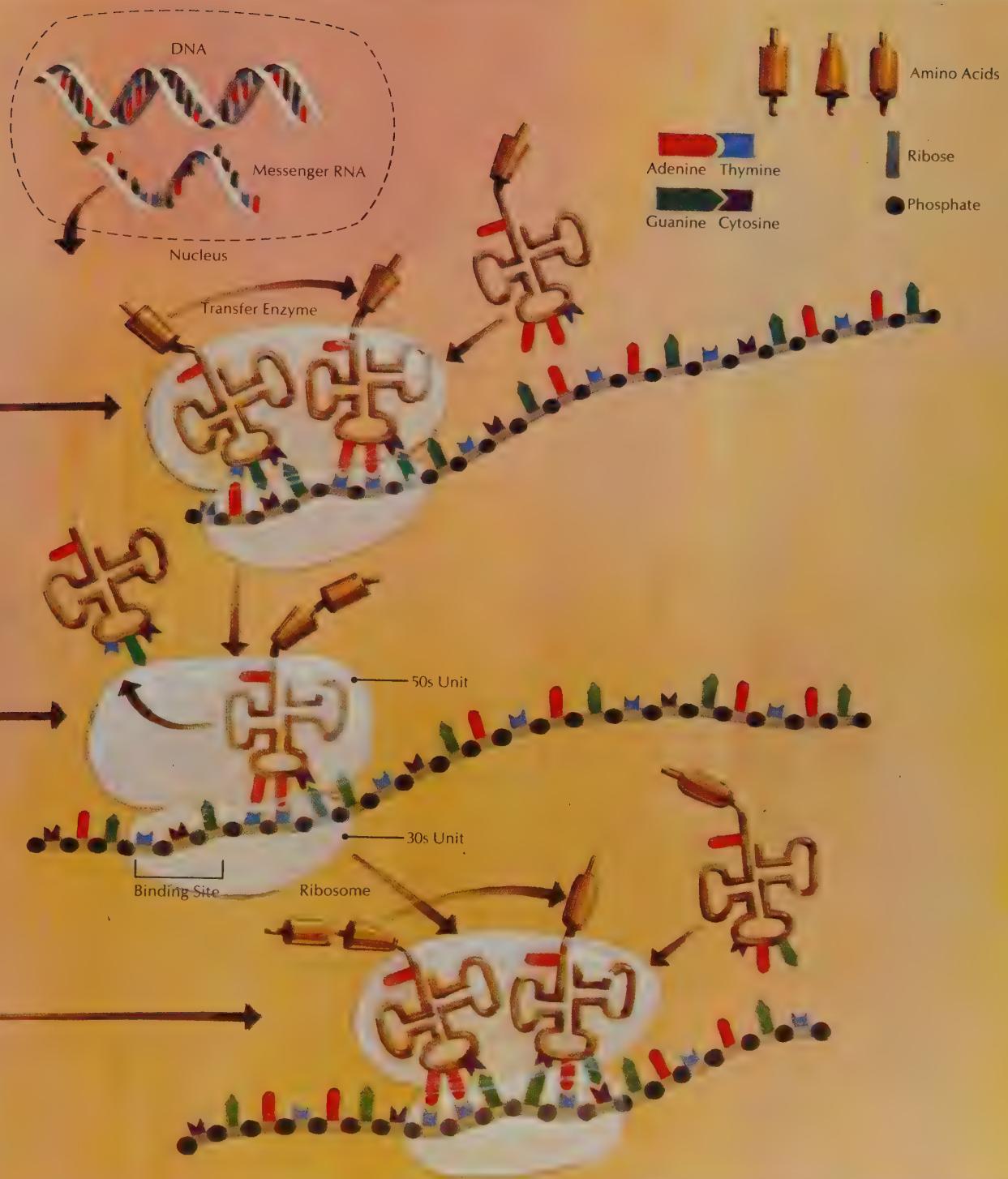


Figure 15.13. Protein synthesis. The left side of the illustration shows the activation of three different tRNAs, with specific anticodons for specific amino acids. ATP provides the energy for this reaction (the ATP's source being the Krebs cycle and oxidative phosphorylation in the mitochondrion). The right side of the illustration depicts the translation process. Each ribosome has two tRNA binding sites. Through enzyme mediation, the polypeptide chain grows by the formation of a peptide bond between, for example, AA-1 and AA-2. After the bond has been formed, the tRNA is ejected from the

AA-tRNA binding site. The movement of the mRNA over the ribosomal surface is still unknown, but the growing chain is translocated to a different site on the ribosome, allowing a different tRNA to bind and the transfer process to repeat itself. In this illustration, the ribosomal subunits attach themselves while the mRNA is moving from left to right.





methionine is later removed from the end of the molecule, presumably by a special enzyme. Recent studies suggest that a similar process involving a special methionine tRNA may also occur in eucaryotic systems. The entire process of synthesizing a polypeptide of 200 amino acids may be accomplished in a fraction of a minute. Several ribosomes may translate a single message at once. Then the ribosomes are strung along the mRNA like beads, forming a structure called a *polyribosome*, or *polysome*. Each ribosome catalyzes synthesis of a polypeptide, so that a single mRNA molecule may direct the synthesis of a number of protein molecules.

The assumption that the linear arrangement of nucleotides in a nucleic acid translates into a matching linear arrangement of amino acids in a protein has been confirmed. The enzyme tryptophan synthetase in *E. coli* is composed of two polypeptide chains that are coded for by two adjacent genes, called the A and B genes. The amino acid sequence of the polypeptide product of the A gene was determined. Several mutant strains exist in which a single amino acid change in the polypeptide chain inactivates the enzyme. Studies using a lysogenic phage made it possible to determine the location of each mutation on the gene. In each case, the position of the mutation is consistent with the position of the altered amino acid in the polypeptide chain. Mutations appear to be caused by a substitution of one nucleotide for another or by deletion of one or more nucleotides in the DNA chain. For example, position 48 in the polypeptide chain is glutamic acid in the normal enzyme, but it is valine in one mutant strain. This alteration is apparently caused by the substitution of the codon GUG for GAG in the corresponding position on the DNA chain of the A gene.

In Vitro SYSTEMS

Much knowledge about molecular mechanisms involved in DNA replication, RNA transcription, and translation has come through the use of *in vitro* systems, which simulate the processes occurring in the cell. These studies involve breaking the cells, then isolating and purifying specific genes, RNA, or special enzymes or complexes. These purified components are then reconstituted under experimental conditions, where specific manipulations of the components often lead to a precise understanding of the individual reactions and their interrelationships.

The enzyme DNA polymerase has been used to synthesize DNA from its constituent nucleotides. At first, there was some doubt as to whether this enzyme made exact copies of the template DNA; recently, however, Kornberg (1960) showed that the enzyme was able to replicate a viral DNA so precisely that it could infect its host just as well as viruses produced in the living cell could have done. Other workers have isolated and purified segments of DNA coding for single sets of genes, including genes for ribosomal RNA, β -galactosidase, and several small viruses. However, these purified genes have not been replicated *in vitro* using DNA polymerase.

RNA polymerase transcribes the DNA base sequence into complementary RNA. It is composed of five subunits plus one additional polypeptide called a sigma factor, which is rather loosely bound to the core enzyme, the five-subunit polymerase. The sigma itself is inactive in transcription, but in combination with the core enzyme it determines which sequences of DNA will be transcribed. Sigma factors are interchangeable so that one sigma can substitute for another to guide the selection of DNA for transcription. Certain viruses are able to direct the synthesis of their own RNA in place of host cell RNA simply by displacing the host's sigma factor and replacing it

with their own, which will only transcribe viral genes. Sigma factors may prove to be important in developmental processes where selective gene expression is necessary.

The proof that this polymerase faithfully transcribes DNA into active RNA is shown by the fact that this *in vitro* synthesized RNA can be translated into active enzymes. There are now several *in vitro* systems (called coupled systems) in which purified genes are simultaneously transcribed into RNA and then translated into proteins.

In vitro protein synthesis involves a combination of mRNA, ribosomes, tRNA, activating enzymes, and other enzymes, along with amino acids and an ATP energy source. Such systems have already been invaluable in elucidating the salient features of protein biosynthesis. In addition, they may soon provide answers to problems of translational controls, such as are known to exist in oocytes, seeds, spores, and slime molds (Chapter 17).

GENE TRANSFER

The work of Griffith and Avery proved that DNA could be transferred from one cell to another, producing a stable genetic change in the recipient. Bacteria are now known to have other methods of gene transfer.

In a process called *bacterial conjugation*, certain strains of *E. coli* are able to directly exchange genetic material by forming a type of cytoplasmic bridge between two cells. In any mating pair, only one cell will act as the DNA donor. The donor, however, is not depleted of its genetic material because conjugation will occur with simultaneous DNA replication, with one double strand going to the recipient and the other remaining with the donor. The donor DNA is fully functional in the recipient cell and will be transmitted to that cell's descendants. In this way, bacteria with new combinations of genes can be produced without the slow process of mutation. This process has been appropriately termed a *sexual mating system*; the donor is called the male and the recipient, the female.

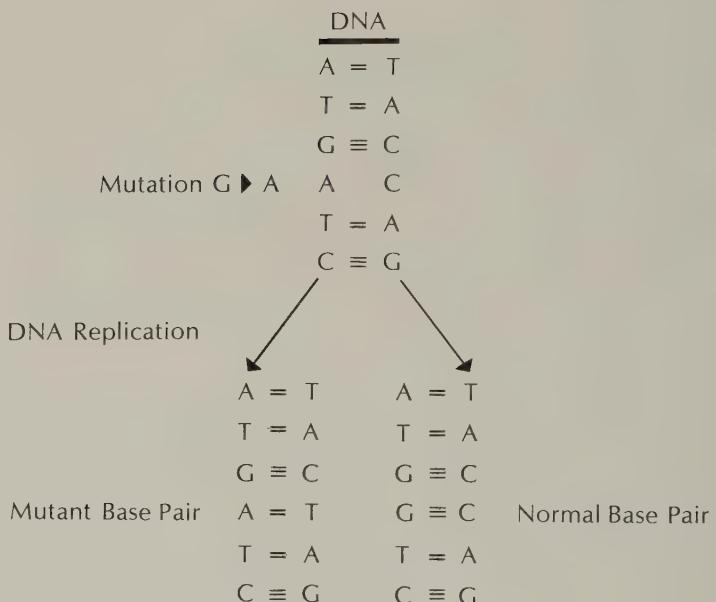
The transfer of DNA begins at a particular region on the bacterial chromosome. Thus, the DNA that codes for certain genes enters the recipient before others do. Because it takes about two hours for complete chromosomal transfer, the amount of DNA injected into the recipient cell will be proportional to the time the two cells are in intimate contact. Obviously, genes that are adjacent or close to each other will be transferred together. The order of the genes can then be determined by gently splitting the pairs apart at different times after the onset of mating and then measuring which new genes are present in the recipient cell. This process is called *genetic mapping*. Maps are expressed in terms of minutes after beginning of mating.

Also useful in mapping relative gene positions is phage transduction, in which a few bacterial genes can be incorporated into a replicating virus. After lysing the cell, the virus may then infect another cell, but because it contains a small piece of bacterial DNA in place of some of the viral genes, the virus is unable to kill the cell. Instead, the bacterial DNA transduced into that cell with the phage becomes active. Genes that are transduced together in a single phage are close to each other. By studying a series of various overlapping transductions, biologists can determine the relative order of the genes on the chromosome.

MUTATIONS

Mutations are heritable changes in the genes other than the direct transfer of genetic material between organisms. In general, they represent deletion

Figure 15.14. Illustration of a mutation arising in DNA replication. Substitution of an A for a G results in the production of a normal DNA molecule and a mutant one after replication. Subsequent replications of the mutant DNA will preserve the mutation at that point.



or modification of one or more of the bases in the DNA. In nature, mutation is a rare event, but the rate of occurrence can be increased by certain mutagenic agents, such as x-rays, ultraviolet light, and a variety of substances that chemically react or interact with DNA.

By definition, a mutation is any change in the base sequence of DNA. However, mutations are usually observable only when they affect the phenotype. Many mutations go unnoticed because the change of a single base may not cause a change in the amino acid specified by the code at that position. For instance, a change from GAC → GAU still results in the insertion of aspartic acid into the protein. Occasionally, *suppressor mutations* occur within a gene; they are able to conceal another mutation in the same gene by compensating for the effect of the first mutation.

Point mutations result from the replacement, insertion, or loss of a single nucleotide. Replacement may occur by the substitution of one purine for another or one pyrimidine for another (called a *transition*). The exchange of a purine for a pyrimidine or vice versa is a *transversion*. Some chemicals cause marked changes in the hydrogen bonding properties of the bases so that the proper base pairing no longer occurs. Thus, base-pairing errors are introduced during DNA replication or during RNA transcription. If a transition from G → A occurs on one strand of DNA, then at replication one daughter duplex will have a proper GC base pair (because the complementary C of the unmutated strand will pair with a G), but the mutation will produce an AT pair at the same place.

The insertion or loss of a nucleotide can lead to a *frame shift mutation*, in which the codons are still read three at a time but not in the proper phase. For instance, a DNA base sequence CCAACGGCCGGA would translate into a peptide with the sequence proline, threonine, alanine, and glycine. But if an additional G were inserted at the beginning of the third

Figure 15.15. A frameshift mutation, in which the codons are read out of phase. Addition or deletion of a base still results in the reading of codons as triplets, but now different triplets are read. This alteration causes the insertion of the wrong amino acids in the polypeptide or may even result in the premature termination of the protein. Printed by permission from J. D. Watson, Molecular Biology of the Gene, © 1970, J. D. Watson

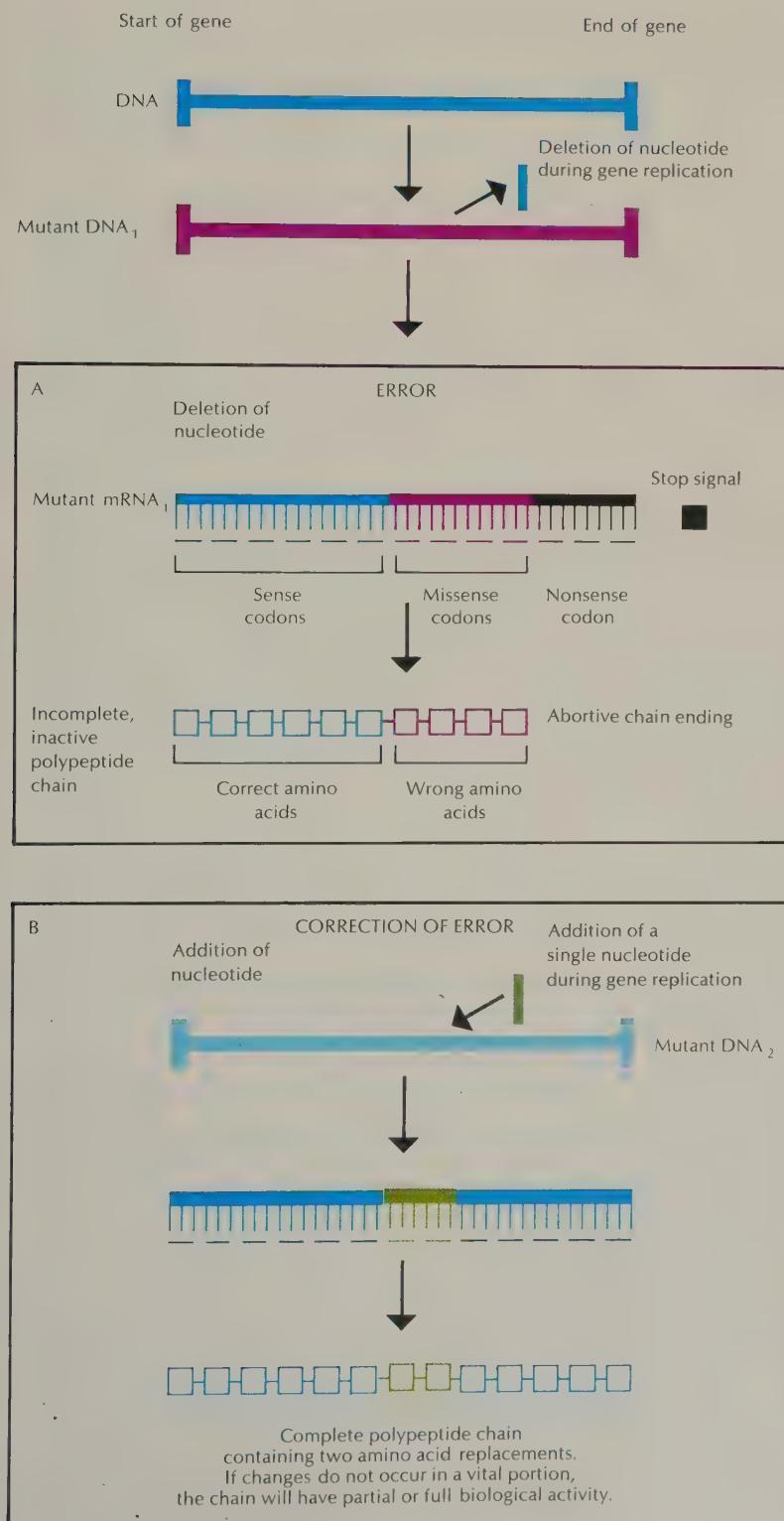


Figure 15.16 (above). Intergenic complementation. In a normal cell, both active gene products 1 and 2 are necessary to produce an active enzyme. A mutation (X) in either gene, which renders either 1 or 2 inactive, leads to the production of an inactive enzyme. If extracts of cells deficient in gene product 1 are mixed with extracts from cells deficient in gene product 2 (A), an active enzyme is produced because the corresponding active gene products may complement each other (B).

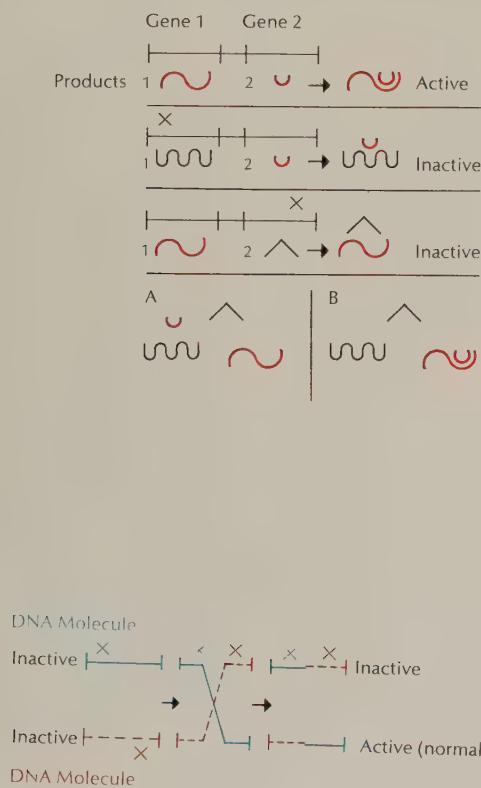


Figure 15.17 (below). Intragenic complementation occurs when two DNA molecules in the same cell, each bearing a mutation (X) within the same single gene, are able to give rise to an active product. This product occurs when the two DNA molecules break and reunite in a new combination, making one gene a double mutant (XX) and the other gene normal.

codon, then the third codon sequence would be GGC and would be read as if it were the intended codon. The result would be a peptide sequence of proline, threonine, glycine, and arginine. Often the change of a single amino acid can lead to the production of a totally inactive gene product. The deletion of a base leads to the same kind of reading frame shift. Mutations often arise by the deletion of many bases, and entire genes may be lost.

Mutations often give rise to proteins that are inactive in a catalytic sense. They are still produced by the cell but are nonfunctional. In diploid organisms, recessive alleles can often be thought of as inactive or partially active gene products.

If an enzyme is composed of two different polypeptide chains, which are coded for by separate genes, mutations in one gene do not affect the other gene. Mutations in either gene 1 or gene 2 result in an inactive enzyme. But if an extract is prepared from cells containing a mutation in gene 1 but normal in gene 2, and another extract having a mutation in gene 2 but normal in gene 1 is prepared, and then the two are mixed, enzymatic activity results. The active product of gene 1 is able to complement the active product of gene 2 to give a fully active product. This *intergenic complementation* occurs at the level of the interaction of the gene products. *Intragenic complementation* also occurs, but it involves interaction of the DNA itself within the living cell. If a cell contains two separate copies of the same gene, each one possessing a mutation somewhere within, sometimes progeny that possess a normal gene arise. The two mutations have complemented each other in a way similar to crossing over in meiosis. The homologous regions of each DNA molecule pair precisely, then there is a breakage and reunion of the strands in the new combination. Very fine genetic mapping reveals that the breakage and reunion, or recombination, can occur even within a single codon.

THE FUTURE

For a decade, it was believed that DNA→RNA→protein represented the exclusive direction of flow of genetic information. In 1970 Howard Temin succeeded in demonstrating that cancer-causing RNA viruses contain an enzyme that reverses the flow of genetic information; it catalyzes DNA synthesis from an RNA template. There is evidence that the same enzyme activity is also found in normal cells, and its occurrence raises new questions concerning gene expression.

The transforming viruses may someday be used to introduce healthy genes into cells that lack a required enzyme; such genes might be isolated from healthy cells or synthesized chemically. It would then be possible to actually change an organism's genetic message.

Genetic information is contained in isolated DNA molecules, and this information is inherent in the sequence of bases. These facts imply that genetic information can be synthesized by chemical means. In fact, the chemical synthesis of a gene has already been achieved. Starting with only small molecules and employing straightforward, well-established, organic reaction schemes, scientists have synthesized a gene coding a yeast tRNA.

The implications of genetic knowledge for the future are obvious. Many diseases of metabolism will be cured only when it is possible to change the genetic message of an organism. It must be concluded that, for good or evil, such advances will not be long in coming. It is possible to create genetic information, as polydeoxynucleotide sequences, in the test tube, and it is

possible to transform the genotype in certain organisms. To some people, transformation of human genotypes is an attractive proposition, for much human misery could be obviated. To others, it is a disconcerting proposition. Where will it lead? Surely the utmost foresight is required to consider the applications of such knowledge.

FURTHER READING

As an example of the rapid progress in molecular genetics and of the skepticism with which one should regard firm statements of "fact" in textbooks, obtain a copy of a genetics text published in the early 1940s and read the sections dealing with the structure and chemistry of genes and chromosomes.

Probably the best current survey of knowledge about the molecular biology of the gene is that given by Watson (1970). More detailed discussion of experiments and principles will be found in books by Drake (1969), Haggis, et al. (1964), Hayes (1968), Herskowitz (1967), Ingram (1963, 1966), and Jacob and Wollman (1961).

For further information about the fine structure of the gene, see articles by Benzer (1962) and Yanofsky (1967).

The many articles published by *Scientific American* over the past few decades provide an accessible and readable review of changing views about nucleic acids and protein synthesis. See particularly the articles by Clark and Marcker (1968), Crick (1954, 1957), Davidson (1965), Deering (1962), Delbrück and Delbrück (1948), Dobzhansky (1950), Doty (1957), Edgar and Epstein (1965), Fraenkel-Conrat (1956), Fruton (1950), Hanawalt and Haynes (1967), Hoagland (1959), Holley (1966), Horowitz (1956), Hurwitz and Furth (1962), Ingram (1958), Isaacs (1963), Jacob and Wollman (1961), Kellenberger (1966), Kendrew (1961), Knight and Fraser (1955), Kornberg (1968), Linderstrom-Lang (1953), Mirsky (1953, 1968), Pauling, Corey, and Hayward (1954), Rich (1963), Sonneborn (1950), and Spiegelman (1964).

16

Reproduction



An adult organism may be logically regarded as a mechanism designed to keep itself functioning until it has contributed to the next generation of its kind. It then deteriorates and dies, removing itself from competition with its offspring for food and space. As the nineteenth-century novelist Samuel Butler put it, "A hen is only an egg's way of making another egg."

Self-reproduction is a basic property of living systems. Because natural selection favors those species that are most successful at reproduction, all other functions of the organism may be regarded as means of enhancing the reproductive function. Reproduction forms new organisms, which embark upon their own careers of growth and development, culminating in their own reproduction. The intertwined processes of reproduction and development may well be considered as the central phenomena of life at the organism level.

TYPES OF ASEXUAL REPRODUCTION

For unicellular organisms, reproduction involves duplication of cellular structures and division of the cell into a pair of daughter organisms. The parent organism does not die but contributes all of its materials to its offspring. There is reason to believe that cell division rejuvenates the unicellular organism, which otherwise would age and die. Even if a cell were immortal, it would still be susceptible to accidental death. The continued survival of a species therefore depends upon its ability to produce new individuals at least as rapidly as old ones die.

For most prokaryotic organisms, reproduction is accomplished through the relatively simple process of *binary fission*. During a brief period of growth, all cellular components—including the single, circular chromosome—are duplicated. The cell then splits into two cells, each receiving exactly half of the chromosomal materials and about half of the other materials from the parent cell. The newly formed cells then begin to grow in preparation for the next division. Under suitable conditions, some species of bacteria repeat this fission at 20-minute intervals.

In some bacterial species, daughter cells do not separate fully after division but cling together to form pairs, clusters, or filaments. Many species of blue-green algae form similar clusters or filaments in which the daughter cells are held together after division by a sheath of gelatinous material. In some species, a rapid series of divisions occurs within a single cell, producing a large number of daughter cells, or *endospores*, within the single parental cell wall. The endospores eventually are released from the parental cell, from their own cell walls, and become unicellular adults.

In all these cases, the offspring cell receives a single copy of the parental chromosome. The single chromosome is duplicated again just before each division. Variations in genetic information can arise only through mutations—through spontaneous changes in the nucleotide sequence or through errors in the process of chromosome replication. Variations from this simple pattern are rare among prokaryotic organisms. The *actinomycetes*, or funguslike bacteria, form long, threadlike, tubular bodies that probably contain several chromosomes. Presumably, these chromosomes are identical copies of the same chromosome, resulting from successive divisions without the formation of cell walls. In certain bacteria, sexual recombination—the production of a daughter cell with genetic material contributed by more than one parent cell—does occur, although rarely. In a

simple form of sexual mating called *conjugation*, one bacterial cell inserts all or part of its chromosome into another cell. The recipient cell later divides to produce daughter cells with single chromosomes created by a recombination of fragments of the parental chromosomes. In another process of genetic transfer called *transduction*, chromosomal fragments are carried from one bacterial cell to another as parts of viral chromosomes. A similar process called *transformation* involves the passage of chromosomal fragments from one bacterium to another, either directly or through the culture medium. These processes of sexual recombination probably are rare in natural populations of bacteria, but they are of importance to geneticists studying the nature of bacterial chromosomes and genes.

Although mechanisms for distributing chromosomes and other parts of the prokaryotic cell between the daughter cells probably exist, these mechanisms remain unknown. In eukaryotic unicells, chromosomes are distributed during the process of mitosis, but little is known about the distribution of the other parts of the cell. For more complex unicellular organisms, a great number of organelles either must be duplicated before division and then distributed properly, or they must be produced after division according to instructions carried by the chromosomes.

For many kinds of eukaryotic unicells, reproduction is a matter of mitotic cell division. In many species, however, meiotic division of the diploid adult cell produces haploid gamete cells. A pair of these gametes (often derived from different parental cells) then fuse to form a new diploid individual cell. In many cases, two kinds of gametes are produced by different parental cells: a flagellated gamete that contains little cytoplasmic material and a nonmotile gamete that contains a large amount of cytoplasm. Fusion occurs only when a flagellated gamete happens to run into a nonmotile gamete. There are several species of algae in which daughter cells remain attached to one another after reproduction.

MULTICELLULAR ORGANISMS: ASEXUAL REPRODUCTION

Few multicellular organisms reproduce by a process equivalent to binary fission or cell division. Most multicellular organisms produce single cells or small multicellular fragments that develop into new individuals. The parent, after completing a period of such reproduction, in most cases enters senescence and eventually dies. Asexual reproduction may involve either the formation of multicellular buds or fragments or the production of unicellular spores. Sexual reproduction usually is accomplished by production of unicellular gametes.

Volvox is a green alga that might be regarded as a multicellular plant or as a colony of unicellular organisms (Figure 16.2). Each of the hundreds or thousands of cells in the spherical colony has two flagella. The flagella of the entire sphere beat in a coordinated pattern, moving the sphere through the water with a rolling motion. One particular side of the sphere always is in front during this motion and thus can be called the anterior end of the colony. Asexual reproduction occurs when one of the cells begins to divide, eventually forming a new sphere of cells within the hollow center of the parent colony. Only cells in the posterior half of the colony produce daughter colonies. The daughter colonies remain inside the parent colony until it dies or is broken apart.

In the process of *budding*, one of the cells of the parent organism begins to grow and divide, much as if it were a newly formed zygote. For a time,

Figure 16.1 (upper left). A paramecium dividing.
(Courtesy Carolina Biological Supply Company)

Figure 16.2 (upper right). Volvox, a colonial protistan.
Note the daughter colonies within the hollow center of
the parent colony.

Figure 16.3a (lower left). The fresh-water coelenterate
Hydra shown here is reproducing by means of budding.
Several buds can be seen attached to the adult's stalk.

Each bud will form a miniature adult, like the one to
the right, and each will eventually pinch off to assume
an independent existence.

Figure 16.3b (lower right). Yeast budding.

this bud continues to grow into a new organism while remaining attached to the parent and drawing nourishment from it. At some stage in its growth, it may become separated from the parent and take up an independent life. In many cases, however, the offspring remain attached to the parent, forming a colony of potential individuals. Each bud is capable of survival if it should be cut off from the colony, but most retain bodily connections and an interchange of nutrients and other materials with the others.

The simple coelenterate *Hydra* is an example of an animal that reproduces by budding. Each bud forms a miniature adult while still attached to the parent organism, then drops off to become independent (Figure 16.3). *Hydra* is of particular interest because the cells of each individual are being continuously and rapidly replaced. Cell division occurs in a zone near the center of the body, and newly formed cells continuously move outward as old cells die and drop off at the base and at the tips of the tentacles. Every few weeks the entire individual is completely replaced. Thus, the individual *Hydra* might be regarded as being in a continuous process of asexual reproduction, which apparently eliminates the process of aging and natural death for the total organism. Other coelenterates, sponges, and flatworms use budding as a means of asexual reproduction.

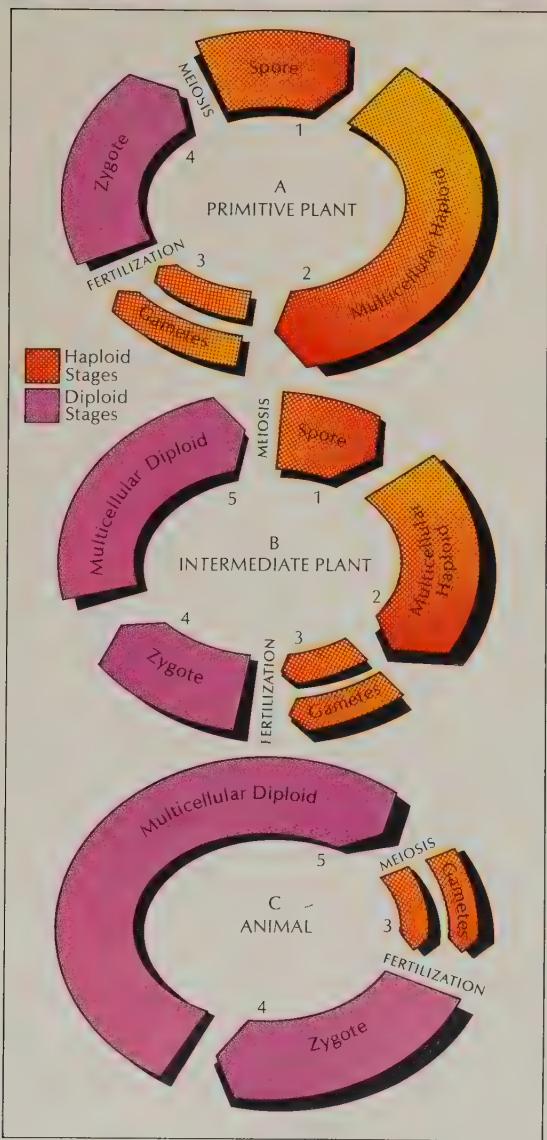
In some kinds of algae and liverworts, older parts of the plant body may die, leaving the tips to develop into new individuals. Among many kinds of plants and fungi as well as some animals, fragments of the body can



Figure 16.4 (above). Planarian spontaneously dividing.
(Courtesy Carolina Biological Supply Company)

Figure 16.5 (lower left). Three types of life cycles.

Figure 16.6 (lower right). The alternation of haploid and diploid generations.



develop into new individuals if some exterior force breaks up the body. Flatworms such as *Planaria* are among the few examples of multicellular organisms that spontaneously divide themselves into more or less equal fragments, each of which develops into a complete new individual.

Asexual reproduction through unicellular spores is found among protists, fungi, and plants, but not among animals. The spores of some species are flagellated; others are immobile and in most cases are transported by wind or water. Thus, the process of reproduction usually involves more-or-less widespread dispersion of the new individuals.

In many species of algae, large numbers of spores are formed by mitotic or meiotic divisions of one or more of the body cells of the parent organism. Each of the *mitospores* formed by mitotic division can develop into an individual similar to the parent plant. A *meiospore* formed by meiotic division will develop into a haploid organism that may be different from the diploid parent in appearance. In such species, the haploid individuals produce gametes, whose sexual fusion creates diploid offspring. Thus, haploid and diploid generations alternate (Figure 16.6). Similar life cycles are found among fungi, ferns, and mosses. In the flowering plants, meiospores are formed within the sexual organs of the adult plant, but they develop within

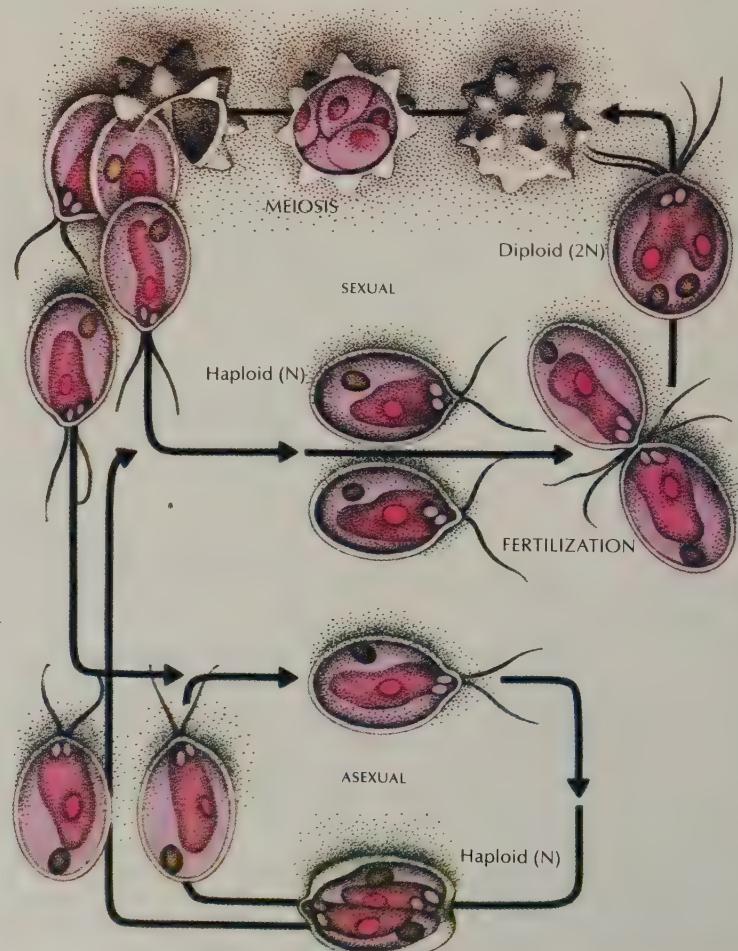


Figure 16.7. An example of parthenogenesis, the development of an unfertilized egg into an adult organism and thus a form of asexual reproduction. In the honeybees shown here, the queen produces eggs that if fertilized become female workers and if not fertilized become male drones.

those organs into small haploid individuals. Until they produce gametes, they remain parasitic upon (and hidden within) the diploid parent.

Another form of asexual reproduction is *parthenogenesis*, the development of an unfertilized egg into an adult organism. It occurs spontaneously as an important means of reproduction in some kinds of organisms. Among honeybees, development of an unfertilized, haploid egg gives rise to a drone, a male adult with haploid cells. Fertilized honeybee eggs form diploid, female adults—either queens or workers, depending upon the nutrients supplied during development.

In some cases of parthenogenesis, a diploid egg is formed either through fusion of the egg cell and one of the polar bodies or through a replication of the egg chromosomes without cell division. In other species where parthenogenesis is common (among the aphids, for example), eggs are formed without meiotic division, thus producing diploid eggs that can develop into diploid individuals with the same genetic information as the parents.

MULTICELLULAR ORGANISMS: SEXUAL REPRODUCTION

Almost every kind of multicellular organism is capable of sexual reproduction, although this process may be supplemented by various forms of asex-

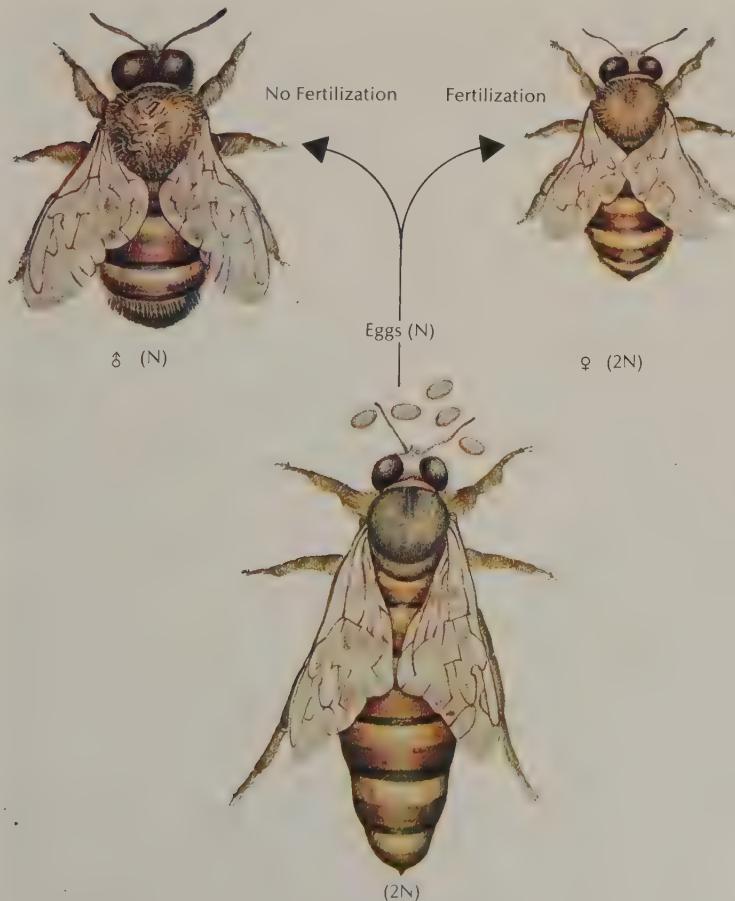
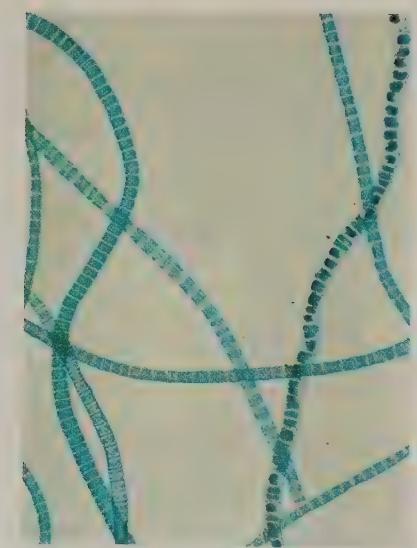


Figure 16.8a. The alga *Ulothrix* is an organism in which all gametes appear identical.



ual reproduction. Sexual reproduction provides a source of genetic variation in the population. Variations arise in any population through mutations—relatively infrequent, spontaneous changes in the genetic message. For example, suppose that two independent mutations, *A* and *B*, each appear on the average in about 1 of each 1,000 individuals. In a population that reproduces only asexually, about 0.1 percent of the population will show the mutant character *A* and another 0.1 percent will show the mutant character *B*. However, only about 1 individual in each 1,000,000 will happen to undergo both *A* and *B* mutations simultaneously. The only other way that individuals with both *A* and *B* mutations can arise is to wait until one of the descendants of an individual with one mutation happens to undergo the other mutation as well.

On the other hand, in a sexually reproducing population, there is a possibility that an individual with the *A* mutation will mate with an individual with the *B* mutation. Thus, the *A-B* combination can be produced within a single generation by sexual recombination. Similar sexual recombinations can be made of all the different characters that happen to arise by mutation, so that a population that reproduces sexually is likely to possess a much wider variety of genotypes than one that reproduces only asexually. The sexually reproducing population is much more likely to contain individuals suited for survival in changed environmental conditions. Sexually reproducing populations may therefore be expected to survive environmental changes more successfully and to undergo more rapid evolutionary changes. It is not surprising to find that most modern multicellular organisms utilize sexual reproduction. The ability of many unicellular organisms to survive with only asexual reproduction is probably due to their very rapid rate of reproduction, which leads to large populations and frequent chromosome replication, so that favorable mutations are likely to appear in a population within a relatively short time.

In theory, there is no compelling reason why sexual recombination would have to be linked with reproduction. Although the process would be complex for any organism with large numbers of cells, it is possible to imagine an interchange of chromosomes between the cells of two individuals, followed at some later time by asexual reproduction of each individual. In all known cases, however, sexual recombination leads to the formation of a single cell, a zygote, which then develops through repeated divisions into a new adult. The result of this universal process of sexual recombination combined with reproduction is that every cell of a multicellular organism contains the same genetic information. This combination of reproduction and sexual recombination has been favored in the evolution of all multicellular organisms presumably because it ensures that all of the cells of the organism will have the same genetic instructions and therefore will function together smoothly. This combination also underlies the process of natural selection, for it ensures that the reproductive cells of an organism will contain the same genetic information as the body cells that determine the phenotype.

In its simplest form, sexual reproduction involves the formation of haploid gametes that fuse to form a diploid zygote. In some algae, such as *Ulothrix*, all gametes appear identical (Figure 16.8). A gamete fuses with any other gamete that it happens to encounter, although in many species fusion will occur only if the gametes are from different parent individuals. In the case of *Ulothrix*, the adult plant is haploid, and the diploid zygote

Figure 16.8b. Each filament of *Ulothrix* is able to reproduce by sexual and asexual means. The adult plant is haploid and the diploid zygote divides meiotically early in development. Only one of the resulting haploid cells survives to give rise to a new individual.

undergoes meiosis early in its development. Only one of the haploid cells resulting from meiosis survives to give rise to the new individual, thus again ensuring that all cells of the adult plant will have the same genetic information. The process of reproduction through identical gametes is called *isogamy*.

Among most multicellular organisms, sexual reproduction involves two kinds of gametes. One kind tends to be small and highly motile; the other kind is less motile and carries a large supply of nutrients. In this case of *heterogamy*, or *anisogamy*, a particular adult usually produces only one kind of gamete. In the extreme development of heterogamy, one kind of gamete is the sperm—small, active, and equipped with powerful flagella—and the other kind is the egg, or ovum—large, immotile, and packed with nutrients. This extreme form of heterogamy is called *oogamy*; the individuals that produce sperm are males and those that produce eggs are females.

Although the pattern of oogamy is by far the most common form of sexual reproduction among multicellular organisms, it is not universal. In some cases of heterogamy, the two kinds of gametes are very similar in appearance, and the designation of one sex as female and the other as male may be arbitrary. In the green alga *Ulva* (the “sea lettuce”), for example,

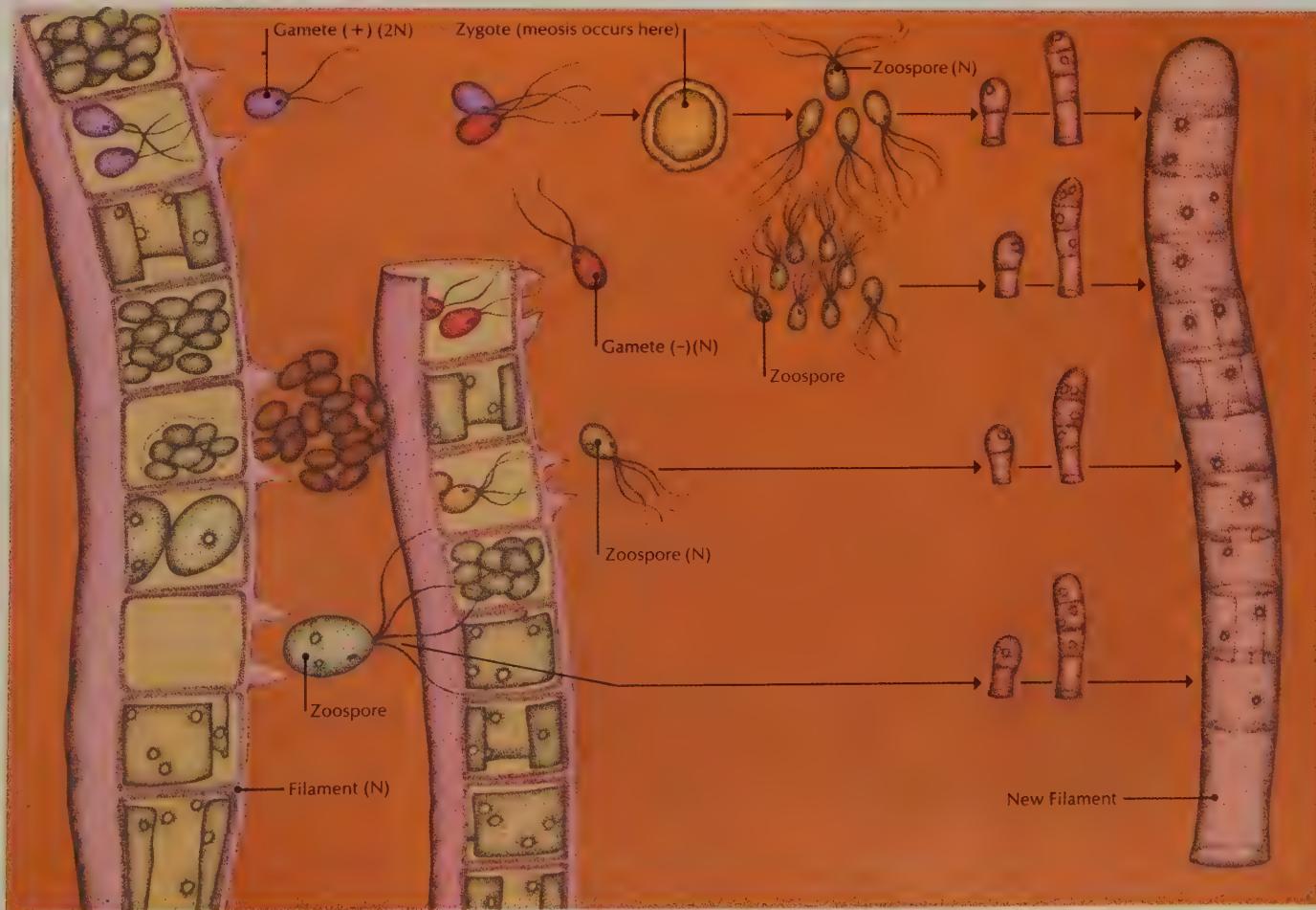


Figure 16.9a (above). The black bread mold *Rhizopus*.
(Courtesy Carolina Biological Supply Company)

Figure 16.9b (below). Formation of a zygote by two differing strains of *Rhizopus*. This haploid organism normally reproduces asexually through the production of haploid spores. Under certain conditions, however, different individuals will develop filaments whose nuclei fuse and form a zygote.

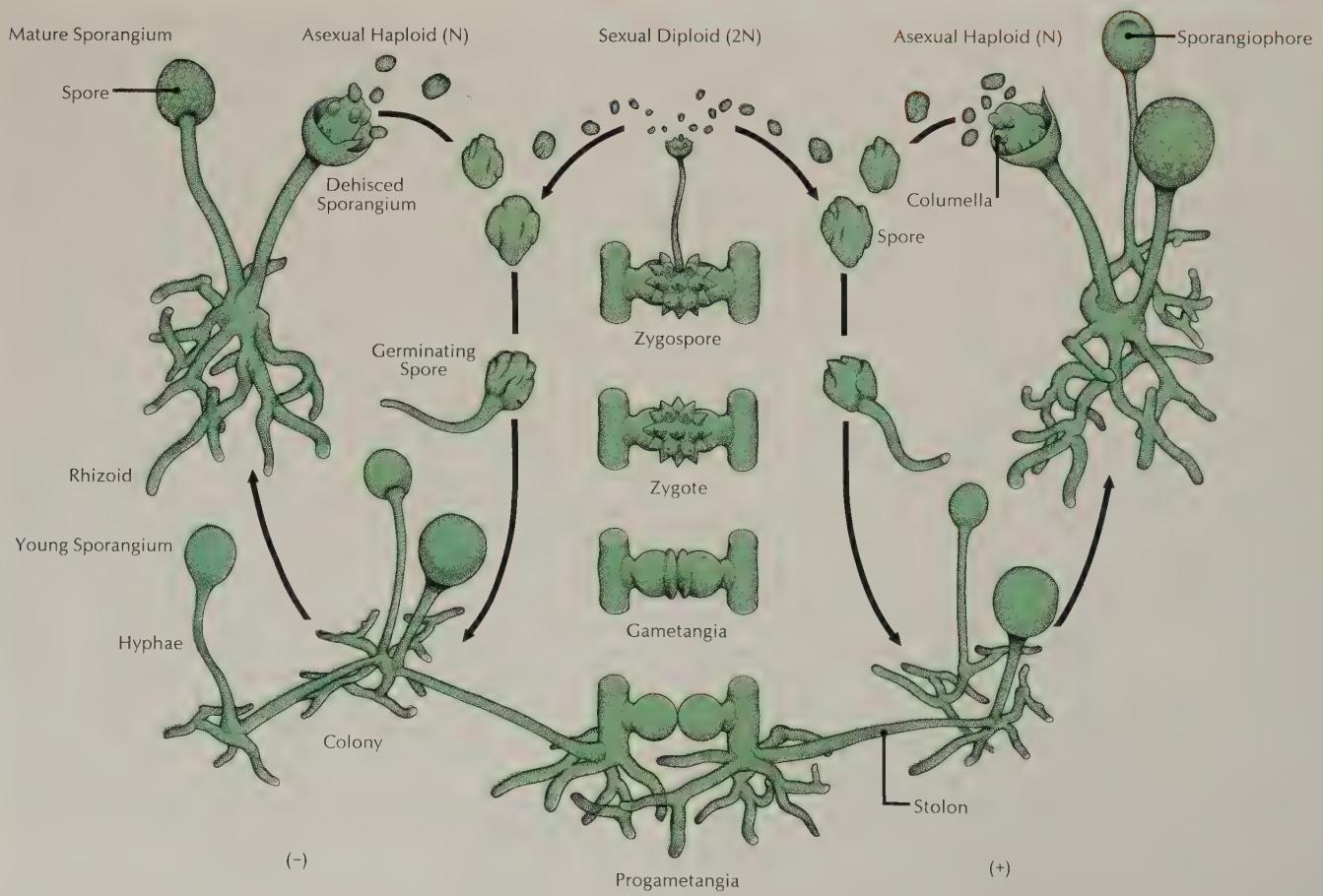


Figure 16.10. Pattern of sexual reproduction in the filamentous green alga *Spirogyra*. Vegetative filament (above). Early conjugation, with protoplasmic bridge being formed between four sets of opposite cells in different filaments (upper middle). Later conjugation, with migration of cellular contents from one cell into the other cell (lower middle). Conjugation is completed (below) with the formation of zygospores (zygotes), each capable of developing a new vegetative filament. (Courtesy Carolina Biological Supply Company)

both kinds of gametes are flagellated, although one kind is larger than the other. Among the red algae, on the other hand, neither kind of gamete is flagellated.

In some fungi, the diploid portion of the life cycle has been nearly eliminated. The black bread mold *Rhizopus* is a haploid organism that normally reproduces asexually through the production of haploid spores (Figure 16.9). Under certain conditions, however, sexual reproduction may take place between individuals of two different types. Because individuals of the two types appear physiologically identical, they are called + and – rather than male and female. Projections grow from the filaments of the two individuals and join. At the juncture, nuclei from the two individuals fuse to form a diploid zygote. The zygote is covered by a resistant wall and may remain independent and dormant for some time. Under suitable environmental conditions, the zygote germinates and undergoes meiotic division to produce new haploid spores. Thus, the only diploid part of the life cycle is the dormant zygote.

A similar pattern of reproduction is found in the filamentous green alga *Spirogyra* (Figure 16.10). In this case, the zygote undergoes meiosis upon germinating, but three of the four nuclei formed by meiotic division disintegrate, and the remaining nucleus undergoes mitotic division to form a new filament.

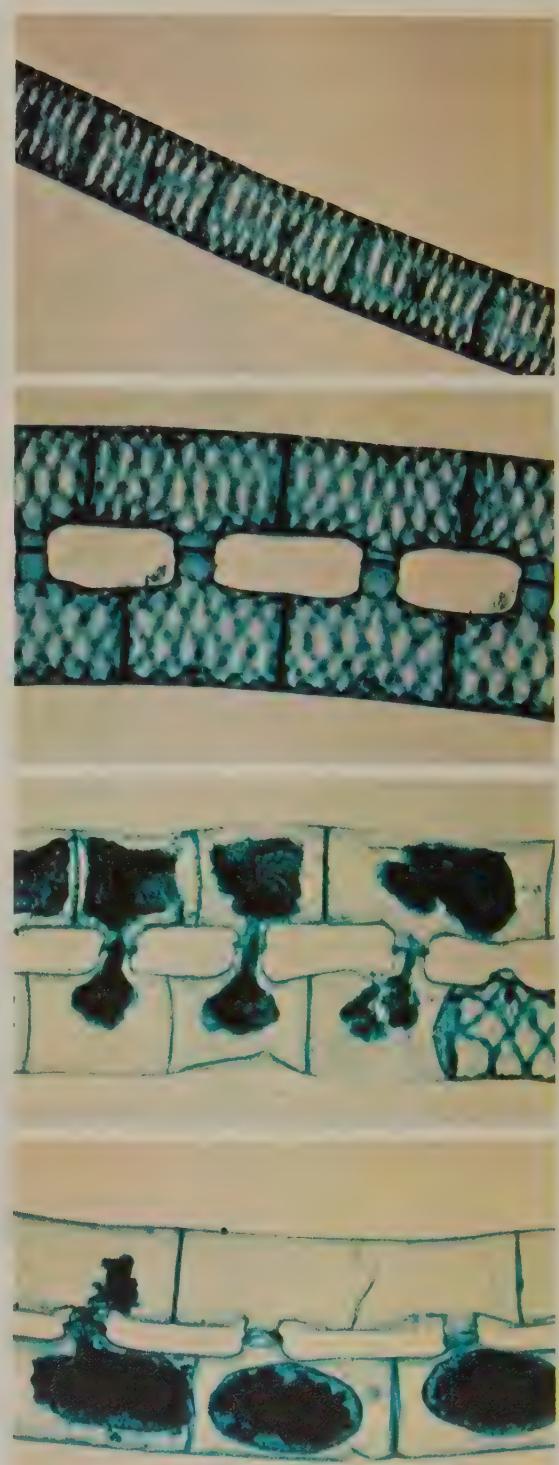
ANIMAL REPRODUCTIVE SYSTEMS

Although asexual reproduction through budding, fission, or fragmentation does occur in some of the invertebrate groups of animals, sexual reproduction is almost universal throughout the animal kingdom. In some of the simpler aquatic organisms, sperms and eggs are released into the water, where fertilization occurs. In most kinds of animals, some form of copulation occurs, with the male injecting sperms into the female's body. The fertilized egg cell, or zygote, may develop for some time within the female's body or may be enclosed with nutrient materials in an egg and released from the female's body for further independent development.

The gametes are produced by meiotic division of specialized germ cells in the organs called gonads. The process of *gametogenesis*, or gamete production, varies somewhat in detail among the different groups of animals, but a generalized description of the process in mammals illustrates the major events.

The reproductive system of a mammal consists of the gonads, the reproductive tract through which the gametes move, and various associated glands. As in most higher animals, each mammalian species has male individuals that produce spermatozoa and female individuals that produce eggs. Hermaphroditism, in which a single individual produces both eggs and sperms, is found among some groups of animals. Fertilization occurs within the reproductive system of the female, and the embryo is retained within the female's body during the early stages of its development. In addition to the differences in the sexual organs of the two sexes (appropriate to their different roles in the formation of the zygote and nurture of the embryo), the sexes can be distinguished by various differences in the form of various other parts of the body—the *secondary sexual characteristics*.

The sex of an individual is determined primarily by genetic inheritance. A mammalian zygote obtaining an X chromosome from each of its parents will normally develop into a female, whereas a zygote obtaining one X and



one Y chromosome will develop into a male (Chapter 14). The Y chromosome must carry various genes involved in the development of male characteristics, for an abnormal individual with only a single X chromosome and no Y chromosome (an XO genotype) develops as a female (normal in the mouse, but sterile in the human). The abnormal XXY genotype results in development as a male, but with some female secondary characteristics and severe mental retardation. The determination of sex through sex chromosomes is widespread among animals but is not universal. Among bees, for example, males are haploid and females are diploid. Even among animals having sex chromosomes, the nature of sex determination varies widely. In birds, for example, males have a pair of similar sex chromosomes, whereas females have a pair of different sex chromosomes.

The genetic inheritance of sex is by no means the complete story. The balance of various hormones in the developing embryo—a balance presumably regulated by the genetic information—appears to play the crucial role in controlling the determination of tissues and organs toward male or female forms (Chapter 21).

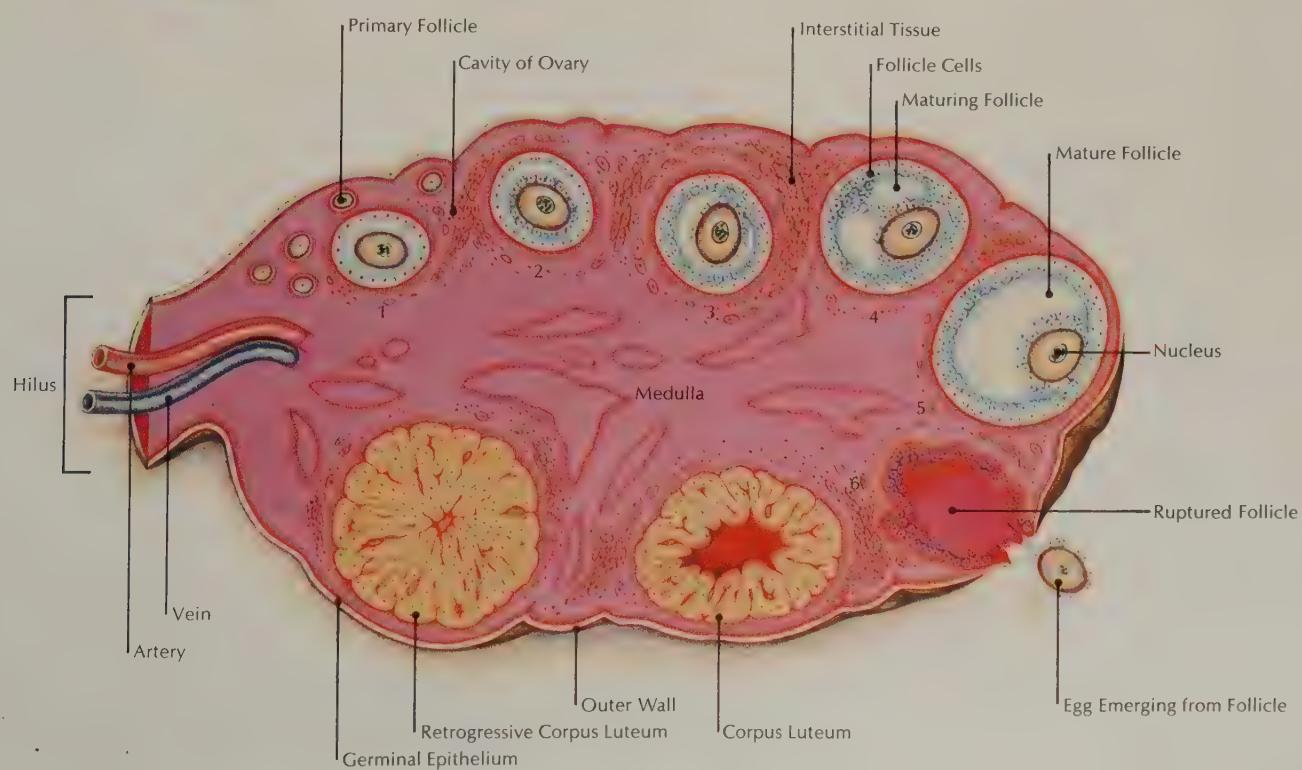
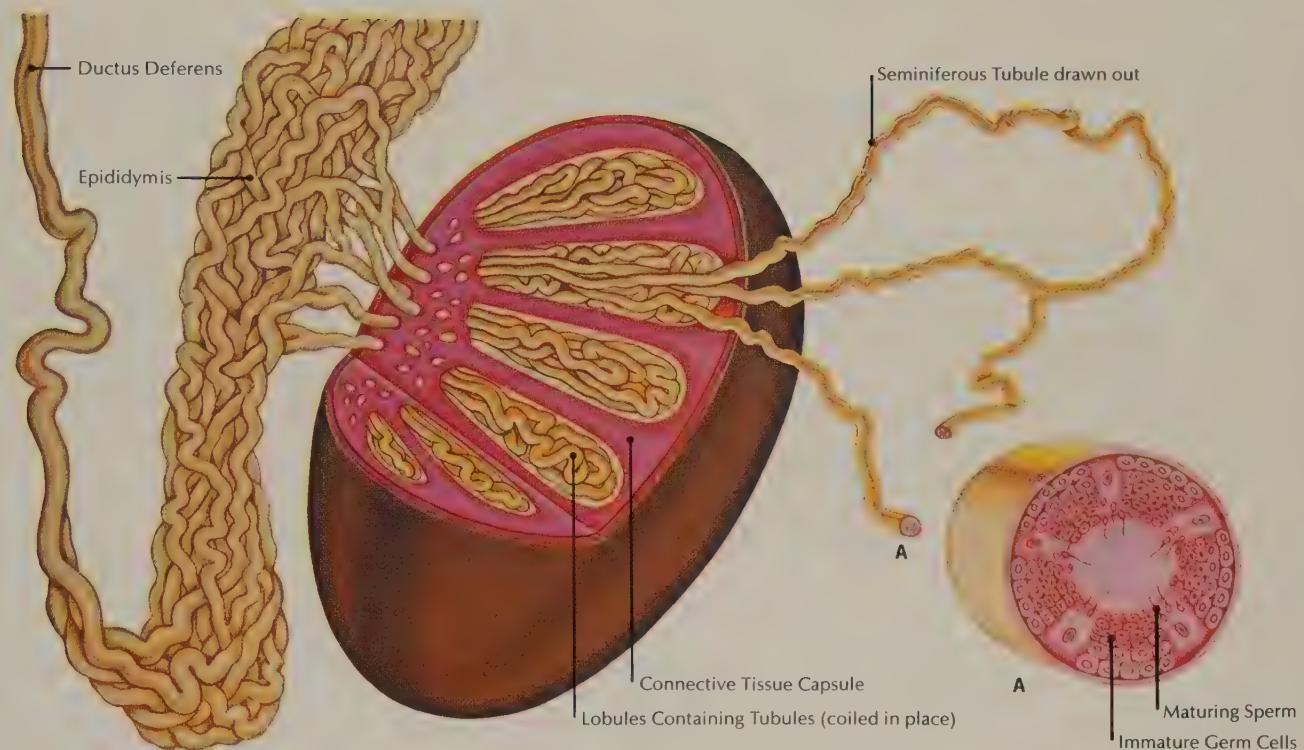
The determination of the primary germ cells, which will later form the eggs or sperm, occurs at a very early stage in the development of the animal embryo. Cells that form the gonads become determined at a much later stage of development. Thus, the primary germ cells are set aside for that fate very early in development, long before the gonadal structures are visible. Even after the gonads have begun to develop, it is some time before they become determined as either male or female sex organs. The primary germ cells migrate into the developing gonads at a relatively late stage of development. This migration occurs either through amoeboid movement or through transport by the blood (Franchi, et al., 1962).

The male gonads, or testes, of a mammal contain a large number of tubules that are made up of germ cells and *Sertoli cells*. The tubules are complexly coiled within the testes and are surrounded by connective tissues and interstitial cells (Figure 16.11). In the mature mammal, the germ cells continue to divide mitotically, producing a continuous new supply. Some of these cells divide meiotically to produce haploid cells that develop into spermatozoa. As the spermatozoa mature, they cluster around the Sertoli cells, whose function is not understood. In some mammals, maturation of spermatozoa occurs only at certain seasons. In others, including man, mature spermatozoa are produced continuously during the reproductive portion of the life cycle. Mature spermatozoa leave the Sertoli cells, move through the tubule, and enter a duct leading to the exterior. The structure of the duct and the nature of the various glands that lubricate the passage of sperm or provide nourishment for sperm cells vary greatly among groups of animals.

The female gonads, or ovaries, of vertebrates consist of oval-shape masses of cells, including vascular and connective tissues, as well as the germ cells, *follicle cells*, and *nurse cells*, which provide nutrients for the development of egg cells, or oocytes (Figure 16.12). The mammalian female germ cells cease mitotic division before birth, so that the newborn female possesses a complete lifetime supply of immature eggs. The germ cells grow considerably larger than the other kinds of cells that surround them. Gametogenesis begins with meiosis, but the meiotic division is halted at prophase of the first division. The developing oocyte then grows extremely large as it absorbs nutrients from the follicle and nurse cells. The various substances

Figure 16.11 (above). Mammalian testes.

Figure 16.12 (below). Mammalian ovary.



that make up *yolk*—including protein, fat, glycogen, and RNA—are pumped into the rapidly expanding cytoplasm of the oocyte.

Among some organisms, such as birds, the growth of the egg cell continues until it is many thousands of times as large as other cells of the body. The follicle cells form a thin layer over the growing egg cell. The mammalian egg is relatively small and contains only a small amount of yolk, but the layer of follicle cells becomes quite large around the egg cell. When accumulation of yolk has been completed, the oocyte resumes meiotic division. However, the division of cytoplasm during meiosis is unequal. The first division results in the pinching-off from the large egg cell of a very small cell containing little cytoplasm. This *first polar body* eventually disintegrates. In most vertebrates, meiosis is again halted after the first division and is not resumed until sometime after the egg has left the ovary. But whenever the second meiotic division occurs, it also results in the pinching-off of a small cell with very little cytoplasm. This *second polar body* also disintegrates. Thus, a single germ cell in the male gives rise to four spermatozoa, whereas a single germ cell in the female gives rise to only one mature egg.

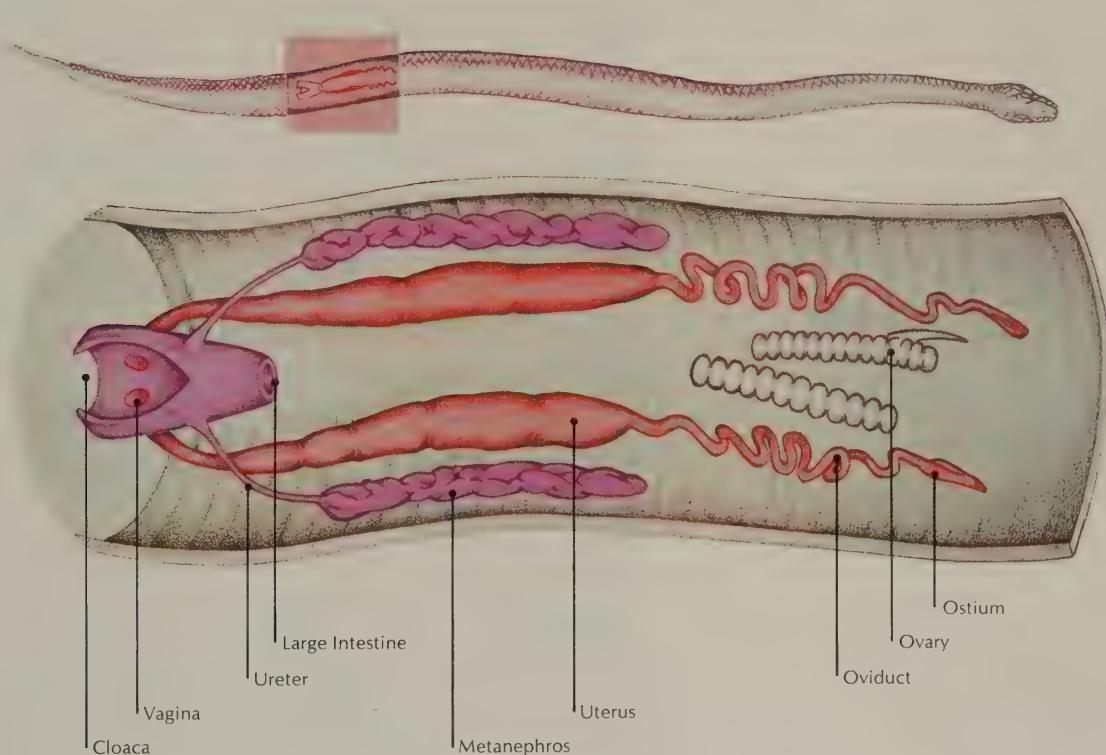
In various vertebrates, large numbers of mature eggs may be produced at a single time in the life cycle of the female or at seasonal intervals. Eggs of birds may be matured individually at about one-day intervals, but maturation of eggs is normally halted when a batch of eggs has been laid and must be incubated. In the human female, one egg is matured each month during her reproductively active life, but egg maturation is suspended during pregnancy.

When the egg is matured, it moves out of the ovary as a result of the breakdown of some of the follicle cells and connective tissues. Moved by the action of cilia, the flow of fluids, or muscular contractions, the mammalian egg moves through the oviduct toward a chamber called the *uterus*. The uterus connects to a passage called the *vagina*, which opens to the exterior and is specialized to accept the male sex organ, or *penis*, permitting the introduction of sperm into the vagina and uterus during copulation. The sperm are carried into the uterus and the oviduct by their own swimming motions and by cilia or muscular contractions of the female reproductive tract. Among mammals, fertilization occurs in the oviduct; among some other groups of vertebrates, it occurs in the uterus. The fertilized mammalian egg becomes implanted in the wall of the uterus, where the early development of the embryo takes place.

Among various groups of animals, there are many variations in the structure of the reproductive organs and tracts. The female insect, for example, has tubules leading directly from the ovaries to genital pores that open to the exterior. In the oviduct of a bird, the egg is successively coated with albumen—a shell membrane—and a hard, limy shell as it moves through the oviduct. Many vertebrates possess paired uteri rather than a single uterus. In most vertebrates and some mammals, the uterus opens into the *cloaca*, which carries excrement and urine to the exterior (Figure 16.13). Only in primates are there separate exterior openings for the vagina, urethra, and anus. Many invertebrates possess an organ called the *spermatheca* (in insects) or *seminal vesicle* (in other groups) in which sperm may be stored within the female reproductive tract so that fertilization of eggs can be carried out over a long period of time after copulation.

The testes of most vertebrates lie inside the body, but in most mammals they are located in a sac called the *scrotum*, which hangs outside the body

Figure 16.13. Diagram of the cloaca of a snake. In reptiles, birds, amphibians, many fishes, and some mammals, the cloaca is the most posterior chamber of the digestive tract; the reproductive, intestinal, and urinary canals empty their products into this cavity.



wall. This arrangement keeps the testes at the relatively low temperature needed for spermatogenesis and survival of the sperm. In birds and other vertebrates, various means of internal cooling are used to accomplish the same results. Only among primates and ungulates (hoofed mammals) do the testes remain permanently in the scrotum; in most other groups of mammals, the testes descend into the scrotum only during the mating season.

Among some groups of vertebrates, the sperm are discharged into the cloaca, which then is partially protruded from the male anus and inserted into the cloaca of the female. Insects possess a wide variety of complex copulatory parts, including various kinds of grasping or clasping structures on both males and females; so that mating in many cases is physically impossible with members of slightly different species. When birds mate, the usual pattern is inverted, for the cloacal end of the oviduct is protruded and inserted into the cloaca of the male. Only mammals and turtles have true penes, which are soft structures that become rigid when pumped full of blood. Grooves along the sides of the turtle's penis become ducts for the passage of sperm when the penis is erect. In mammals, the sperm passes through the urethral duct, which extends through the length of the penis. In some mammals, including cattle, a bone adds permanent rigidity to the penile structure.

MATING BEHAVIOR

In the simplest form of sexual reproduction, gametes are shed into the environment, and fertilization occurs whenever two gametes of the proper types happen to meet. Such random fertilization is highly inefficient, with

Figure 16.14a (lower left). The female Surinam toad has "pouches" on her back in which baby toads develop. Just prior to the time of egg-laying, the back of the female toad becomes very spongy. When the eggs are released, the male toad scoops them up and deposits them on her back. He then mounts the female toad and fertilizes the eggs. The pressure of his body forces the eggs down into the spongy layer where they remain until birth.

Figure 16.14b (upper right). Ladybug beetles mating. In

many arthropods, sperm from the male is transferred directly to the female by specially modified appendages.

Figure 16.14c (lower right). Mating behavior in amphibians. Shown here are male and female chorus frogs in the mating position (amplexus) and during egg-laying. Fertilization is external; the male elicits egg-laying of the female by grasping her sides and by applying slight pressure. Note the egg mass in the water.



most of the gametes dying before fertilization occurs. A great variety of behavior patterns exist in different species to increase the probability that any given gamete will participate in fertilization. The release of gametes into the environment often is synchronized by chemical means (for example, a substance released with the eggs may trigger other females to release their eggs and males to release sperm) or by synchronization to some external stimulus (such as the tidal cycle). Among many species of fish, the complex behavior pattern involved in mating causes the male to shed the sperm directly over the eggs that have just been laid by the female. The process of copulation achieves the greatest efficiency in guiding spermatozoa to the eggs, but it requires very complex patterns of behavior to synchronize the actions and gametogenesis of the two individuals involved.

Various kinds of courtship behavior help to bring together two individuals of opposite sexes and of the same species, to synchronize the reproductive cycles of the prospective mates, and in some cases to make initial preparations for the care of the offspring. The actual behavior of copulation also involves complex patterns, both instinctive and learned, that ensure that the sperm will successfully be transferred to the reproductive tract of the female and that an egg or eggs will be ready for fertilization. The nature and functions of mating behavior are discussed in more detail in Chapter 31.

CARE OF OFFSPRING

In cases where gametes or spores simply are shed into the environment, the new individual is on its own. It must provide its own protection and nutrition throughout its period of growth and development. In most such cases, vast numbers of new zygotes are produced for each one that manages to survive to maturity. In most species, parents provide some protection or nutrition for the offspring. Various forms of spores, seeds, and eggs provide nutrients and protective coatings for the young individual during the most critical early stage of its growth. Organisms that retain the embryo within the body of the female adult during early growth provide even more protection and nourishment.

Parents (or other adult members of the species) may care for the offspring in a variety of ways that depend upon behavior patterns as well as upon physiological structures. Burrows or nests may be prepared. The adults may carry or cover the young or the unhatched eggs to provide body heat or protection from predators and parasites. Food may be brought to the young, provided from special glands or other sources on the adult body, or stored in the nest or burrow for the use of the young. Such behavior by adults involves complex patterns of reaction to stimuli provided by the presence and the behavior of the young themselves. Behavior patterns that create families and other social groupings provide adult care for the young of many species.

Among some species—particularly mammals—the young remain with the adults for a considerable period of time, learning a variety of behavior patterns that are not genetically determined. This transmission of learned as well as genetic information reaches its peak in some primate species, including the human species.

It is difficult to draw boundaries around the study of reproduction because almost every structure and behavior of a living organism has some relation to the perpetuation of the species through the production of offspring. The process of evolution through natural selection favors the continued

Figure 16.15. Care of offspring. Female scorpion (above) with newly-born young riding on her back. Scorpion courtship consists of a "dance" between partners with the male depositing a germ sac, or spermatheca, on the ground, then maneuvering the female over it. Siamang and baby (middle left). Three-week-old baby cougar cubs with their mother (middle right). As with most mammals, these cougar cubs are dependent on their mother for warmth, protection, food, guidance, and orientation. Gnu and baby (bottom). Giraffe and baby (opposite).



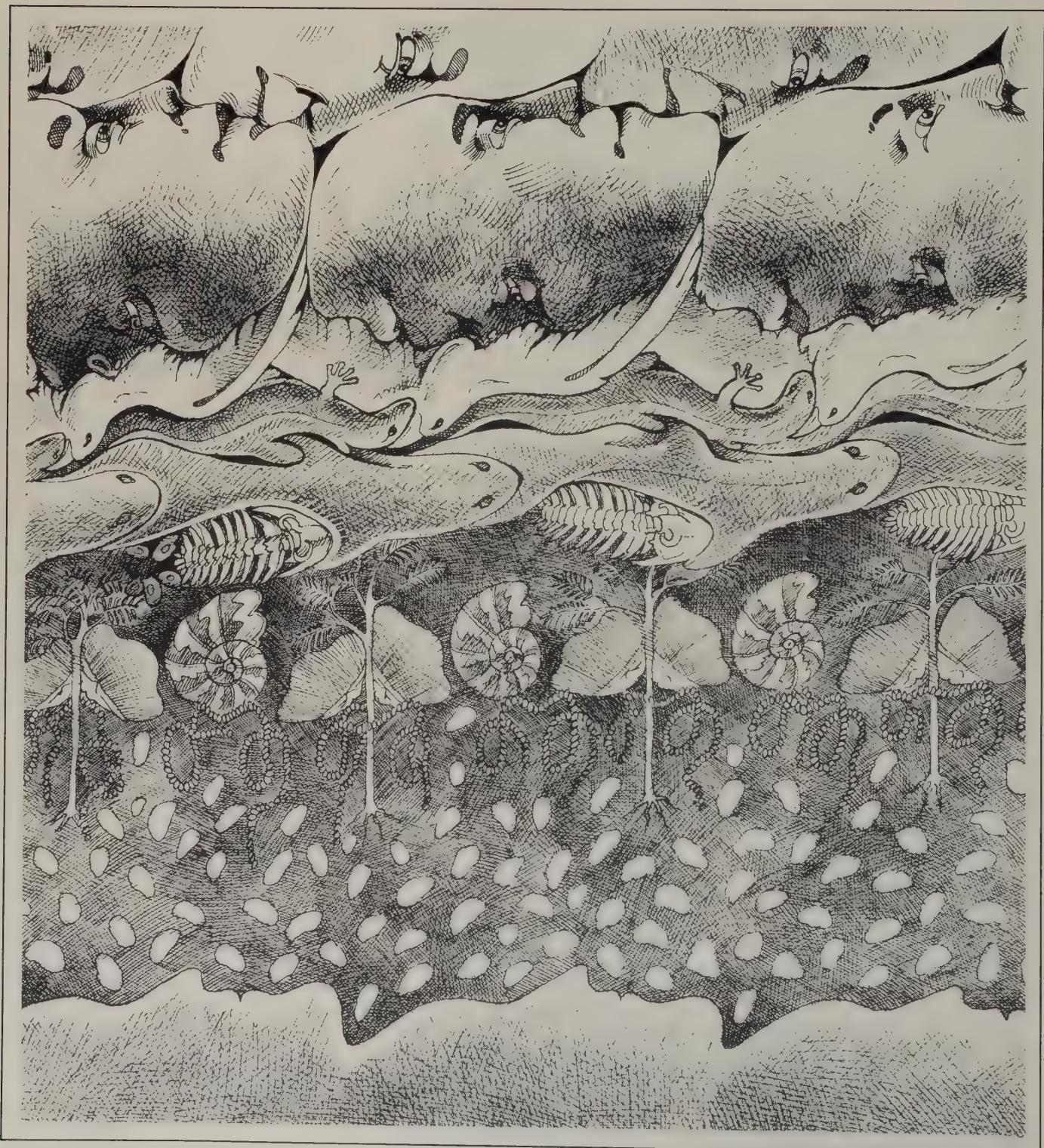


existence of those species most successful in reproducing themselves. In the long run, every characteristic that has been developed and retained through evolution probably contributes to the process of reproduction in one way or another. The picture of the natural world that is consistent with Darwinian theories of natural selection is not so much the often-depicted tooth-and-claw struggle to the death but a continuous effort to produce and protect offspring.

FURTHER READING

More details of reproduction in a wide variety of organisms will be found in many general books on biology, zoology, and botany. The discussions of these matters in books by Hardin (1966), Jessop (1970), and Telfer and Kennedy (1965) are particularly useful. For more extensive discussions of sexual reproduction, see books by Asdell (1964), Berrill (1953), Michelmore (1964), and Van Tienhoven (1968).

Articles of interest in relation to this chapter include those by Jones (1968), Roth and Barth (1967), Rothschild (1956), and Zahl (1949).



V

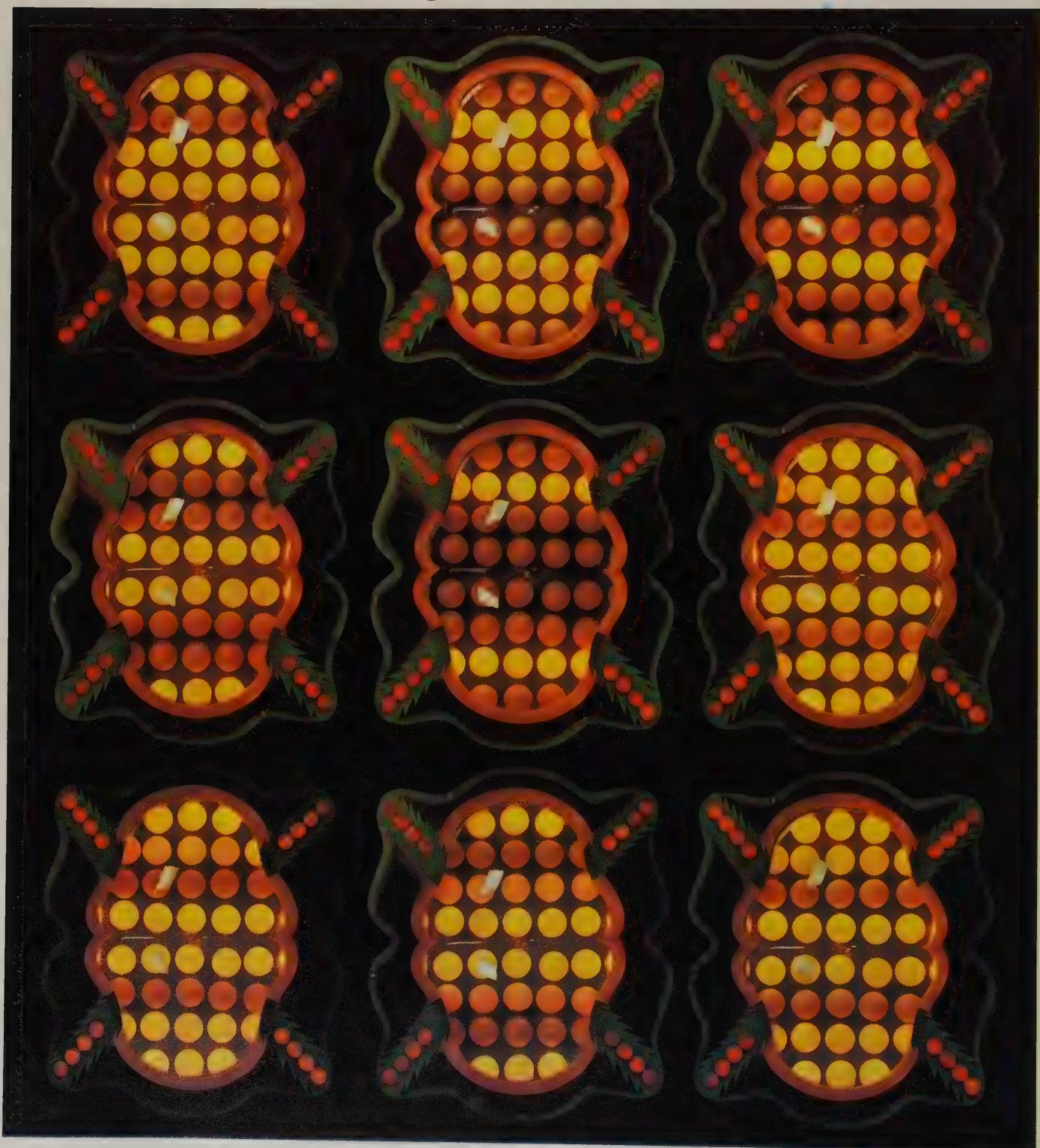
Integration

A cell will eventually divide in two, and that is an impressive enough sight, but still all it is doing is to produce another cell like itself. The striking achievement of an egg is to produce things – roots, leaves, legs, eyes, backbones, and so on – which were not in it originally. It does more than merely reproduce itself; it produces something new. Even if you have a certain degree of biological knowledge when you start looking at it – knowing perhaps what everyone seems to know nowadays, that the fundamental characteristics of organisms are determined by the genes inherited from their parents, and that these genes are made of nucleic acid (DNA) – even so, merely to say that the lump of jelly you are looking at contains the right DNA to produce a rabbit leaves an enormous amount unaccounted for. Exactly how does the egg produce legs, head, eyes, intestine, and get up and start running about?

—C. H. Waddington (1966)

17

Cellular Regulation and Control



The cell is an exceedingly complex biochemical system. Its life processes require the synthesis and utilization of millions of macromolecules and billions of smaller molecules and ions. Thousands of different kinds of molecules must be produced in the proper numbers and brought together at the appropriate times and places within the cell to serve their functions in metabolic processes. How is this complex system controlled and coordinated?

Part of the controlling mechanism is described by the "central dogma" of molecular genetics. Information specifying the amino acid sequence for a polypeptide chain is encoded in the sequence of base pairs in a gene—a segment of the chromosomal DNA molecule. This information is transcribed into the sequence of base groups in a molecule of messenger RNA, which moves from the gene to a ribosome. At the ribosome, with the aid of transfer RNA, ribosomal RNA, and various enzymes, the polypeptide chain is constructed according to the directions specified by the base sequence. The polypeptide chain thus constructed may act as an enzyme, or it may become part of an enzyme or structural complex.

Because the chromosome contains many genes, each coding for the production of a different polypeptide chain, the cell is able to construct the many different enzymes and structural proteins needed for its life processes. Manufactured enzymes, in turn, can guide the synthesis of necessary nonprotein molecules.

However, the genetic specification of proteins alone is not sufficient to explain the life history of either an individual cell or a multicellular organism. Except for rare cases of mutations, a cell retains the same genes throughout its life. A multicellular organism develops by repeated mitotic divisions of a single zygote, so that each cell of the mature organism possesses at least one complete copy of the zygote's genetic information. If each cell possesses the same set of instructions for protein synthesis, how can different cells synthesize different proteins and exhibit specialized functions? How can a variety of cellular structures and functions develop from a single set of genetic instructions? Obviously, a cell needs a method of selective gene expression. It must be able to control which genes it will express and how much of each gene product it will make.

The cell could exert control at any one of several points along the route from gene to gene product. The duplication of the gene itself could provide many templates for messenger RNA transcription. Direct control over the transcription mechanism could determine which genes would be transcribed and regulate the number of mRNA molecules made from each gene. Likewise, the cell could control the kinds and amounts of mRNA molecules that are translated into proteins and control the assembly of those proteins into functional structures. Even after a protein has been synthesized, it is not beyond the cell's control; there are numerous ways in which a cell could alter and regulate the function of a gene product.

The control of the cellular mechanism is not always a one-way process, with prerecorded instructions in chromosomal DNA blindly guiding the cell along a predestined course. Cellular controls result from interactions of the cell's genetic material with the cytoplasm and, indirectly, with the external environment. Obviously, the living system possesses feedback capability—it reacts to changes in its surroundings and in its own internal status. Although a complete understanding of such genetic control mechanisms lies far in the future, experimental work done in the past few decades has

Figure 17.1. A broad interpretation of the aphorism "What is true of *E. coli* is true of the elephant."

revealed the general nature and some details of the cellular control systems.

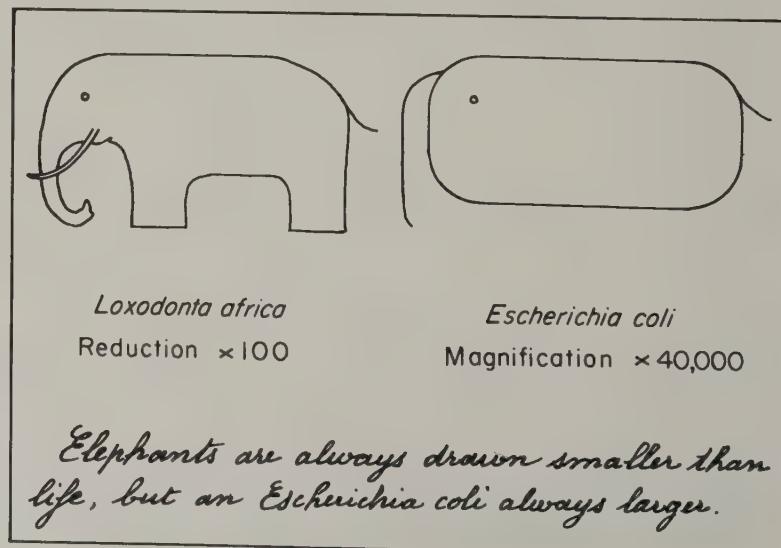
Biologists and biochemists have faced many obstacles in their attempts to understand the processes of cellular control in animals. The technical difficulties involved in trying to formulate meaningful answers from the vast array of specialized cells in an adult are staggering. Yet an individual cell removed from an embryo or adult does not always act in the way that it would as a part of the multicellular organism.

THE COLON BACILLUS

Much can be learned from cells grown in tissue-culture media, but such an environment is highly artificial. For this reason, many researchers have turned to simpler organisms for research on cellular control mechanisms, hoping that the fundamental biochemical similarities of all living systems will permit generalization of their findings to more complex organisms. A particularly useful and popular organism for such study has been the colon bacillus *Escherichia coli*, a bacterium normally found in the human intestines (Interleaf 17.1). This bacterium multiplies rapidly, can easily be isolated or grown in large populations, and survives on a simple nutrient medium of glucose, water, and a few inorganic salts. Because a new generation of bacteria can be produced as rapidly as every 20 minutes, *E. coli* are particularly useful for genetic studies. As a result, this bacterium and the bacteriophage that infest it are by far the best-understood organisms in the biosphere.

A surprising number of biochemical mechanisms discovered in the simple cells of *E. coli* have been found in complex eucaryotic cells as well. Some biologists who have studied *E. coli* extensively have humorously overstated the case by coining the aphorism "What is true of *E. coli* is true of the elephant."

Today, however, a growing number of biologists are advising caution in the uncritical generalization of the results of *E. coli* research to all cells. There is evidence that prokaryotes and eucaryotes began following separate evolutionary paths very early in the history of life on earth. Although both kinds of cells may share many mechanisms inherited from their com-



mon ancestors, it is reasonable to expect that evolution has produced many differences between them. Despite the basic differences, there seems to be good reason to hope that the basic principles learned from studies of *E. coli* will point in the right direction for studies of more complex organisms.

Although relatively simple in comparison to eucaryotic organisms, the *E. coli* cell itself is a complex system. Within this microscopic cell are thousands of different kinds of small and large molecules (Table 17.1), many of

Table 17.1

Approximate Chemical Composition of a Rapidly Dividing *E. coli* Cell

COMPONENT	Number of Different Kinds	Average Molecular Weight	Approximate Number of Molecules Per Cell	Percentage of Total Cell Weight
Water (H_2O)	1	18	40,000,000,000	70%
Inorganic ions	20	40	250,000,000	1
Carbohydrates*	200	150	200,000,000	3
Amino acids*	100	120	30,000,000	0.4
Nucleotides*	200	300	12,000,000	0.4
Lipids*	50	750	25,000,000	2
Other small molecules	200	150	15,000,000	0.2
Proteins	2,000–3,000	40,000	1,000,000	15
Nucleic acids				
DNA	1	2,500,000,000	4	1
RNA				6
16s rRNA	1	500,000	30,000	
23s rRNA	1	1,000,000	30,000	
tRNA	40	25,000	400,000	
mRNA	1,000	1,000,000	1,000	

*Including precursors.

Source: James D. Watson, *Molecular Biology of the Gene*, 2nd ed. (New York: Benjamin, 1970) p. 85.

which have not been fully identified. Even with modern techniques, the determination of the three-dimensional structure of a single protein molecule requires many man-years of difficult research. Therefore, the complete molecular structure of the *E. coli* cell may not be known for many decades. Yet enzymes can be recognized in the cell by their catalysis of metabolic reactions, even if their detailed structures remain unknown. Of the approximately 1,000 enzymes needed to catalyze the metabolic reactions known to occur in *E. coli*, many have been isolated and characterized. The *E. coli* chromosome is only large enough to code for about 4,000 proteins, which indicates that 25 percent of the organism's biochemistry is understood.

Because the genetics and biochemistry of *E. coli* are so well known, they have provided most of the available knowledge of cellular regulatory mechanisms. Thus, by necessity, any discussion of regulation must center around bacterial mechanisms. As will be seen, however, bacteria cannot provide the full story.

GENE AMPLIFICATION

One of the most dramatic examples of the regulated expression of genes occurs during the maturation of amphibian oocytes (Chapter 18). The genes

Biologists today estimate that they are aware of about 25 percent of all the specific chemical reactions occurring in *E. coli* and, in many cases, how the cell controls the rates of these reactions. They also have a good understanding of how large molecules such as proteins and nucleic acids are made and, frequently, how these syntheses are controlled. Many of these findings are based on experiments in which the living cells are physically or chemically fractionated into nonliving components. Such results must then be demonstrated in the intact, living cell to prove that they are significant. Similarly, when experiments are performed on whole cells in the laboratory, these results should be interpreted as they apply to the organism in its natural environment, for the demands of this environment restrict and ultimately dictate to the organism what it must do in order to survive. This environment is the mammalian intestine and not the test tube.

E. coli and its ancestors have probably occupied this same relatively static environment for 100 million years, during which time there have been perhaps 10^{30} times as many *E. coli* as mammals. Each of these cells has been subject to the selection pressures of the environment. Because of their rapid growth rate, as compared to mammals, and the fact that they have occupied the same environment for so long, it is reasonable to assume that they are by now optimally adapted—that is, *E. coli* are probably as good at growing in their environment as they could be.

When studied in the laboratory, however, *E. coli* are grown under conditions quite unlike those in the intestine, where food is supplied in intermittent and unpredictable windfalls. The bacteria may experience long periods of near starvation if the host cannot obtain a meal; thus, they normally alternate between periods of feast and famine. In nature they usually divide only once a day, in contrast to once an hour under laboratory conditions where food is both abundant and continuous.

When dividing rapidly in the laboratory, *E. coli* direct their energies very efficiently toward growing and dividing, but when dividing at rates like those in the intestine, they do not appear to allocate their resources as wisely. A cell that wastes any of its limited resources will have a slow growth rate and will soon be outgrown by its more efficient competitors. For example, when *E. coli* are grown under conditions where they divide only once every 24 hours, they contain 7 times as many ribosomes as they need. The large and complex ribosomes are an expensive investment in terms of the cells' materials and energy. Between each division, a cell must synthesize the same total amount of protein regardless of how rapidly it divides. Because a ribosome can assemble the same number of proteins per hour at any growth rate, a cell that must double itself once a day needs only $1/24$ as many ribosomes as a cell doubling once every hour. Therefore, it is surprising to find that slowly growing *E. coli* have such an excess of ribosomes. These extra ribosomes represent about 10 percent of the dry weight of the cells. Each time they divide, they must direct 10 percent of their metabolic raw materials and energy toward the production of these extra ribosomes. Clearly, *E. coli* that did not make the extra ribosomes would be able to divide 10 percent more rapidly under the same culture conditions and would soon outgrow the cells containing the extra ribosomes.

Why hasn't this adaption occurred in nature? There has been ample time. A closer look at the environment provides a probable answer. When the host eats, the *E. coli* are suddenly presented with food for which each cell must compete. Each cell then tries to utilize as much of the food and grow as quickly as possible. The rate at which it can grow is limited by the availability of the synthetic machinery—that is, DNA and RNA polymerases but primarily ribosomes. A cell with excess ribosomes could immediately begin to make protein more rapidly. In contrast, a cell that did not contain excess ribosomes would have to make more ribosomes before it could synthesize protein at the faster rate.

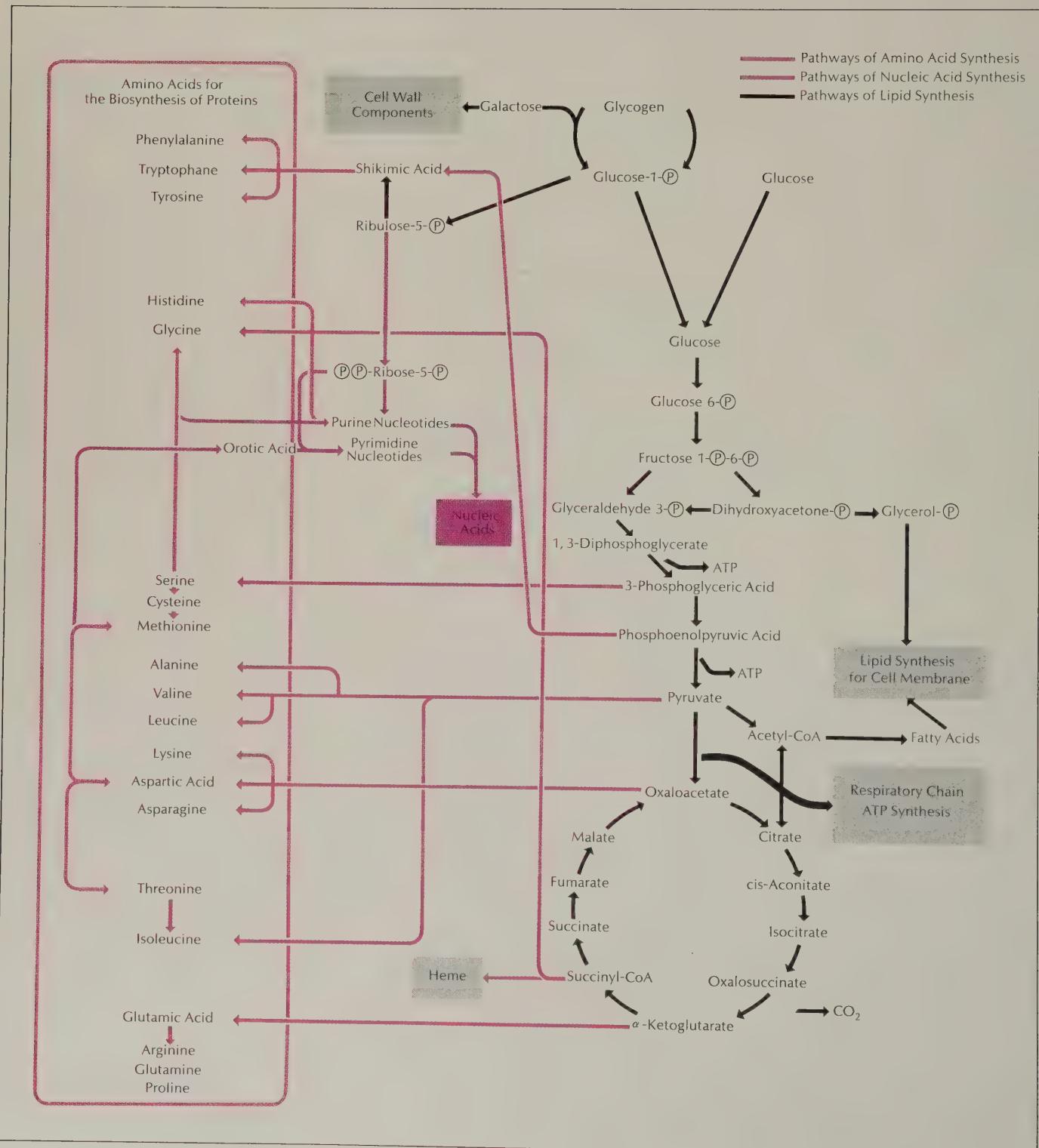
If cells with and without the excess ribosomes were grown with a 24-hour doubling time and were suddenly given unlimited food, calculations show that the *E. coli* with a sevenfold excess will have divided once before the hypothetical cells with no excess have even begun to grow significantly faster. Thus, between meals the bacteria with no excess ribosomes would grow about 10 percent faster, but every time the

host eats, the bacteria with excess ribosomes will gain a 100 percent advantage. The host need eat only once every 9 days for the bacteria with excess ribosomes to have a 10 percent selective advantage. Therefore, *E. coli* with extra ribosomes are not inefficient but are very "wise" indeed.

The transport systems of *E. coli* provide a further demonstration that laboratory studies of an organism often need to be correlated to its natural environment to be fully understood. If *E. coli* are grown on lactose at concentrations that occur in the intestine, the growth rate of the cells is limited by the rate at which lactose enters the cell, not by the rate at which it is metabolized. So, why haven't *E. coli* developed a more efficient transport system for lactose? To answer the question, it is necessary to look again at their native environment. The viscosity of intestinal contents is approximately that of lightweight motor oil. In such viscous material, the rate of diffusion of lactose is about 100 times slower than in water. In laboratory media, diffusion is fast relative to the speed at which the "permease" can transport lactose into the cell. However, in the intestine, diffusion is so slow that the cell is able to transport the lactose inside as fast as it can diffuse up to the cell. Thus, the cellular growth rate is limited not by lactose transport from the cell surface to the interior but by the rate of diffusion of lactose through the intestinal contents. Obviously, improvements in the efficiency of the transport system in the intestine would not increase the growth rate and would have had no selective advantage.

An organism must be studied in relation to its natural environment to understand fully and to appreciate its biochemistry, for it is the environment that has shaped the organism's destiny. The phenomena observed at the molecular level are but the organism's methods of coping with the demands placed on it by the environment. In a very real sense, biochemistry is subordinate to environment.

Figure 17.2. The metabolic pathways of *E. coli*.



coding for ribosomal RNA are specifically duplicated (as many as 10^3 times), and each copy serves as a template for ribosomal RNA synthesis. Ribosomal RNA synthesis thus proceeds at many times the rate as in other cells, a rate that would be physically impossible with only four sets of ribosomal genes.

Although this increase is the only clearly demonstrated example of gene amplification known at present, there is speculation that it might occur (although less dramatically) in other cell types. Many cells in eucaryotic organisms must produce large quantities of a few gene products, and it is possible that amplification of those genes facilitates the synthesis. In most cases, however, if amplification does occur, the number of extra copies of the gene would probably be small (10 to 100) and, at present, very difficult to detect.

CONTROL OF RNA TRANSCRIPTION

The regulatory mechanism best understood today is the control of transcription of specific messenger RNA in bacteria. Of the number of possible ways in which a cell could control the rate of synthesis of specific mRNA molecules, several have been shown to occur in various organisms. The most extensively studied mechanism controls the synthesis of *inducible* and *repressible* enzymes in *E. coli*.

β -galactosidase, the enzyme that cleaves lactose into glucose and galactose, is the classic example of an inducible enzyme. When no lactose is present, *E. coli* contain only about two molecules of β -galactosidase. When lactose (which must be cleaved to be further metabolized) is added, the bacteria rapidly produce more β -galactosidase—as much as 3,000 molecules per cell. If the lactose concentration is lowered, the cells make intermediate amounts of β -galactosidase. If lactose is subsequently removed from the medium, the cells rapidly cease production of new enzyme molecules. This very sensitive mechanism allows the expression of a gene product only when the cell has need for it and only in the amounts required.

The elucidation of this control mechanism during the last two decades by François Jacob, Jacques Monod, and many others has proved to be one of the major advances in molecular biology. The key component of this regulatory system is a protein known as the *repressor*. When lactose, the *inducer*, is absent, the repressor binds tightly to part of the β -galactosidase gene, physically preventing RNA polymerase from making β -galactosidase mRNA. When lactose (or a synthetic molecule that mimics lactose) is present, it binds to the repressor and changes its conformation so that it can no longer bind to the β -galactosidase gene. The polymerase is then able to make β -galactosidase mRNA, which will serve as the template for many enzyme molecules.

The entire process takes place very rapidly. Within seconds after the inducer enters the cell, the gene releases the repressor and RNA synthesis begins. The first active enzyme molecule appears about two minutes later. When lower concentrations of the inducer are present, the repressor remains bound to the gene part of the time and RNA is made less frequently.

Normal cells contain only about ten molecules of the β -galactosidase repressor, but the recent isolation of mutants that produce a large excess of repressor have made possible its purification and characterization. The binding between the repressor and the inducer is not covalent but is a relatively weak hydrogen bond interaction. Such bonds break and re-form

Figure 17.3. Jacob-Monod model of gene control via a DNA section known as the operon. The operon consists of a regulator gene, an operator gene, and structural genes. Without lactose present in the system, the regulator gene produces a repressor protein. In the absence of an inducer molecule, such as lactose, the repressor binds onto the operator region. This action prevents the activation of the structural genes, and no unnecessary enzyme synthesis results. With lactose present in the system, repressor molecules are synthesized as above, but lactose acts as an inducer for

its own enzymatic breakdown. Lactose and repressor combine to form a complex that is unable to bind to the operator region. The "switch" is now turned on, and the structural genes transcribe mRNA, which in turn guides the synthesis of the three enzymes necessary for lactose breakdown.

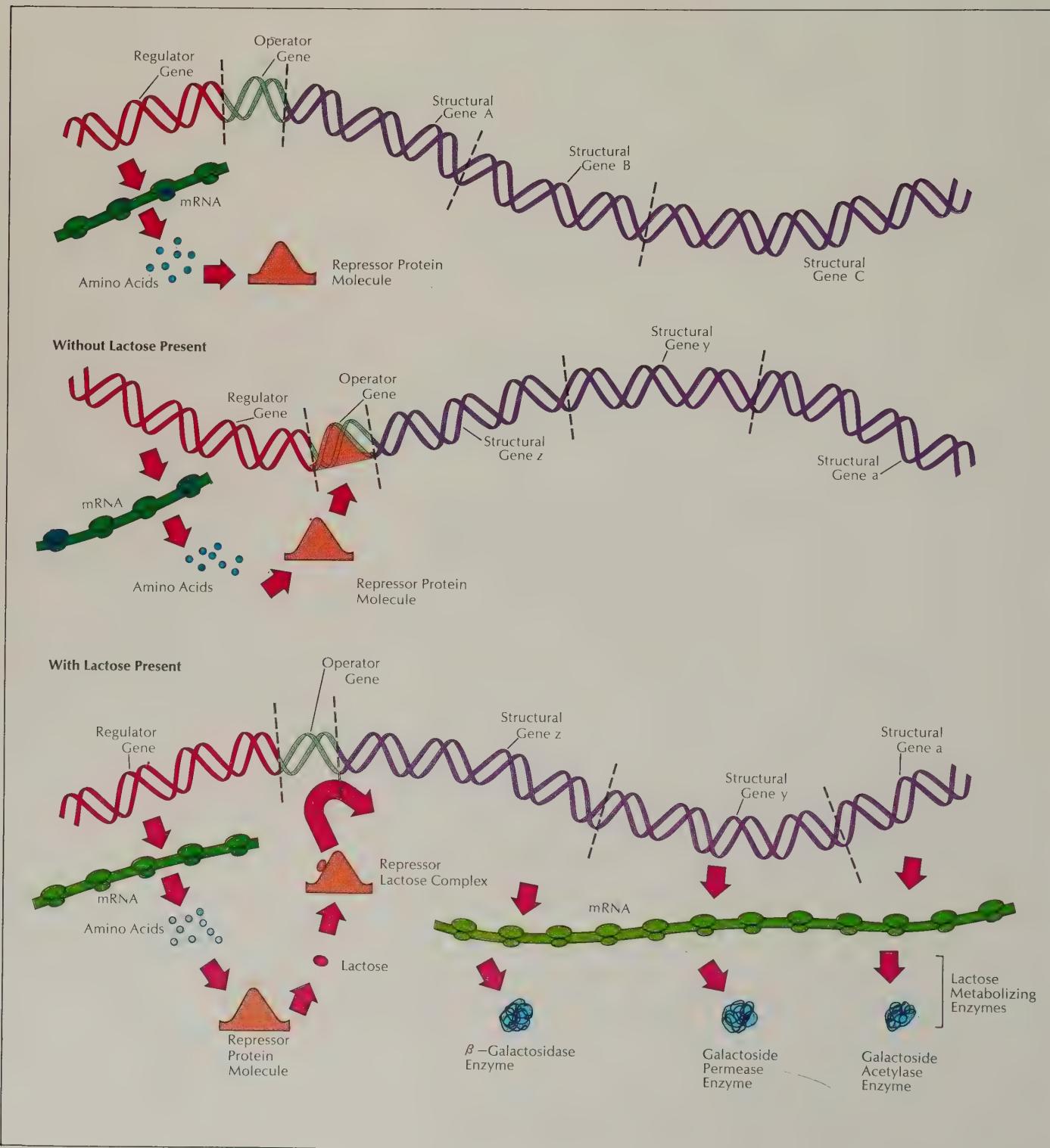
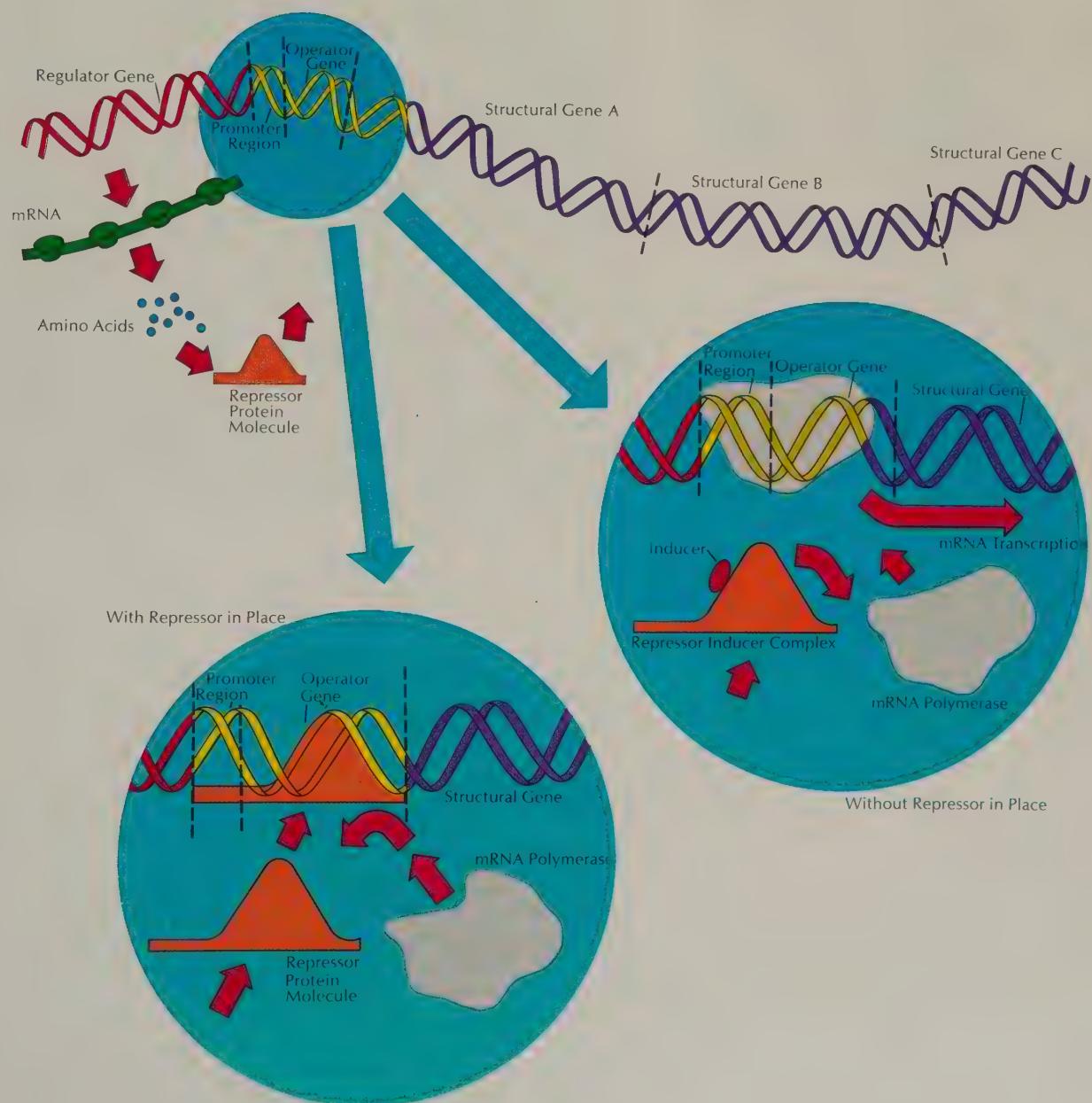


Figure 17.4. Current operon theory. Recent investigations indicate that there is a small region of DNA called the promoter next to the operator gene. The attachment site of the enzyme mRNA polymerase, which is necessary for mRNA transcription, is located at this promoter region. With the repressor molecule in place, the promoter region is blocked, and no transcription takes place.



easily, allowing the repressor to adjust quickly to changes in the concentration of the inducer. Enzyme production can thus "turn on" or "turn off" rapidly.

The active repressor (one that is not combined with an inducer) binds to a specific region of DNA called the *operator*. This region, which is about 20 nucleotides long, is near the point at which transcription of the β -galactosidase gene begins. The repressor forms many hydrogen bonds with this nucleotide sequence, which bind it tightly to the operator. Thus, a cell needs only a few active repressor molecules to ensure that at least one molecule is bound to the operator at all times.

A single repressor-operator mechanism can control the expression of more than one gene. The β -galactosidase repressor, for example, also regulates the production of β -galactosidase permease, a protein (located on the cell membrane) that facilitates the passage of lactose into the cell. The two genes are adjacent to each other and the mRNA for both genes is transcribed as one molecule. When the mRNA is translated, separate molecules of the two proteins are produced. A block of two or more adjacent genes, controlled by the same regulatory system, is known as an *operon*. (The operon containing the β -galactosidase gene is known as the lactose operon.) The operon is a very convenient method for coordinating the regulation of enzymes involved, for example, in a single sequence of metabolic reactions. A bacteria such as *E. coli* must regulate quite a few metabolic pathways in order to respond to various nutrients in its environment, and it therefore makes extensive use of operons.

It is also possible for a small molecule to turn off an operon rather than turn it on. The repressors for many operons require the presence of a small molecule called a *corepressor* in order to bind to the operator. In the absence of the corepressor, the repressor is unable to bind and the genes in the operon are expressed. The enzymes involved in the synthesis of many amino acids are regulated in this fashion. In such cases, the corepressor of the operon is an amino acid produced by a set of enzymes. The cell stops synthesis of these enzymes when the amino acid is already present in the medium.

The point at which RNA polymerase attaches to a gene and begins transcribing RNA has recently been shown to have an important regulatory function. This region, known as the *promoter*, does not appear to code for any part of the protein, but it does have a high affinity for the RNA polymerase. The nucleotide sequence of the promoter determines how well the polymerase binds to it and thus how frequently the polymerase transcribes the gene. For example, the promoter controlling the synthesis of mRNA for β -galactosidase repressor has a low affinity for the polymerase, and the gene is transcribed only once or twice every cell generation.

Many genes in bacteria have no repressor mechanism. They are expressed at a constant rate predetermined by the nucleotide sequence of the promoter. Such *constitutive* genes generally code for proteins, which the cell always needs in fairly constant amounts. Inducible or repressible genes also have promoters, however, in which case the promoters control the maximum rate at which the gene can be expressed.

RNA polymerase is a large component consisting of five different sub-units, only one of which, the σ (sigma) factor, recognizes and binds to the promoter region. Recent work has shown that different σ factors have different promoter specificities and may play a significant role in regulation.

Figure 17.5. Electron micrograph (left) showing characteristic attachment of RNA polymerase molecules to DNA strands; (right) DNA-dependent polymerase molecules from *E. coli*. ($\times 400,000$)



When *Bacillus subtilis* sporulate, for example, a new factor that is specific for genes involved in sporulation appears while at least one of the σ factors specific for the vegetative growth of these cells disappears.

CONTROL OF TRANSLATION

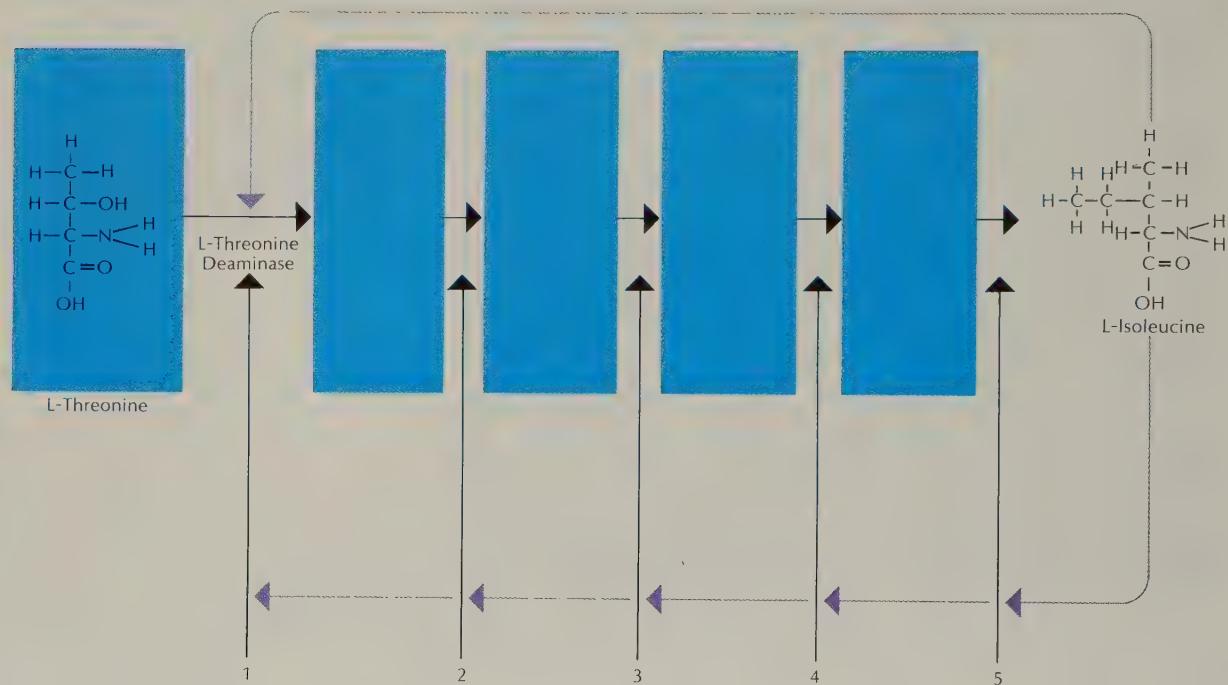
Control of gene expression at the level of protein synthesis is a widespread phenomenon, but the significance and mechanisms of such control are very poorly understood. For example, translational control occurs widely in plant spores and seeds and in animal oocytes. Some of the mRNA for proteins produced early in the development of the organism is present in spores, seeds, and oocytes, but actual protein synthesis by these messages does not begin until germination or fertilization takes place.

Translational control is also important in later developmental sequences, such as in the slime molds. Many new enzymes are made as slime molds aggregate and form fruiting bodies. In some cases, the synthesis of the mRNA for these enzymes begins or is even completed several hours before synthesis of the enzyme begins. Although the mechanism controlling the timing of the translational signals remains a mystery, recent work with viral RNA suggests that the secondary structure (for example, hairpin-type loops formed by base pairing between short nucleotide sequences) can determine whether or not a ribosome recognizes a "start" codon and that changes in secondary structure can affect the translation rate of a protein.

FEEDBACK INHIBITION

In addition to controlling the synthesis of a gene product, it is also possible to control the expression of the product itself. For example, *E. coli* normally synthesizes the amino acid isoleucine by a five-step pathway starting with

Figure 17.6. Isoleucine synthesis.



another amino acid, threonine (Figure 17.6). When too much isoleucine is produced or when it is added to the medium, it inactivates the first enzyme of the synthetic pathway, threonine deaminase, thus halting the biosynthesis and accumulation of isoleucine. As excess isoleucine becomes incorporated into proteins and the concentration of the free amino acid drops, threonine deaminase gradually becomes active again. This inactivation, known as *feedback inhibition*, occurs in much the same way as inducer inactivation of a repressor. In this case, isoleucine binds to a specific part of the enzyme molecule and changes its conformation (tertiary structure) so that it is no longer an active enzyme. This freely reversible process enables the enzyme to be active again when isoleucine is removed. Only the first enzyme of a synthetic pathway needs to be inactivated in order to stop synthesis of the amino acid and all of its precursors.

Feedback inhibition allows very rapid, sensitive control over the cells' metabolism. Whenever a product begins to accumulate more rapidly than it is being used, its synthesis is slowed down or halted. As the supply is used up, the synthesis is speeded up again. In contrast, induction or repression provides a somewhat slower adjustment to long-lasting changes in the cells' environment. Many enzymes are subject to feedback inhibition and repression of synthesis by the same end product.

REGULATION IN EUKARYOTIC ORGANISMS

Most of the available knowledge of regulation is virtually limited to bacteri-

al regulation. The big question now is what differences and similarities should be expected in cells of eucaryotic organisms. The requirements a eucaryotic cell places on its regulatory systems are quite different and, in many ways, more complex than the requirements of a bacterial cell.

Most eucaryotic cells undergo a precisely ordered development from an undifferentiated embryonic cell to a highly specialized cell in the adult organism. Many genes must be turned on and off in the right sequence during such differentiations. This situation is quite different than most bacteria experience. On the other hand, most eucaryotic cells live in a rather constant environment. The composition of human blood, for example, remains relatively constant, so that most cells in the body do not need to make large adjustments in their basic metabolic pathways. Eucaryotic cells must be regulated by a wide variety of external factors, not just by small molecules as in bacteria. Many changes that occur during differentiation are triggered by chemical effectors that are liberated by one cell or tissue and travel to a *responder cell*, whose metabolism is affected by the chemical signal. In some cases, the biochemical change triggered by the signal is permanent; in others, the change is transitory.

A number of regulatory mechanisms not commonly found in bacteria have already been described in various eucaryotes. Control exerted at the level of protein synthesis seems to play an important role in the timing of enzyme synthesis during differentiation. It now appears that σ factors can also perform this function. For example, a series of genes expressed early in development may include the gene for a new σ factor that is specific for genes to be expressed at a later time. Thus, the second set of genes cannot be transcribed until after the first set. And this second set may include yet another σ factor for a third set of genes, and so on. Another new type of control appears to regulate the degradation of mRNA and proteins. There is now evidence that a cell can vary the amount of an enzyme by varying the rate at which it (or its mRNA) is degraded, keeping the rate of synthesis constant. This mechanism may be more efficient for cells that need to vary the content of an enzyme over only a fivefold or tenfold range.

Almost every observed biochemical differentiation can be explained by variations on basic models such as that diagrammed in Figure 17.4. At the present time, however, such models are merely theoretical constructions.

There has been greater progress in the study of the chemical nature of effectors that pass from one cell to another. From these studies comes evidence to indicate the existence of several different mechanisms for transmitting a signal from one cell to another: (1) exchange of genes; (2) cytoplasmic fusion; (3) exchange of small molecules; (4) control of hormone effectors; and (5) interactions through contact of cell surfaces.

Genetic Exchange

Perhaps the most direct mechanism of cell-cell interaction is the injection of certain genes of one cell into another cell. In this case, DNA acts as the effector, directly transcribing mRNA in the responder cell. Viral transduction, bacterial transformation, and bacterial conjugation are the best-studied examples of this form of interaction.

Direct exchange of genetic information in mammalian systems has been suggested to exist only in a part of the immune response mechanism. There is evidence that when the cell recognizes a foreign particle (antigen), it synthesizes an mRNA molecule coding for an antibody and transmits this

Figure 17.7. The development of a muscle colony from a single myoblast at 3, 6, and 13 days. The multinuclear cells that are formed by the sixth day probably involve cell fusion. Differentiation of myoblasts from a single spindle shape to an amoeboid shape is also shown.

mRNA to secondary cells, where the antibody protein is translated. If subsequent research should support this model, then it is a clear case of cell-cell interaction via nucleic acid.

Cytoplasmic Fusion

Another common form of cell-cell interaction involves the fusion of the cytoplasm of two or more cells to form a multinucleate, *syncytial* tissue. In such a tissue, molecules can travel from one nucleus to another without passing through a cell membrane. Cell fusion occurs in the forest mold *Physarum*, forming a large, yellowish mass containing thousands of nuclei. The amazing property of this organism, or syncytium, is that all the nuclei divide simultaneously.

During chick embryogenesis (development of the embryo), large numbers of cells aggregate within the primordial blood vessels and fuse to form syncytial "blood islands." The nuclei in this tissue divide simultaneously about once an hour. As the nuclei multiply, the tissue begins to synthesize hemoglobin rapidly. After a period of hemoglobin accumulation, the blood islands slowly separate into individual cells that make up the original blood cells of the chick embryo. This natural mechanism apparently serves as an

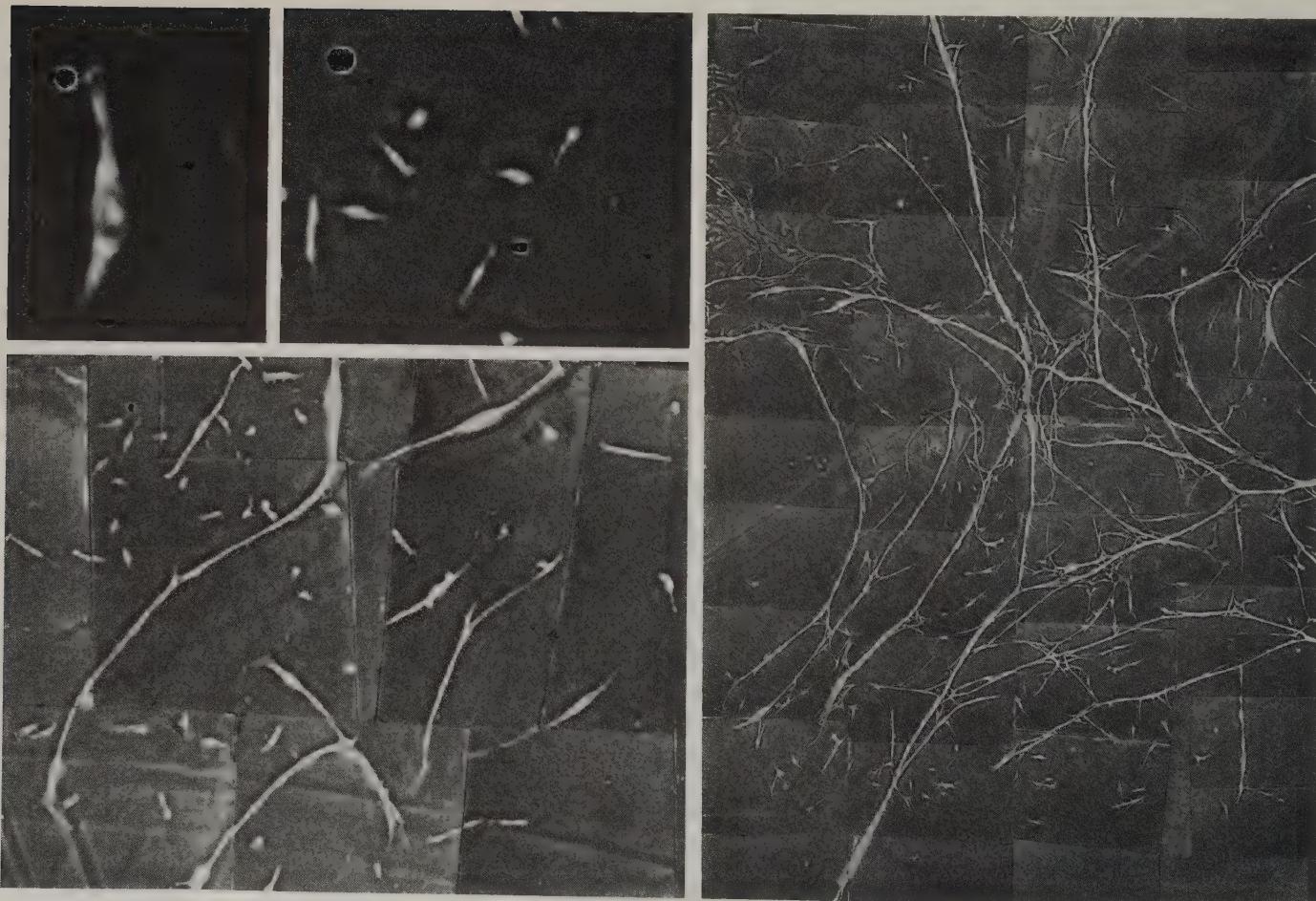


Figure 17.8. Cross-feeding of nutrients in bacteria.

efficient means of initiating hemoglobin synthesis. The genes for hemoglobin synthesis can be turned on simultaneously within the single multinucleate mass, rather than in separate blood cells scattered throughout the embryo.

Cross-Feeding

Most cells, from bacteria to mammalian cells, must communicate with other cells across plasma membranes. A fairly large number of different mechanisms has evolved for accomplishing such communication.

A simple form of cell-cell interaction involving diffusible molecules can be demonstrated with mutant strains of bacteria—for example, with strains that are deficient in enzymes required for the synthesis of arginine from ornithine (\rightarrow ornithine \rightarrow citrulline \rightarrow arginine). One strain is unable to convert ornithine to citrulline and therefore can survive only in a medium containing citrulline or arginine. A second strain is unable to convert citrulline to arginine and therefore can survive only in a medium containing arginine. However, if the two strains are mixed together, both can survive in a minimal medium. The first strain accumulates ornithine, some of which diffuses out of the cell and into the medium. The second strain converts this

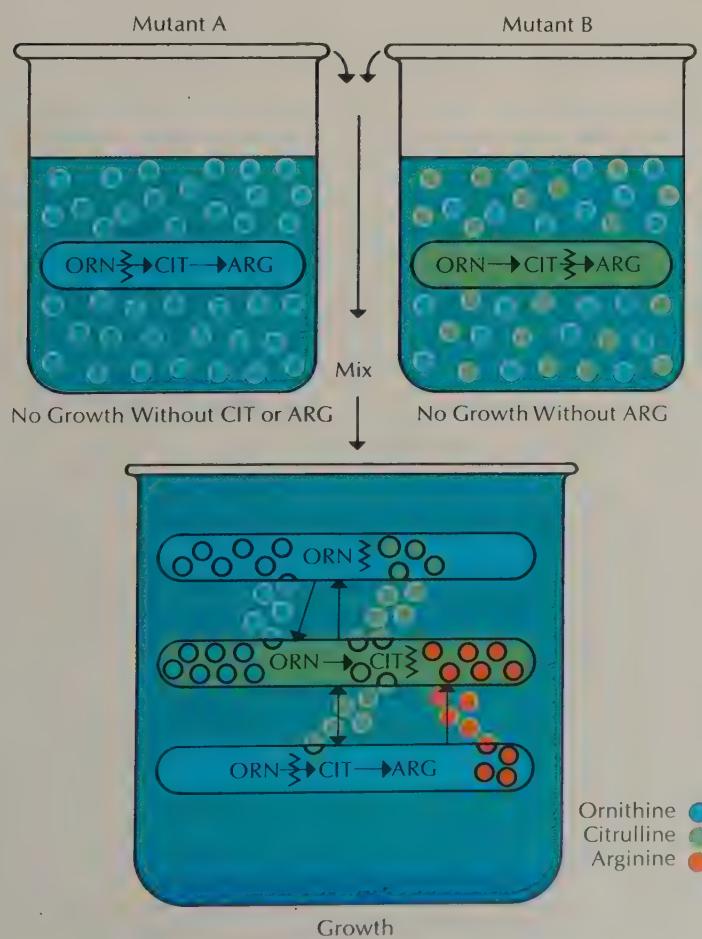
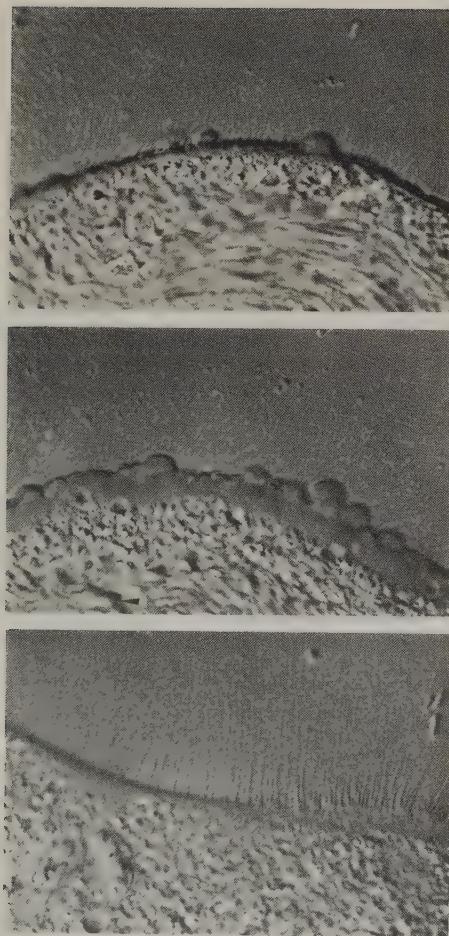


Figure 17.9. The advancing end of this slime mold is moving by amoeboid motion.



ornithine to citrulline and accumulates citrulline, which diffuses into the medium. The first strain converts the citrulline to arginine, and some arginine diffuses back to feed the second strain (Davis, 1950).

Cross-feeding of nutrients also occurs among mammalian cells in culture. Many necessary nutrients are synthesized by cells and diffuse into the medium. When the population density of cells in a culture is low, the nutrients may be so dilute in the medium that some cells starve. Only when sufficient population density is reached can deficient cells grow without the addition of nutrients. This "mass effect" results from the combined nutrient leakage of many cells, conditioning the medium with a sufficient concentration of nutrients so that all cells may grow. Thus, the growth of one cell depends on the presence of all the others. Interactions of this type in cultures of human cells involve cross-feeding of several different substances (Eagle, 1965).

The cross-feeding of nutrients may be regarded as a simple control signal carrying only the message "grow" or "don't grow," but it is an important control mechanism for tissue development, at least in cultures. In the intact organism, cellular interaction via cross-feeding may be the basis for many mass effects that restrict cellular growth or development until a certain population of cells is achieved. This mechanism guarantees that differentiation of organs will proceed only when there are enough cells to form the required tissue.

Hormone Effectors

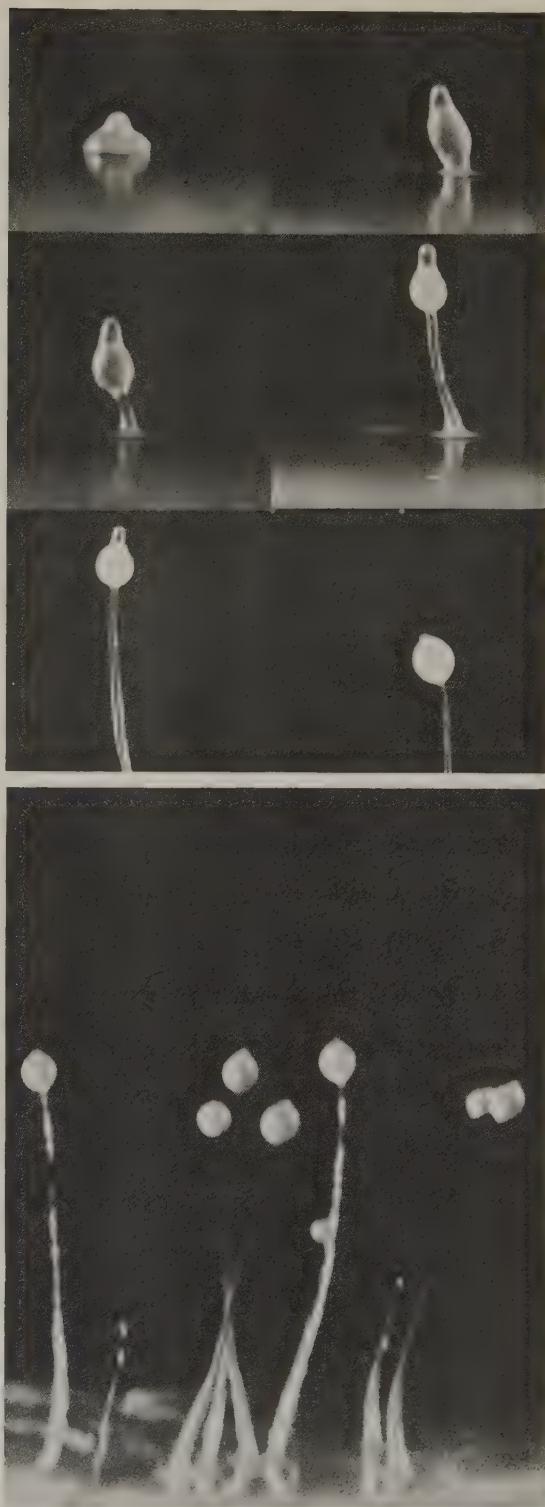
Hormonal induction of differentiation occurs during embryogenesis as well as in adult life. Complex organisms have evolved circulatory systems that transport oxygen and food products to the cells and remove CO_2 and waste products. The circulatory system also carries chemical signals among the cells of the organism, thus integrating the functions of various organs and adapting their biochemical properties to changing conditions. Certain specialized cells secrete hormones into the blood, which eventually carries them to responsive "target" cells. When a hormone reaches a target cell, it stimulates a series of biochemical events that result in a specific response. The exact response depends on the specific hormone involved and the nature of the target cell (Chapter 21).

Surface Phenomena

Many cases of cell-cell interaction apparently involve either nondiffusible molecules or direct contact between cells. Such a case is found in the growth and differentiation of myoblasts in tissue culture. Myoblasts differentiate only in the presence of the insoluble protein collagen, which is secreted by fibroblasts. Growth and differentiation of muscle tissue in intact embryos may also depend upon either direct contact with collagen or "conditioning" of parts of the embryo by fibroblast secretions.

As normal cells multiply mitotically in tissue culture, they separate and creep along the surface of the culture chamber by amoeboid motion. This kind of motion is characteristic not only of cultured cells and of the amoeba (from which it derives its name) but also of many embryonic cells and of white blood cells. The cell membrane is distorted by the formation of slender projections, or *pseudopodia*, and by a sort of undulating action. Cytoplasm often streams away from the cell body into the pseudopodia, which then may flare out at their ends or branch to form other pseudopodia.

Figure 17.10. A sequence in the development of the fruiting bodies of the cellular slime mold *Dictyostelium discoideum*.



Whichever side of the cell displays the greatest activity of this kind is the "front" of the cell, because this activity tends to pull the cell along with it. If two cells make contact with each other in tissue culture, all amoeboid motion along the surfaces of contact ceases—a phenomenon known as *contact inhibition*. Amoeboid activity on the opposite side of each cell then increases and the cells move apart.

The amoeboid cells in tissue cultures are very active metabolically, synthesizing new DNA, RNA, and proteins. Mitotic division occurs frequently. When the cell population eventually covers all the available surface as a single layer, each cell is necessarily in contact with other cells at all points on its horizontal perimeter. Contact inhibition stops all amoeboid motion, whereupon the rate of synthesis of nucleic acids and proteins decreases. Growth and division are halted dramatically (Abercrombie and Ambrose, 1958).

It appears that contact inhibition plays a major role in normal animal development, causing cells to cease growing and dividing when a tissue has been formed. A tumor may be formed by cancerous cells that continue to grow and divide despite close contact between cells. In some cases, cells become cancerous after infection by an oncogenic, or cancer-producing, virus. In culture, cells infected by such a virus continue to grow and divide even after a complete layer has been formed in the culture dish. Because contact inhibition apparently plays a central role in differentiation and failure of this process may be crucial to the development of cancerous tumors, many biochemical studies of the process are being actively pursued (Egylud and Szent-Györgyi, 1966; Rubin, 1970).

A MODEL SYSTEM

Analysis of cell-cell interaction in embryogenesis is technically difficult because so few cells are involved. In many cases, the interactions of primary interest may occur among fewer than 100 cells. Thus, the biochemist has only a few hundredths of a microgram of material per embryo to work with, and important molecules may be present in such minute amounts that he cannot detect them. A few unusual cases of extreme differentiation—such as the formation of feathers, eye lens, blood, and muscle—have been studied successfully by biochemical techniques, but it has been difficult to relate knowledge about these unusual systems back to the overall development of the organism.

Another approach involves the study of simpler organisms that undergo some sort of developmental processes. One organism that has been extensively studied is the cellular slime mold *Dictyostelium discoideum*. Single cells of this organism grow on decaying forest leaves and are almost identical to amoebae (Raper, 1935). They ingest bacteria and divide mitotically. When the individual cells run out of food, however, an amazing sequence of structural changes transforms a group of the cells into a multicellular organism. The cells gather together to form a fruiting body, within which some cells are transformed into encapsulated spores. The spores can survive extended periods of drought or cold without food. They later germinate to form new, individual, amoeboid cells (Figure 17.10).

The process of differentiation, or specialization, that transforms individual amoeboid cells into the specialized cells of the fruiting body and spores has been extensively studied. Various effectors are exchanged among cells to coordinate the differentiation processes. These effectors act within the

Figure 17.11. The mouse in this photograph was inoculated subcutaneously with a small number of mouse cells transformed by the animal virus SV-40. Within three weeks, these cells grew to produce the large mass visible in the mouse.



cells to exert specific controls over the activation of genes and the timing and rates of translation of various proteins. The precise nature and mode of action of the effectors is still unknown.

CANCER AND CELLULAR CONTROL

Any study of cellular control inevitably comes face to face with its malevolent extreme—the lack of control underlying the wild proliferation of cancer cells. Like normal cells, cancer cells presumably contain a normal complement of DNA. Their abnormal behavior probably is a result of some malfunction of the cellular control mechanism, which removes normal restraints upon growth and division. The cancerous cells then multiply to such an extent that they crowd out and starve other body cells.

Cancer cells may form from almost any kind of living animal cell—cells of the brain, liver, kidney, bone, blood, skin, or other tissues and organs. Most cancer cells formed from specialized cells continue to perform the specialized function of the parent cell. Cancerous hormone-producing cells still produce hormones; cancerous cells of the immune system continue to manufacture antibodies. In almost all respects, the cancer cell is functionally similar to its normal ancestor, but it grows and divides uncontrollably.

Many different influences have been shown to cause a normal cell to lose its restraints upon growth and division and to become cancerous. Among the cancer-causing agents, or *carcinogens*, are atomic radiation, chemicals of many different varieties (including certain combinations of otherwise beneficial drugs), certain hormones, and viruses. The causes vary, but the effects are thought to be essentially the same. In both man and other animals, some individuals apparently inherit a genetic susceptibility to cancer. This hereditary tendency probably is caused by malfunctioning mechanisms that normally counteract the effects of carcinogens.

When a carcinogen acts upon a normal cell, it somehow must disrupt the cell's genetic machinery in such a way that it either alters or destroys the normal checks on growth. The change is permanent. Once the genetic machinery has been altered, each cancer cell produces cancerous daughter cells, and all following generations are cancer cells. Without restraints on growth and division, the cancer cells spread rapidly through the organism and, if they are not controlled, eventually kill it.

Cancer cells clearly differ from normal cells in the way that they interact with other cells, both in the live animal and in tissue culture. Cells in benign tumors are contact inhibited by normal cells but not by other tumor cells. The tumor continues to enlarge because the cells inside it grow without restraint, but it does not spread to other parts of the body because contact with normal cells inhibits cell growth on the surface of the tumor. Malignant cancers, on the other hand, are not contact inhibited by cancer cells or by normal cells. Thus, they grow outwardly from the periphery of the tumor, as well as within it, and can spread to other areas of the body. As mentioned earlier, the loss of contact inhibition is also observed among cancer cells in tissue culture.

Some researchers also suspect that cancer cells are less "sticky" than normal cells. The cancer cells move more freely among one another in culture than do normal cells. This freedom of movement may be related to the failure of contact inhibition and other processes that inhibit growth and division in normal cells. The stickiness of normal cells is very selective. If

Figure 17.12. Cancer cells are the result of some malfunction in the cellular control mechanism. Normal growth restraints are lacking, and the cells multiply in a wild fashion.

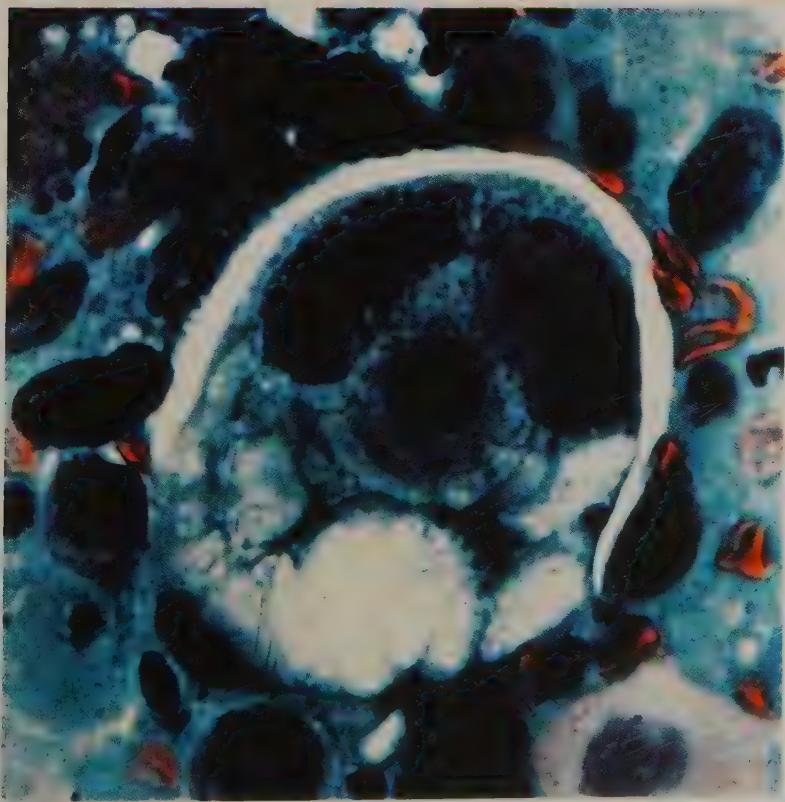


Figure 17.13a (left). Mouse embryo cells. These normal cells grow in monolayers and stop growth when they are contact inhibited.

Figure 17.13b (right). Same cells transformed by a polyoma virus. These cancerous cells are growing wildly because contact inhibition does not occur.

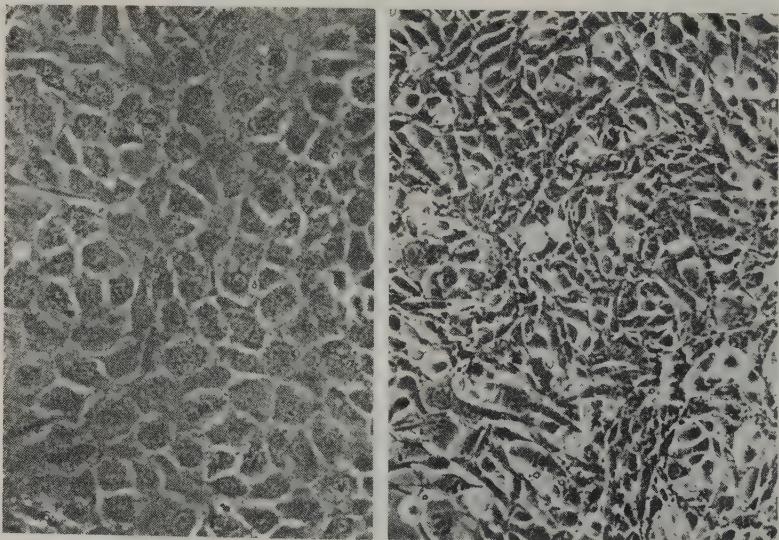
kidney and liver cells are mixed in a culture, the two kinds of cells will separate and form clusters of kidney tissue and clusters of liver tissue. When cancerous liver cells are mixed with cancerous kidney cells, this separation does not occur. These observations support the conclusion that cancer cells have abnormal surfaces that fail to carry out normal interactions with adjacent cells.

VIRUSES AND CANCER

Tissue-culture techniques are used to determine how normal cells differ in their interactions from cancerous cells. Bacterial cells, such as *E. coli*, cannot be used in these studies because they do not form tissues; they separate from one another after division, rather than forming clumps as animal cells do. The cultivation of normal mammalian cells in the laboratory, however, is tedious. Only cells from embryos or from organs such as the kidney and liver—where cell growth is normally relatively unrestrained—can be cultured readily. Cancer cells, on the other hand, can easily be grown for generation after generation in cultures.

Radiation and chemicals cause cancer in many types of cells, but the mechanism by which they do so is difficult to study in cell cultures because these carcinogens affect a number of genes and other cellular components at the same time. Thus, many molecular biologists have come to rely on cancer-causing viruses as a means of exploring the cellular control mechanisms that cancer disrupts.

Viruses are known to act by introducing new genes into a cell, causing the cell machinery to produce new viruses at the expense of its normal structure and functions, and eventually destroying the cell as the new viruses are liberated. A cancer-causing virus also introduces new genes into the cell and causes the cell to produce new viruses, but it does not kill the cell. Instead, it causes the cell to multiply rapidly, with the virus being contained in each new cell that is formed. Experiments carried out in 1968 by Renato Dulbecco and his group showed unequivocally that an animal virus—known as SV-40—introduces a small group of genes into the chromosomes of a normal cell. These genes become irrevocably bound there and cause



the cell to multiply as a cancer cell, replicating the viral genes in each division. Dulbecco and other researchers chose the SV-40 virus and others very much like it because the number of genes in the viral DNA is very small. Because new research methods make it possible to turn off or delete one gene at a time, it should not be too long before the gene that initiates the cancer process is identified.

The Jacob-Monod theory of cellular control has been used to suggest a model for the cause of cancer. For example, the cancer process might involve production of an inducer that combines with the repressors normally controlling the production of proteins involved in cell growth and division. In late 1969, Robert J. Huebner of the viral carcinogenesis branch of the National Cancer Institute announced his own theory, which is related to the theory of Jacob and Monod. According to the Huebner theory, all normal cells contain a type of RNA virus that stays with the cell from the period of embryonic development to maturity. This RNA virus—called a C-type particle by cancer researchers—contains an oncogene that can be triggered into action by built-in genetic defects, cellular aging, or any number of outside factors including carcinogenic chemicals, viruses, or radiation. When the oncogene is activated, suggests Huebner, the substance that it produces releases the inhibitions on the normal DNA of the cell, converting it to a wildly reproducing cancer cell.

Although Huebner's theory has not been definitely proven, RNA C-type viruses are known to cause cancer in a number of different animal species and have been linked, at least by association, with certain human leukemias.

More support for the Huebner theory comes from much earlier work by Henry Kaplan and Ludwik Gross, who showed that irradiation of normal rat cells causes production of carcinogenic RNA viruses. These viruses produce cancer tumors when they are injected into other mice that have not been irradiated. These results suggest that the RNA viruses were present all along, but they were unrecognized until the radiation triggered an inactive gene that caused the cell to become cancerous.

Only a small sampling of the many theories and experimental approaches being used in the study of cancer has been represented here. Cancer research and research on cellular control mechanisms are closely interrelated, and an advance in either area is almost certain to be useful to researchers in the other.

FURTHER READING

The classic paper by Jacob and Monod (1961) sets forth the original version of the operon theory and discusses many of its implications. Further discussions of cellular control processes will be found in articles by Britten and Kohne (1970), D. D. Brown (1967), Changeux (1965), Davidson (1965), Gurdon (1968), Martin and Ames (1964), Miller and Beatty (1969), Moscona (1961), and Ptashne and Gilbert (1970).

Figure 17.14. RNA type C virus particles, showing mature type C particles in an intercellular space and budding of these particles in the insert at upper right. ($\times 50,000$)



18

Development



How does a single cell, the fertilized egg, differentiate into a multicellular organism? It is clear that the fertilized egg of both plants and animals carries in its chromosomes a complete set of genes for all cells in the mature organism. During development, each cell in the organism, excluding the gametes, contains a complete set of these genes. Although the genetic content remains constant, different sets of specialized cells arise during development, producing specific tissues and organs. But how does this process occur?

As indicated in Chapter 17, the process of cellular differentiation is now interpreted in terms of selective gene expression. The fertilized egg of a plant or an animal develops into a multicellular organism by turning on and off specific genes at particular times during development. Although biologists have learned much about this control system, its major mechanisms are far from fully understood, and research in this area of development is being pursued by many biologists today.

Although the basic questions concerning the molecular mechanisms of development remain unanswered, much is known at the cellular level about the sequences of events that occur during the development of a large number of organisms. For many years, developmental biologists have described the orderly and sequential changes occurring in an organism from the time it "begins life" until it dies. In higher plants and animals, this developmental cycle begins with gametogenesis; continues through fertilization, embryogenesis, and maturation into adulthood; and ends with aging and death.

DEVELOPMENTAL PROCESSES

The processes of development are obviously different in plants and animals. Because these processes have been studied by specialists in totally separate fields of research, different concepts and different vocabularies exist for the description of plant development and animal development. Nevertheless, it must be emphasized that both plant and animal developmental biologists are concerned with many of the same questions. In general terms, both attempt to trace the sequence in which various parts of the adult organism are developed and to understand the interactions that cause particular cells to differentiate in particular ways at particular times. More specifically, problems such as wound healing, regeneration, cellular differentiation, cancer, and aging are developmental problems common to both plants and animals.

Plant and animal developmental biologists have been largely concerned with the processes by which a newly formed individual acquires structure, specialized cells, and a net size. These processes fall into three major categories: *morphogenesis*, *differentiation*, and *growth*. Because the information in each of these areas is voluminous, in this chapter it will be possible to present only a sampling of the available information about development, concentrating chiefly upon the flowering plants and vertebrate animals.

Morphogenesis

Morphogenesis is a general term used to describe processes by which tissues or germ layers are shaped into organs and by which the organism acquires its overall adult shape and form. In plants, morphogenesis is accomplished chiefly through differential growth—that is, through tendencies for cells to elongate or to divide along particular planes and axes. In animal

development, movements of cells—either by individual migration from one place to another or as sheets of cells—play a major role in morphogenesis.

One major difference between morphogenesis in plants and animals is that plants contain groups of relatively undifferentiated cells that continue to form new tissues and organs. These embryoniclike cells continue to divide throughout the life of the plant, retaining the capability of adding new tissues and organs continuously and indefinitely in response to environmental changes. On the other hand, morphogenetic movements in animals establish tissue and organ primordia relatively early in development. Once the organ primordia are fixed, the overall shape of the animal is determined, and, except for regeneration or developmental abnormalities, no new organs are produced in the animal.

The ability of a plant to develop new organs throughout its life is called *indeterminate growth*. As a result of this morphogenetic potential, the shape and form of an adult plant varies greatly with the environmental conditions under which the plant grows. Although the individual organs and tissues show forms unique to the species, even the number of organs (such as leaves) may vary greatly from individual to individual. In contrast, because of the way morphogenesis occurs in animals, two individuals of the same species are apt to be quite similar in size and shape and certainly will have the same numbers of various organs.

Differentiation

Adult plants and animals are not simply enlarged copies of the fertilized egg. In a multicellular organism, the thousands or millions of cells produced by divisions of the zygote must become differentiated into many different kinds of specialized cells. Not only must the proper kinds of cells be produced but they must be produced in the proper numbers and assorted into the proper locations in the developing embryo. Each group of cells destined to produce a specific adult tissue passes through a series of biochemical and structural alterations that culminate in the formation of a tissue appropriately specialized for its function. This process is called *differentiation*.

During differentiation in a multicellular organism, cells acquire more and more specific determinations, and the paths open to each cell and its descendants become more and more restricted. In some species, determination occurs at very early stages of embryonic development, apparently as a result of unequal distribution of cytoplasmic components during the early cleavages of the zygote. In other species, determination occurs at a later stage of development, apparently as a result of interactions among neighboring cells. Such control of cell differentiation by influences from neighboring cells is called *induction*.

In most cases, the process of determination cannot be detected by biochemical or structural changes in the cell. The cell in which determination has occurred appears identical to other nonspecialized cells, but observation shows that it now is committed to a particular course of development, which can be modified only partially by outside influences. Specialized structures or chemicals within the cell may not become apparent until many division cycles after determination.

In most cases, once a cell has become determined upon a particular course of differentiation it will pass this tendency on to its descendants. Such cells pass on not only their genetic information but also the regulatory

agents that control the use of the genetic information. These instructions might be contained in the portion of the cytoplasm obtained during division, or they might be inherited in the form of certain genes that are more-or-less permanently activated or inhibited.

Although the actual mechanisms of differentiation are not known, it is clear that the course of differentiation in both plants and animals is controlled in time by hereditary influences and by substances entering the cell from its environment.

Growth

Growth is a universal feature of the development of an individual organism. In simple unicellular organisms, binary fission produces daughter cells that are quite similar in structure to the parent cells. After a period of growth in the daughter cells, during which time structures within the cell are duplicated or enlarged, the cell reaches a size at which it is prepared to divide again. In multicellular organisms, far more impressive feats of growth take place in the development from a single cell to the large body of the mature individual. Growth can be measured in terms of length, weight, number of cells, or amounts of various substances. Whatever the measure used, the growth rate varies with time, in most cases reaching a maximum at some point during early life and becoming nearly zero (or even negative) in the mature organism (Figure 18.1). Growth is accomplished both by cell division and by enlargement of cells.

Variations in growth patterns among various organisms are numerous. Many organisms have a simple sigmoid (S-shape) growth curve. Organisms that pass through various abrupt changes in the life cycle—for example, the moltings and metamorphosis of an insect—may have several periods of rapid growth separated by intervals of zero or negative growth. Similarly, a woody plant such as a tree grows each year during favorable seasons.

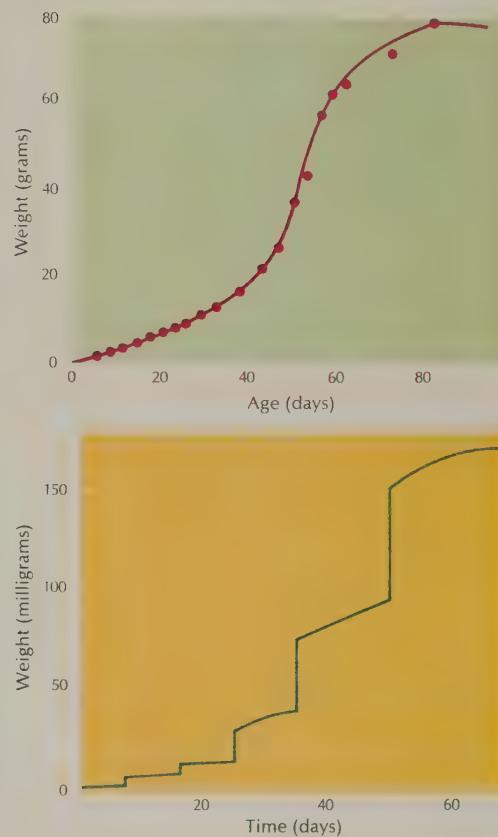
In most multicellular organisms, the early stages of growth are marked by repeated mitoses of all the cells in the organism. As the number of cells increases, the amount of growth accomplished with each set of cell divisions increases. As development progresses, however, more and more cells become differentiated to serve specialized functions, and they cease to grow and divide. Thus, more and more of the machinery of the organism is diverted from growth to other specialized tasks, and the growth rate decreases. Even in the mature organism, some cells continue to grow and divide, but the rate at which new materials are added is barely sufficient to compensate for the loss of materials through processes of aging.

The source of nutrients providing the energy necessary for growth during embryogenesis varies with different species. In most higher plants, insects, fish, amphibians, and birds, the energy reserves are stored materials such as plant endosperm and animal yolk. In mammals, nutrients from the mother via the placenta nourish the embryo during intrauterine growth. Once the organism begins its development into a mature individual, nutrients for growth are provided from photosynthesis in plants and by active feeding by most animals.

Events that occur during growth are closely interrelated. Changes occurring in one part of the organism often trigger changes in other parts. This complex network of *correlative effects* results in the organized growth of an adult organism. In most cases, these interactions involve chemical messengers, or *hormones*, that can move from cells in one region of the organism

Figure 18.1a (above). A typical S-shape growth curve. This curve shows the increase in weight of a young corn plant.

Figure 18.1b (below). The growth rate of an insect.



to cells in another—for example, growth hormone in man, growth and molting hormones in insects, and the hormone gibberellin in plants.

DEVELOPMENT OF THE FLOWERING PLANT

As higher plants develop, they maintain regions of growth and development throughout the entire life of the organism. Stems and roots are extended and new organs are formed through cell division, elongation, and differentiation in certain growth regions. Growth and development can be regulated by hormones that interact with environmental factors such as heat and light, or that cause specific changes elsewhere in the plant. Although the plant is relatively limited in its ability to respond rapidly to changes in external conditions, it can easily shed old organs and grow new ones as conditions change.

Embryonic Development

Although much less is known about plant development than about animal development, several exciting experiments carried out in recent years have revealed new aspects of the sequences in the embryology of flowering plants that eventually produce the adult.

The embryonic development of the flowering plant occurs in the ovule of the adult. After pollination, a double fertilization occurs, forming a diploid zygote and a triploid endosperm nucleus. The zygote nucleus remains inactive while the endosperm nucleus divides rapidly. During this time, the ovule tissues synthesize and transport material into the zygote to form the surrounding endosperm.

After the endosperm is well developed, the zygote nucleus, which is now surrounded by the endosperm, begins to divide mitotically. The zygote becomes polarized and divides unequally to yield two cells that differ both in size and in contents. In the zygote, vacuoles tend to cluster at one end of the cell, while most of the cytoplasm and organelles move to the other end. The wall formed during the first division of the zygote separates a small, densely cytoplasmic *terminal cell* from a larger, more vacuolate *basal cell*.

The developmental fate of these two cells is quite different. The terminal cell produces cells that differentiate into the embryo itself, whereas the basal cell divides to form a *suspensor cell* and a new basal cell. Further divisions of the suspensor cell produce the stalklike *suspensor*, which attaches the embryo to the rest of the seed.

It is clear that the determination of these two cells is established prior to the completion of the first division. Although there are no experimental data, the obvious unequal division of cytoplasmic components in this case is strikingly similar to the unequal distribution of cytoplasmic components involved in some cases of cell specialization in animals.

The first few divisions of the terminal cell produce a spherical or globular embryo, composed of several cells. As a result of morphogenesis (differential growth) and cellular differentiation, the cells in the embryo become organized into the basic organ primordia of the adult—the shoot apical meristem, the root apical meristem, and, in the case of dicots, the two cotyledons.

The word “meristem” refers to those cells in the plant that continue to divide mitotically. The *apical meristems* are located at the tip (apex) of shoots and roots, and the *lateral meristems* are positioned around the cir-

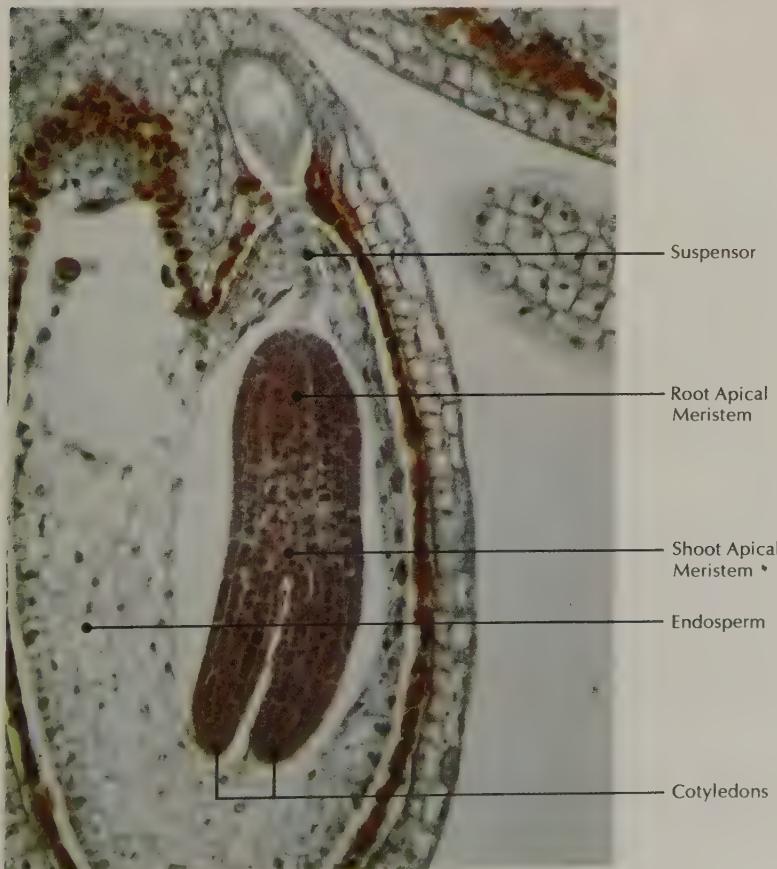
cumference of the shoots and roots. These are the embryoniclike cells that have the capability of forming new organs continuously throughout the life of the plant.

Growth regions that will form the cotyledons become established in the globular stage of the embryo. The dicots have two growth regions, producing two cotyledons that grow vertically upward and make the embryo somewhat "heart-shaped." Between the two cotyledons of dicots, or adjacent to the single cotyledon of monocots, is a growth region that forms the apical meristem, or growing tip, of the young shoot. At the other (suspensor) end of the embryo, the apical meristem of the radicle, or primary root, becomes established. As the embryo grows, the apical meristems of root and shoot become increasingly separated, pushed apart by the cells they form. Elongation of the axis between the two apical meristems produces the "torpedo-shape" stage of embryo development.

During this torpedo-shape stage, cellular differentiation in the embryo becomes readily apparent. A central core of elongated, densely cytoplasmic cells—the procambium—is surrounded by more vacuolate cells of the ground meristem (Figure 18.2). Differentiation occurs among the cells that have been formed by divisions of the meristems and left behind in the central part of the embryo as the meristems grow outward.

The extent to which the embryo develops within the ovule varies from species to species, but growth, division, and most metabolic activities are

Figure 18.2. Longitudinal section of a *Capsella* embryo. The darkly stained embryo is surrounded by endosperm. Note the suspensor (tissue strand) connecting the plant embryo to the surrounding wall of the ovule.



halted when the embryo is enclosed in the seed coat. The embryo remains dormant while the seed is transported away from the parent plant, and in many species dormancy continues for some time after the seed has come to rest. When the seed germinates, growth and development of the embryo resume. The apical meristem of the root divides to form the root tissues. The apical meristem of the shoot gives rise not only to the tissues of the stem but to young leaves, *leaf primordia*, and in some cases, also to *bud primordia*. The procambium develops into the vascular tissues of the plant—the xylem and phloem systems—which carry fluids through the plant body. The ground meristem forms the ground tissue, which may differentiate into various specialized tissues that serve functions of storage, mechanical support, and photosynthesis.

During the 1930s, several experimenters attempted to isolate embryos from ovules and grow them in tissue culture to determine how far the embryo would develop when removed from the adult tissues. Very early embryos would not develop if isolated in a defined liquid culture medium containing minerals, sugars, vitamins, yeast extract, light, carbon dioxide, and oxygen. However, if embryos were not isolated until they had reached the torpedo-shape stage, they would differentiate into normal seedlings. The older the embryo was prior to its isolation, the simpler the culture medium could be to support normal development. For example, if embryos were isolated just after the torpedo-shape stage, they survived and differentiated in medium without vitamins or yeast extract. Embryos isolated at the onset of dormancy develop normally in culture medium containing only minerals.

Johannes van Overbeek and his coworkers (1941) succeeded in culturing very young embryos (in the heart-shape stage) by adding coconut milk, which is actually the liquid endosperm of the coconut seed. Since that time, extracts of the endosperm of other kinds of seeds have been shown to have similar effects in supporting *in vitro* development of very young embryos. An analysis of coconut milk shows that it contains a mixture of basic nutrients such as amino acids and sugars, but more importantly, it contains a complex mixture of hormones.

It is now clear that a mixture of hormones, normally present in the endosperm, is essential for proper development of the early embryo in culture. For example, heart-shape embryos of shepherd's purse (*Capsella*) can be grown in a medium containing various nutrients and a balance of three hormones. In the absence of these hormones, the embryos show little or no growth. An imbalanced mixture of the hormones may cause the embryo to grow into a shapeless, tumorlike mass of cells (Overbeek, et al., 1942; Raghavan and Torrey, 1963).

These experiments demonstrate that hormones present in the endosperm of the zygote are essential for the differentiation of the embryo into the adult plant. Because the endosperm does not begin forming until after fertilization, the influence of the endosperm upon the embryo is not expressed until the early stages of embryogenesis. Therefore, when an early embryo is isolated in a defined medium, without the hormones, it fails to develop.

Single cells taken from carrot embryos can be cultured similarly, and under proper conditions these cells form embryolike structures, or *embryoids*, which pass through development stages resembling those of a normal embryo (Figure 18.3). A single cell from an embryo, isolated from

Figure 18.3. Stages in the development of carrot embryos. Shown below are carrot cells.

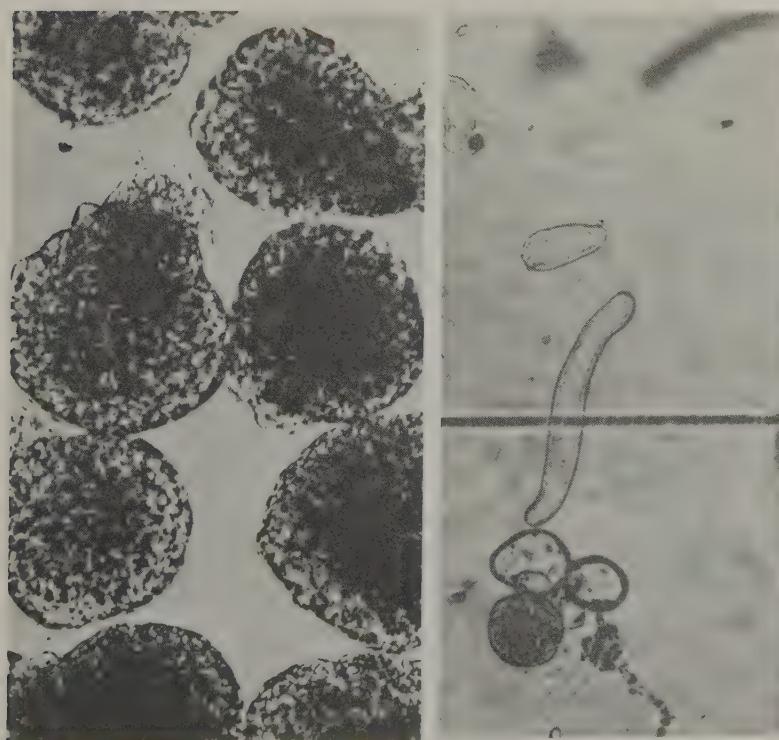
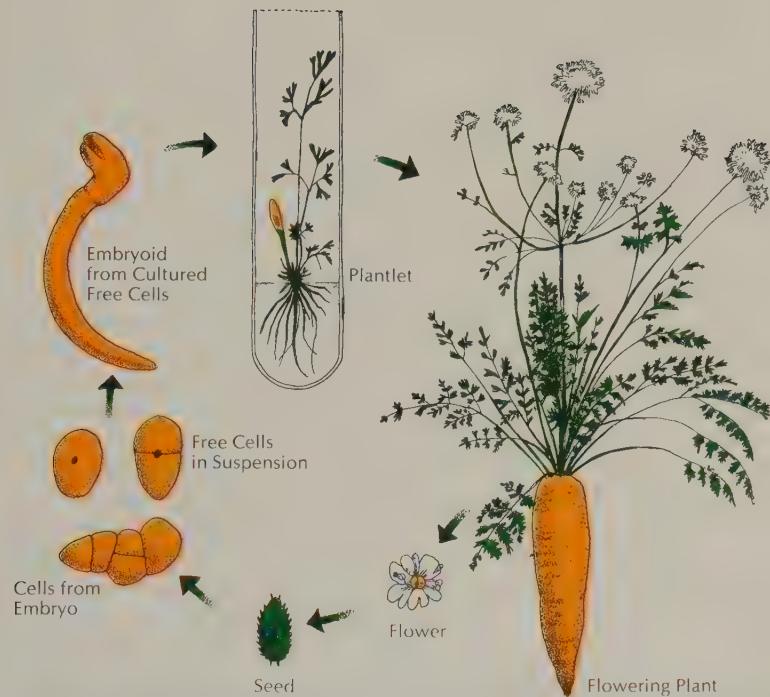


Figure 18.4a (upper left). Surface view of a shoot apex and leaf primordia of the fern *Dryopteris dilatata*. Sixteen leaf primordia can be seen.

Figure 18.4b (lower left). Side view of the shoot apex. Note the compound leaf primordia. A main shoot apex such as this exerts apical dominance over the development of lateral buds.

Figure 18.4c. (right). A scanning electron micrograph of the shoot apex. Primordia of compound leaves are arranged spirally around the apex.

its neighbors and supplied with a medium containing coconut milk, can develop into an apparently normal carrot plant that will flower.

Embryoids have been produced from cells taken from various tissues of many different kinds of plants. Even pollen grains have been cultured to produce embryoids that develop into haploid plants, which are considerably smaller than normal adult plants and which flower but do not produce seeds (Nitsch and Nitsch, 1969).

There has been great interest in the process by which the embryo becomes dormant inside the seed and then resumes growth upon germination (Amen, 1968). The immediate trigger for germination is the absorption of water by the seed under suitable environmental conditions. The tissues of the endosperm swell up as water is taken in, bursting the seed coat and causing hydrolysis of starches and formation of sugars in the endosperm. Growth of the embryo begins as the sugars are transported to it from the endosperm. However, if the embryo end of the seed is cut off before wetting, hydrolysis of starch does not occur in the endosperm. The hydrolysis of starches is catalyzed by the enzyme α -amylase, which is produced by the aleurone cells, a group of cells near the base of the embryo. The activity of the aleurone cells is stimulated by a hormone produced by the embryo.

Some seeds—such as wild oats (*Avena fatua*)—that normally remain in the ground ungerminated for years can be germinated quickly by treating the soil with hormones. Seeds that normally germinate only in the light—some varieties of lettuce, for example—will germinate perfectly in the dark if supplied with hormones. Other seeds that normally require darkness for

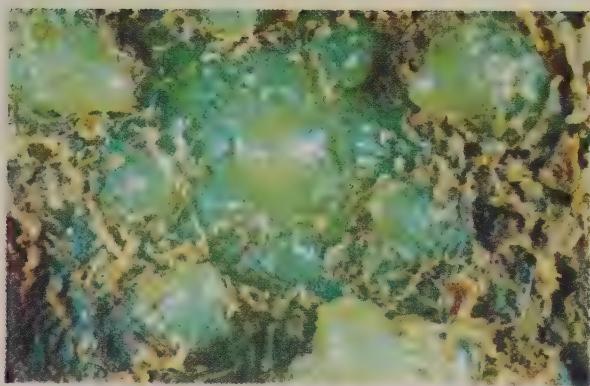


Figure 18.5. A scanning electron micrograph and photograph of the apical dome of *Equisetum* (a horsetail).

germination will germinate in the light if treated with hormones. Seed dormancy in some cases may be nothing more than the failure of the embryo to produce this hormone.

Development of the Shoot Apex

The shoot apex is primarily involved in the initiation of leaf development. The cells in the apical meristem divide mitotically, producing patterns of cells that differentiate into the leaves. Of particular interest to developmental biologists are (1) what initiates leaf development in the daughter cells of the apical meristem and (2) what determines the particular phyllotactic pattern in the plant.

The apical meristem of the shoot is surrounded by leaf primordia that have been formed in a regular sequence by the meristem and are in various stages of development. The location of the primordia on the meristem and the size of the primordia relative to that of the apex vary from species to species. For example, they may be formed singly, in pairs, or in whorls of a greater number.

The tip of the shoot of a flowering plant can be removed and grown in a nutrient medium. If the excised apex bears a few leaf primordia, it will grow in a relatively simple nutrient medium and will eventually give rise to a complete plant with roots and leaves. If the apical dome alone is removed with no leaf primordia, it will only grow in a much more complex medium, indicating that the differentiation of the cells of the apical dome may be dependent on various substances obtained from the cells of the primordia.

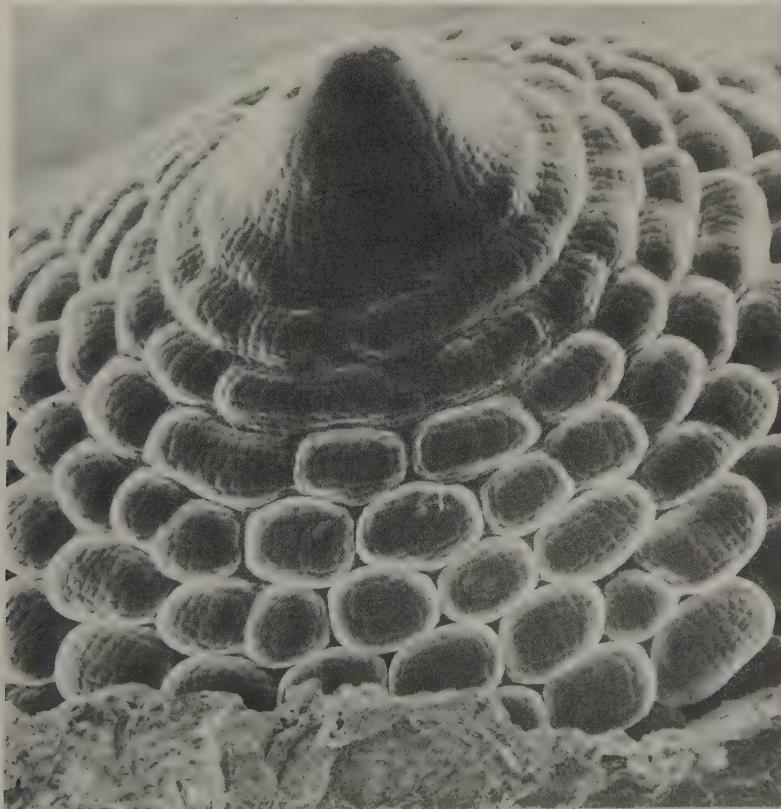


Figure 18.6a (above). Longitudinal view of a shoot apex. Note the formation of a bud primordium (densely stained cells) in the axil of a young leaf.

Figure 18.6b (below). A scanning electron micrograph of young leaf primordium, showing 5 (possibly 7) leaflet primordia.

In ferns and other simpler plants, however, the apical cone itself, without leaf primordia, is capable of normal growth and development in a simple medium (Wetmore, 1954).

Many experiments have shown that the pattern of development of primordia can be modified—for example, by incisions in certain places on the growing apex. If the tip of the shoot apex of a flowering plant is bisected, cells along the flanks can develop into new apical meristems, suggesting that the central cells of the apical dome normally exert some influence that inhibits the development of new apices from flank cells. Therefore, it appears that the phyllotactic pattern is affected by the growth of the apex and is regulated by interactions among existing primordia.

Branches of the main stem originate as *axillary buds*, which form from primordia that appear in the axils of the leaf primordia (Figure 18.6). The potential pattern of branching in a plant is closely related to the pattern in which leaf primordia form around the flanks of the apical meristem.

If the site of an as-yet-invisible new leaf primordium of the fern *Dryopteris* is isolated from older primordia by deep cuts, the primordium becomes much larger than normal, indicating that neighboring leaf primordia inhibit the growth of younger ones. If the site of the developing primordium is isolated from the apical cone by a similar cut, the primordium develops into a bud rather than a leaf (Wardlaw, 1949). In fact, even a partially developed primordium will form a bud if it is isolated from the apical cone before it develops the lens-shape apical cell typical of young leaves (Cutter, 1956). This single, apical cell of the leaf develops from one of a group of cells at the surface of very young leaf primordia. However, if the isolating cuts are shallow and only penetrate the surface cell layer of the apical cone, the primordium continues to develop as a leaf rather than a bud.

These experiments suggest that in ferns there is a period in the early development of the prospective leaf primordium in which it can be switched into another path of development. After a certain stage of development is reached, however, this switch can no longer be made. At this point, the cells are determined to differentiate as a leaf. After determination has occurred, a young leaf primordium can be excised completely from the shoot apex and other shoot tissues and grown on a sterile nutrient medium; under these conditions, it will still develop into a recognizable leaf (Feldman and Cutter, 1970). Evidently, the change that takes place in the primordium at the time of its determination is a profound one, but its nature is not yet understood.

The factors that cause particular regions of the apical meristem to begin rapid division and to differentiate into leaf primordia are not understood. It is clear, however, that most of the cells of the meristem possess the potential for becoming primordia and that complex interactions of inhibitory effects from the apical meristem and from older primordia prevent this potential from being expressed except at certain regularly spaced positions.

Branching in the Shoot

In most flowering plants, the lateral buds that may develop into branches are formed in the axil of each leaf primordium. In some species, however, the buds may form only in the axils of certain leaves (for example, every second leaf) or not at all. The degree to which the buds grow out as branches depends largely on a phenomenon called *apical dominance*. Outgrowth of the buds is inhibited or completely prevented by the main shoot apex. If

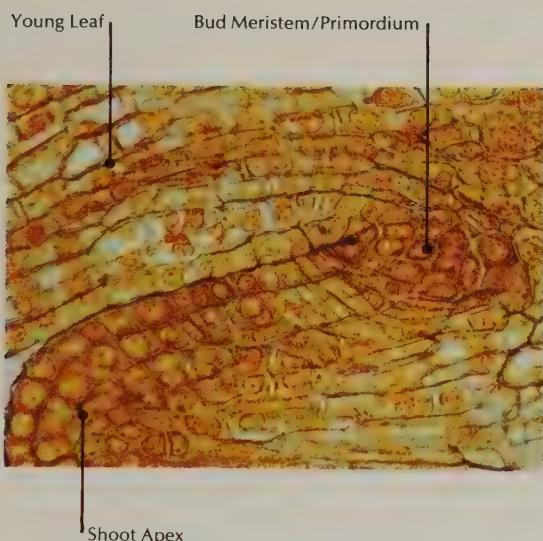


Figure 18.7a (left). Experiments illustrating the concept of apical dominance. (A) Normal plant growth with lateral buds inhibited by hormones secreted by the apical bud. (B) Plant with apical bud removed, thereby allowing lateral bud growth. (C) Plant with apical bud removed and sealed with a plain agar block—lateral buds develop. (D) Plant with apical bud removed and stump sealed with an agar block containing indoleacetic acid. IAA acts to suppress lateral bud development. (From Principles of Plant Physiology by James Bonner and Arthur W. Galston. W. H. Freeman and Company. © 1952)

the apex is cut off or damaged, buds along the shoot begin to grow. Gardeners know that removal of the terminal bud on a shoot almost invariably causes the growth of a branch from the next lateral bud below; this knowledge is the basis of pruning. If certain hormones are applied to the cut surface where the terminal bud was removed (in amounts comparable with what the apical bud would have produced), the lateral buds do not grow (Figure 18.7) (Thimann and Skoog, 1933). Similar inhibitions can be observed in a sprouting potato, where a bud that develops first may inhibit all other buds from developing. Although there is still much controversy about the exact mechanism, hormones secreted by the terminal bud play a major role in inhibition of further development of lateral buds on the same shoot (Phillips, 1969).

Stem Elongation

In some plant species, the portions of stem between the leaves (*internodes*) remain much the same length throughout growth, but in most plants the internodes become longer, and an extended stem is formed. Cell divisions responsible for stem elongation occur mainly in a region just below the

Figure 18.7b (right). Decapitation of shoot apices results in a significant increase in the growth of lateral branches. Application of a paste of IAA to the severed stump acts to inhibit lateral branch growth, thus replacing the function of the normal plant with an intact apex.

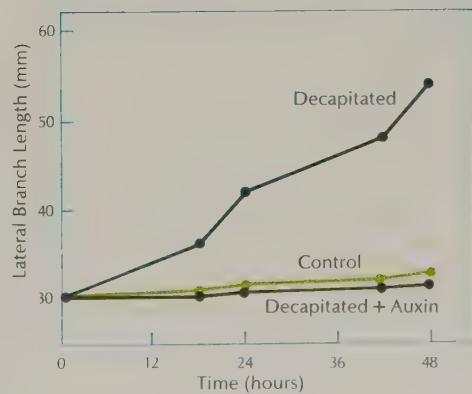
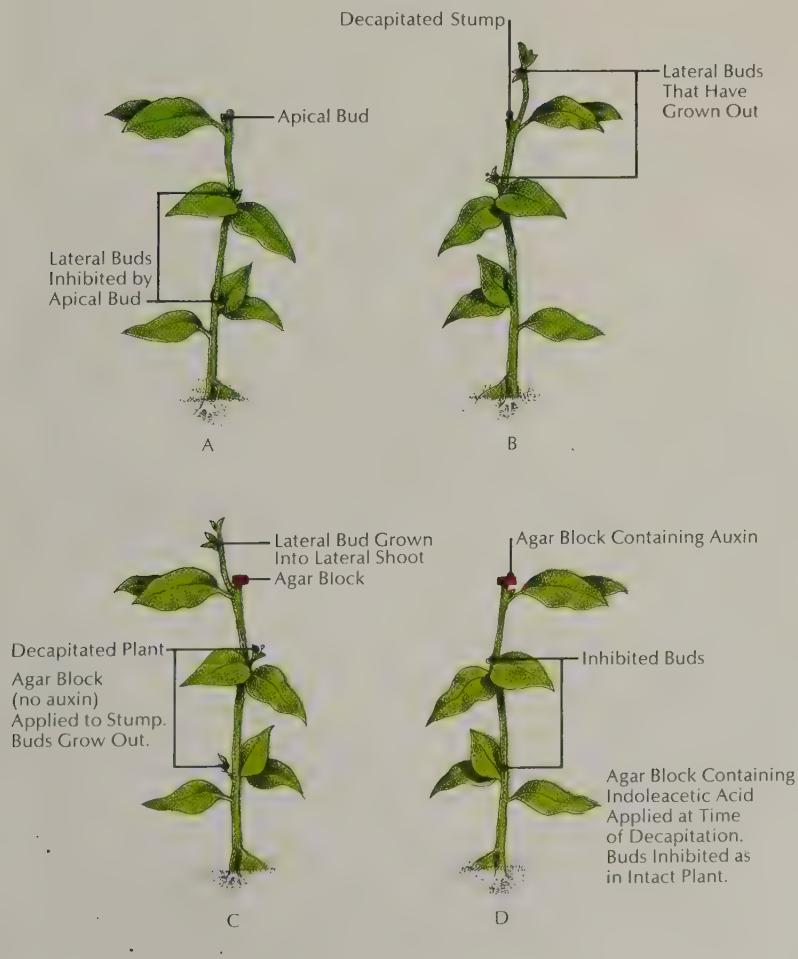
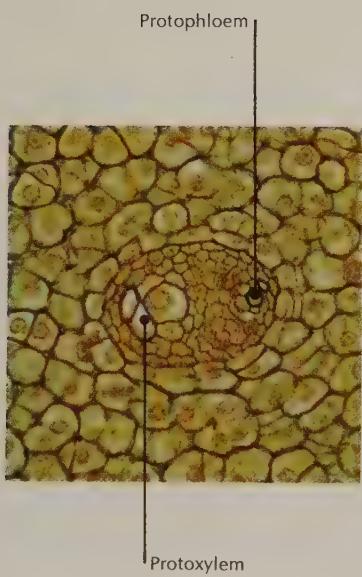


Figure 18.8. Differentiation of vascular tissue. Groups of densely stained orange cells, the procambium, differentiate into xylem (red) and phloem (gray). Only protoxylem and protophloem have been differentiated so far.



apex—a region sometimes called the *primary elongation meristem* (Sachs, 1965). In at least some cases, it has been shown that hormones stimulate the activity of these meristems by causing elongation and enlargement of these plant cells. If long-stem plants are treated with substances that inhibit the synthesis of hormones, they fail to develop elongated internodes.

Differentiation of Primary Shoot Tissues

The cells formed by division in the apical meristem gradually enlarge and become differentiated to form the various tissues of the shoot. In a longitudinal section through a shoot apex, a region of cellular differentiation is visible just behind the apical meristem. The first sign of differentiation is seen near the meristem, where an outer layer of vacuolate ground tissue cells surrounds a cylinder of more densely staining cells. At a slightly lower level in the shoot, groups of very densely staining cells are visible at positions around the cylinder corresponding to the leaf primordia. The densely staining cells, which are elongated along the axis of the shoot, are *procambium* cells, which will differentiate into conducting, or vascular, tissues. A little farther down the shoot, the procambium cells toward the outside of the stem have differentiated to form the first cells of the phloem, which will conduct nutrients and other substances from the photosynthetic tissues to the rest of the plant. The innermost procambium cells have differentiated as elements of the xylem, which will conduct water and dissolved substances from the roots to the rest of the plant.

Phloem tissue begins to form in older, more mature tissues and grows upward into the developing leaf primordia. Differentiation of xylem tissue begins at the level of attachment of a leaf primordium and proceeds both upward into the young primordium and downward to join the mature elements in the stem.

A transverse section of most dicot stems shows a cylinder of separate vascular bundles or a continuous cylinder of vascular tissue (Figure 18.9). In most monocot stems, the number of vascular bundles is much greater, and they are scattered through the ground tissue (Figure 18.10).

If one of the vascular bundles in the stem is severed, the wound is healed when nonspecialized parenchyma cells begin to differentiate and form a new strand of xylem around the wound. Evidence from many experiments now suggests that parenchyma or procambium cells can only begin to differentiate and form xylem or phloem tissue when they are exposed to sucrose and a hormone that is normally secreted by buds or young growing leaves. The proportion of xylem to phloem is related to the concentration of sucrose in the tissues.

Secondary Growth in the Shoot

In most dicots, a meristematic tissue, the *vascular cambium*, differentiates from procambium remaining between the xylem and phloem—and from parenchyma cells between the vascular bundles—forming a complete cylinder (a lateral meristem) around the xylem portion of the stem. The cambium cells divide predominantly by the formation of walls parallel to the surface of the stem. Tissue formed on the inner side of the cambium differentiates to become xylem, whereas that formed on the outer side becomes phloem. In woody plants that survive over many years, the cambium becomes active in the spring (in temperate climates) and ceases activity in the fall of each year. Because reactivation of the cambium closely follows the

Figure 18.9 (above). Cross section of a typical dicot stem showing the concentric ringlike arrangement of vascular tissue. (Courtesy Carolina Biological Supply Company)

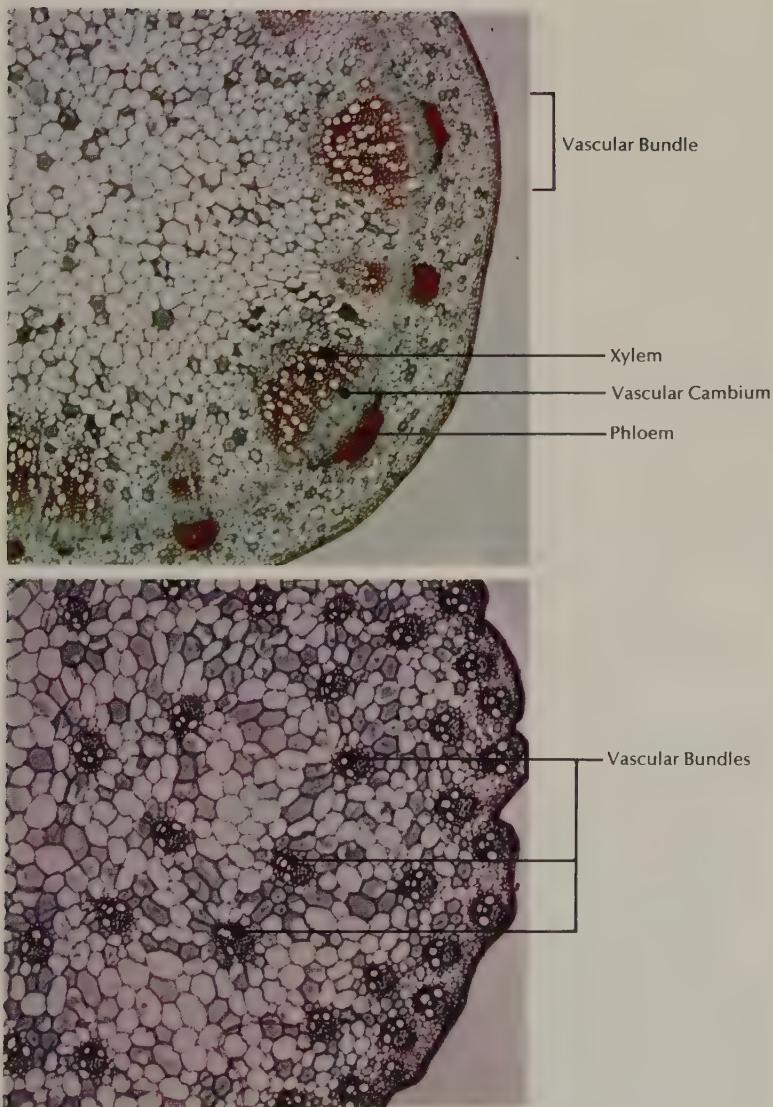


Figure 18.10 (below). Cross section of a typical monocot stem showing the well-developed vascular bundles scattered within the ground tissue. (Courtesy Carolina Biological Supply Company)

outgrowth of new buds on the shoot system, it was long suspected that some substance secreted by the growing buds might be responsible for activation of the cambium. The activation of the cambium begins near the new buds and progresses along the branches and down the trunk. Removal of the buds before their growth has begun in the spring causes the cambium layer to remain inactive. Relatively normal activity of the cambium layer can be stimulated by applying a mixture of hormones to the sites from which the buds were cut. In nonwoody plants, removal of the growing tip causes failure or cessation of cambial activity. The application of hormones to the cut surface causes the initiation or resumption of cambial activity (Digby and Wareing, 1966).

The effects of light upon shoot growth were demonstrated by Charles and Francis Darwin in 1880. They found that a seedling of the grass family would curve toward the light if illuminated from one side but that curvature

would not occur if the extreme tip was covered with an opaque cap. Such patterns of growth toward or away from light sources are *phototropisms*. Because the zone of curvature was well below the tip, the Darwins concluded that some "influence" came down from the tip to the parts below to direct the curvature.

Through a number of ingenious experiments, later researchers demonstrated that the curvature is caused by unequal growth rates on the sides of the stem, that the amount of growth is proportional to the amount of certain hormones received from the tip, and that the amount of hormones produced in various parts of the tip is proportional to the amount of sunlight reaching the part. Some shoots demonstrate *geotropism*—that is, the direction of growth or curvature is determined by gravity. Similar effects of hormones produced in varying amounts by different parts of the tip were shown to account for geotropism.

Flowering

Factors that cause a plant to develop flowers vary from species to species. One important factor is day length—the relative lengths of light and dark periods in each 24-hour day. In temperate regions, the days are longer in the summer and shorter in the winter, and the effects of day length tend to produce seasonal flowering.

It appears that changes in day length lead to production of a hormone in the leaves. This hormone then travels to the shoot apical meristem, where it triggers the beginning of flower production. This hormone, however, has not yet been isolated or identified. Flower production begins with a transformation of the shoot apex. The changes of size and shape vary from species to species. The flowers may develop from flower primordia that form around the flanks of the apex or through a conversion of the whole shoot apex into a floral apex. The floral apex or floral primordium forms the various parts of the flower as lateral appendages.

In many cases, increased cell division in the central apex region is one of the earliest changes observed after induction of flowering; it may occur as soon as 16 hours after treatment to induce flowering has been given. Increased cell division leads to changes in the size and shape of the apex. The eventual differentiation of the flower parts is apparently due to complex interactions of hormones or other factors that alter the environment of individual cells within the floral apex.

Various experiments indicate that the presence of leaves is crucial to the induction of flowering and that the leaves must be exposed to the day-length conditions that trigger flowering. (In some cases, flowering will be induced if as little as 1 square centimeter of a single leaf is exposed to the proper day-length conditions.) Removal of the leaves within a few hours after exposure to the triggering stimulus prevents the induction of flowering, but after a day or two the removal of leaves has little effect upon the further development of the flowers. Stems with leaves that have been exposed to triggering influences can be grafted onto other plants that have not been exposed, and the host plants will flower. These and other experiments strongly support the idea that a substance moves from the leaves to the shoot apex to stimulate flower production.

Although many details of the flowering-induction mechanism remain to be discovered, florists have put the present knowledge to commercial use. By manipulating periods of darkness in their greenhouses, they can make

sure that all their poinsettia plants will be in full bloom for Christmas sales and that their Easter lilies will flower at the proper time. Some plant species respond to particular conditions of temperature for the onset of flowering, rather than demonstrating sensitivity to day length.

The Root

In many dicots, the root system consists of a main, or tap, root with lateral branches. In most monocots, the root system is made up of a number of fibrous roots of fairly similar size. The apical meristem of the root is covered by a root cap of parenchyma cells. Cells on the surface of the root cap are worn away as the growing root pushes through the soil but are replaced by new cells added to the inner surface of the root cap through divisions in the apical meristem. At the other side of the meristem, new cells become elongated in a region behind the area of active division. Just in back of this region of elongation, single-cell extensions called root hairs grow out into the soil, and differentiation of the root tissues occurs.

The cells of the root cap control the geotropic response of the root. Removal of the root cap causes a root kept in a horizontal position to grow straight rather than to curve downward. Apparently, the cells of the root cap control the distribution of substances that affect the growth rates of the other root cells (Juniper, et al., 1966).

The hormones that stimulate stem elongation have only a slight elongation effect—or in some cases an inhibiting effect—on root growth. Cell division and elongation occur chiefly in the region relatively near the root apex. The process of root elongation is apparently subject to a less intricate pattern of hormonal and environmental control than is the process of stem elongation.

In the region just below the zone of elongation, extensive differentiation of tissues takes place. Within the epidermal layer of some species, certain cells become more densely cytoplasmic than others, and these trichoblasts develop into the root hairs (Figure 18.11). The trichoblasts are

Figure 18.11. Transverse section (left) and longitudinal sections (middle and right) of a root tip of the frog-bit *Hydrocharis*. The formation of trichoblasts, or hair cells, by unequal divisions of immature epidermal cells is illustrated. The smaller trichoblasts stain more densely.

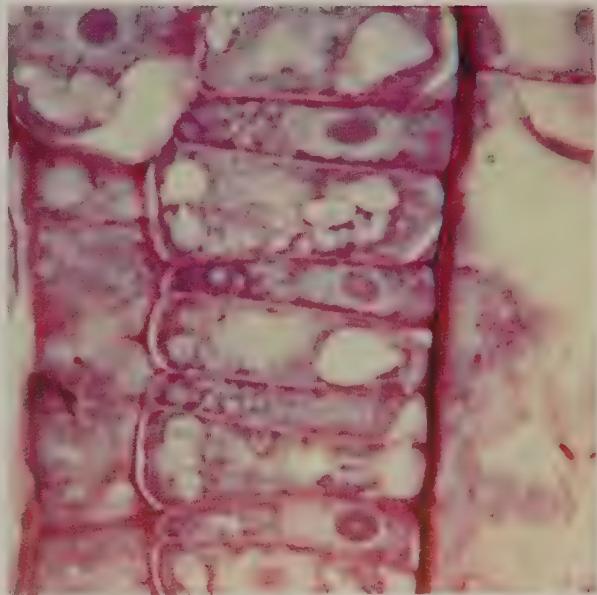
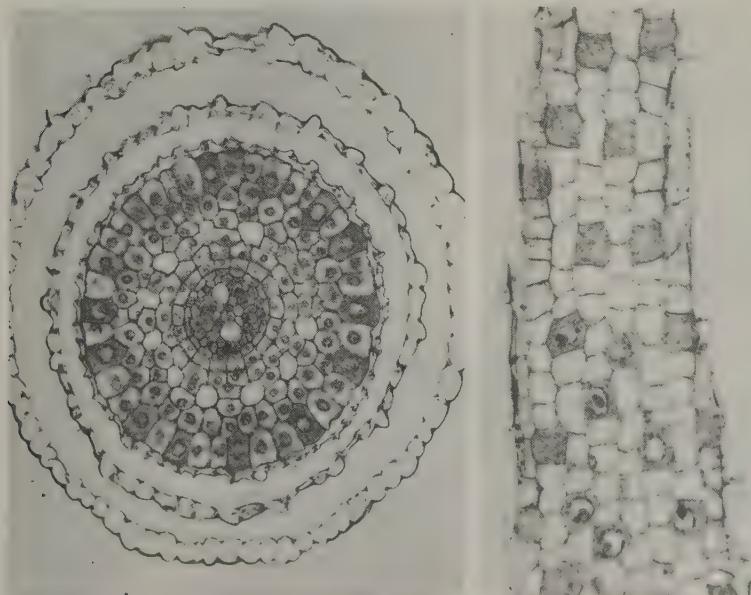
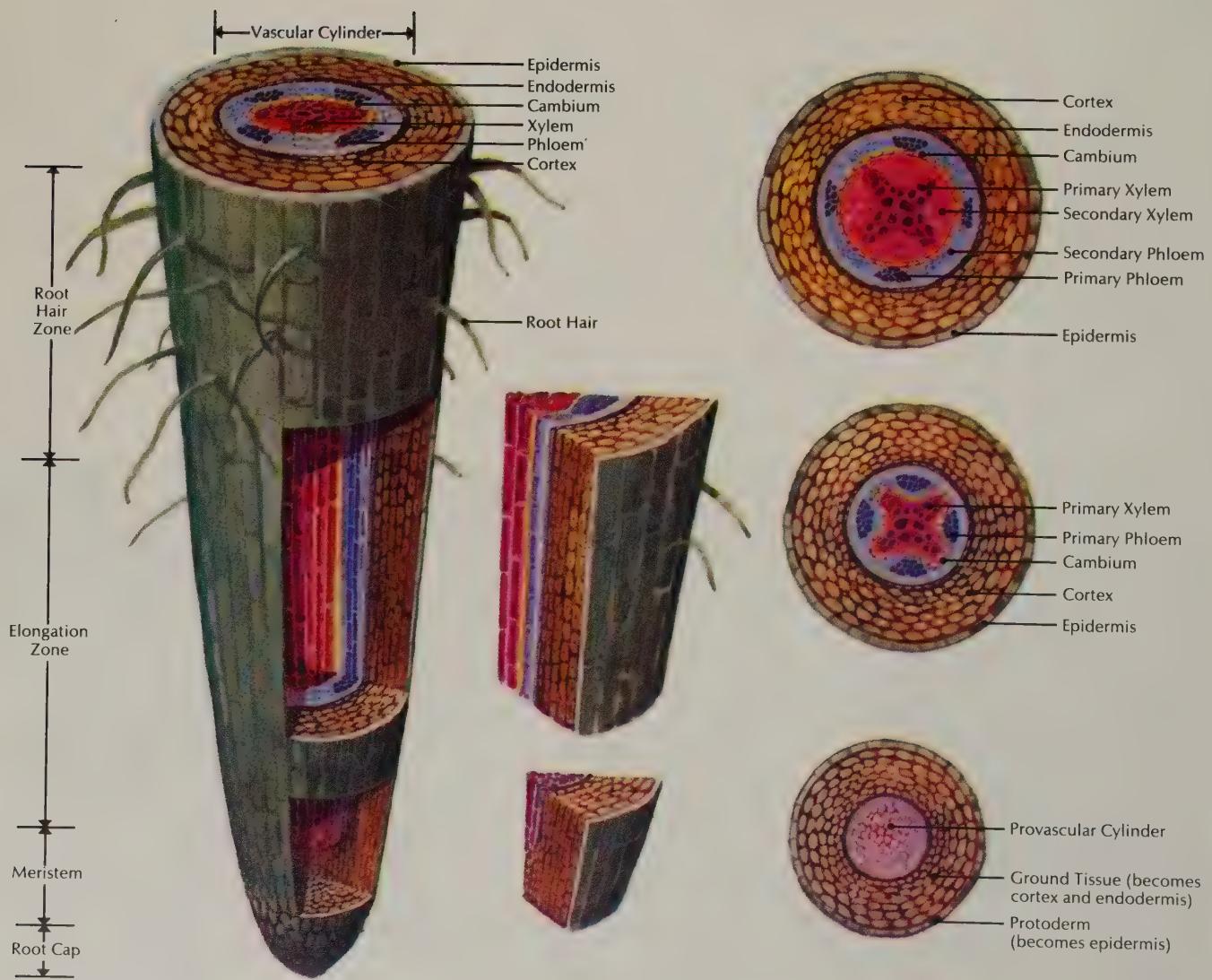


Figure 18.12. The differentiation regions of the root.

characterized by a greater concentration of various enzymes from an early stage of development (Avers, 1958, 1961). Various complex patterns of epidermal differentiation have been observed in several plant species.

As in the shoot, differentiation of ground tissue and a central core of procambium occurs near the apical meristem. Slightly farther back from the tip of the root, the procambium differentiates to form alternating xylem and phloem strands (Figure 18.12). The pattern of the vascular tissues, including the number of strands of xylem, is one of the most characteristic features of the roots of a particular plant species. This pattern is apparently influenced both by the diameter of the root and by concentrations of various hormones.

Cells taken from the outer phloem tissue of adult carrot roots have been cultured in a medium containing coconut milk (Steward, et al., 1958).



These cells grow, divide, and eventually form structures with small roots. If the structures are then transferred from the liquid medium to a solid medium, they form shoots and eventually whole plants.

This highly significant experiment shows that a single differentiated cell from an adult plant has the capacity to produce through morphogenesis, differentiation, and growth all the cells in an adult plant. Thus, if a fully differentiated cell is isolated from its neighbors and supplied with the appropriate chemical environment, the orderly sequences of selective gene expression necessary for embryogenesis can be reinitiated and maintained. Another important conclusion is that the establishment of the differentiated state in a cell is not an irreversible event.

Lateral organs or branches of the root are not formed at the apex but at some distance back from the root tip. In most flowering plants, the lateral root primordia originate by cell divisions in the pericycle—the outermost layer of the central cylinder. The positions of lateral root formation are related to the positions of vascular tissue in the center of the root. After division of the pericycle, the new cells become organized into a primordium that resembles the main root apex. This structure grows through the cortex to the exterior. The primordium secretes enzymes that dissolve the cortex cells as it makes its way toward the root surface. Relatively little research has been done on the interactions among root primordia, but there is some evidence of interactions similar to those observed in the formation of leaf primordia on the stem.

Major Features of Plant Development

The development of higher plants is characterized by the maintenance of regions of growth and development throughout the life of the organism. Stems and roots are extended, and new organs are initiated as a result of cell division, elongation, and differentiation in the growth regions. Growth and development are regulated by hormonal interactions, some of which are responses to environmental conditions and others of which are responses to development elsewhere in the organism. Although the plant is relatively limited in its ability to respond rapidly to changes in external conditions, it is extremely flexible in its ability to shed old organs or grow new ones as conditions change.

STUDY OF ANIMAL DEVELOPMENT

Animal developmental biologists choose an organism for study because of its convenience and suitability for observation or experimentation on a particular part of the developmental process. As a result, a great deal has been learned about the development of animals such as the sea urchin, frog, fruitfly, bird, and mouse. For example, oogenesis (egg formation) is studied more conveniently in frogs, which continue to produce oocytes throughout their reproductive lives, than in mammals, which produce all their oocytes before birth. Fertilization and early development are conveniently studied in the sea urchin, whose eggs can be readily fertilized in a dish of sea water, whereas fertilization in mammals normally occurs within the fallopian tube, where observation is exceedingly difficult. Also, the study of embryonic development is far easier with a frog or an insect, which completes its embryonic life in a few days or less, than with a mammal, which takes months to develop. Although the understanding of mammalian and human development is not complete, enough information is available to make it

Figure 18.13. Successive stages in the embryological development of the frog. From left to right, sequences depicted include: 1–6, early cleavage; 7–9, blastula; 10–11, gastrula; 12–15, the formation of the neural tube. (Courtesy Carolina Biological Supply Company)

1	2	3	4	5
6	7	8	9	10
11	12	13	14	15





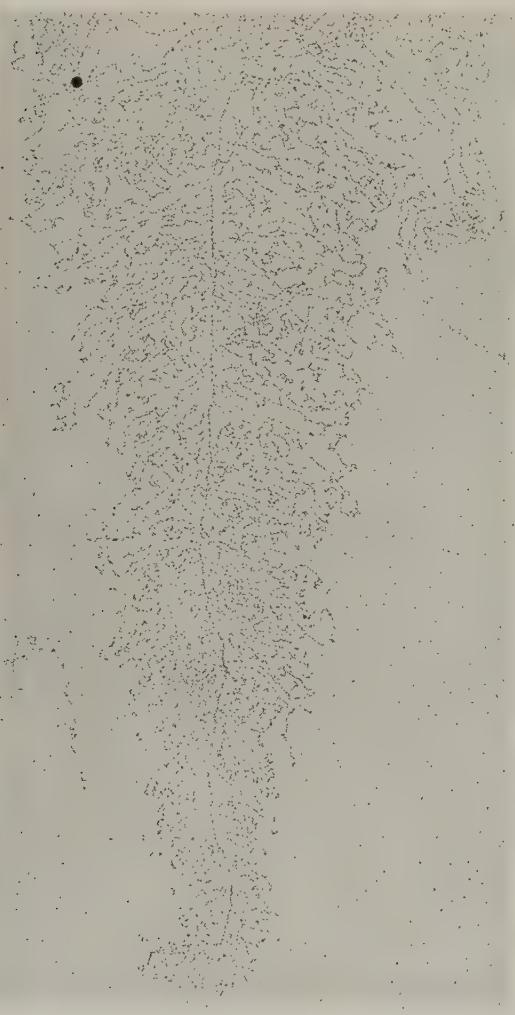
Figure 18.14. Successive stages in the embryological development of the chick. From left to right, sequences depicted include: 1–3, primitive streak; 4–6, neural tube and somite development; 7, beginning of cephalization; 8–11, increased cephalization.

1	2	3	4
5	6	7	8
9	10	11	





Figure 18.15. Portion of a lampbrush chromosome isolated from a newt oocyte. Note the loops of DNA.



clear that many of the same basic principles involved in the development of lower animals are also valid for mammals.

After many years of observation and experimentation, developmental biologists have described many phenomena of animal development. However, these descriptions—although often quite complex and fascinating—seldom explain the mechanisms involved. Only when the molecular basis of development is thoroughly understood will meaningful control of development become possible. As in plant development, a few of the basic control mechanisms are known, but the unsolved problems remain numerous and extremely important. It is perhaps not incorrect to say that studies by developmental biologists have progressed just far enough for them to begin to discover what they do not know.

Oogenesis

The development of the oocyte plays a crucial role in the determination of the future embryonic development. All eggs contain substances that influence the course of later development (Davidson, 1969). These substances include RNA and a group of molecules called *cytoplasmic determinants*, which are of unknown composition but have a profound influence on development.

The maturation of the frog oocyte takes several months, and for part of this time the oocyte nucleus actively synthesizes various types of RNA. Nearly all the RNA synthesized during this period is ribosomal RNA (rRNA). The great demand for the synthesis of rRNA is enhanced in the oocyte by gene amplification—that is, the genes for two of the three types of rRNA are replicated hundreds of times and exist free in the nucleus as short strands of DNA (Brown and Dawid, 1968). These replicated genes form the cores of many extra nucleoli, in which rRNA is transcribed. Sufficient ribosomes are produced during oogenesis to supply the needs of the embryo through its development up to the time of hatching as a tadpole (Brown and Gurdon, 1964).

In the frog oocyte—and in the oocytes of many other kinds of animals—the chromosomes take on an unusual appearance during oogenesis. The homologous chromosomes have synapsed (paired up) at the beginning of the first meiotic prophase, but further steps in meiosis have been suspended. The chromosome strands are thrown into a series of loops of DNA, giving the whole structure a “lampbrush” appearance (Figure 18.15). This lampbrush phase coincides with a period of active messenger RNA (mRNA) transcription, during which about 6 percent of the total genes in the chromosomes are being transcribed. Only about 0.14 percent of the total being transcribed is unique, nonrepetitive DNA (Davidson and Hough, 1969). It is estimated that this relatively small percentage actually represents about 10,000 different genes being transcribed.

The existence of cytoplasmic determinants has been recognized for half a century. When portions of the cytoplasm from a fertilized egg are removed, certain reproducible defects in the developed organism are produced. For example, if portions of the vegetal pole cytoplasm—presumably RNA molecules—are removed or destroyed by ultraviolet irradiation in the frog egg, the resulting frog is normal but sterile (Smith, 1966). Fertility can be reinstated in this case by injecting a small amount of cytoplasm from the vegetal pole region of a normal egg into the irradiated egg. The cytoplasmic substance destroyed by the ultraviolet light suggests that these molecules

are RNA, but its identity has not yet been firmly established. No other cytoplasmic factors are capable of producing the germ cells when these cytoplasmic determinants are removed and, in these eggs, at no time in the subsequent development of the animal are the genes for germ cell formation turned on. Although little is known of the chemistry or mode of action of such cytoplasmic determinants, research is being directed toward this problem.

Induction

Cell-cell interactions clearly are important in developmental processes. In the frog embryo, for example, after the major cell movements of gastrulation, cells in a certain region of the embryo begin to fold in and to form the various elements of the nervous system. These cells differentiate into nervous structures only after they make contact with cells that lie directly beneath them, cells that are forming part of the embryonic backbone (Ebert and Sussex, 1970). This process, in which one group of cells influences the development of a second group, is known as *embryonic induction*. Induction takes place even if contact between the two tissues is prevented by a porous filter. Apparently, the induction is caused by a chemical substance passing from the inducing to the induced cells.

The nature of the inducing influence and the mechanism by which it triggers a specific differentiation is unknown. However, once the potentialities of a cell have been restricted to certain differentiations—either by cytoplasmic factors inherited from the egg or by the action of inducers—this specification persists long after the inducing stimulus or conditions have disappeared, and it is passed on by the cell to its descendants. The cells of the embryonic backbone (notochord) in the frog are capable of inducing differentiation of neural structures only during a brief period of their development. During this time, they can even induce development of neural structures from cells that would ordinarily form skin (Ebert and Sussex, 1970). After the period of induction, the affected cells and their descendants remain differentiated and produce the various elements of the nervous system, even though the notochord cells degenerate. The change in the induced cells is so permanent that they can be removed from the embryo and grown in a culture medium, where the induced cells and their descendants continue to form nervous tissue.

Determination

As indicated earlier in this chapter, a cell that has entered upon a particular course of differentiation will pass that tendency on to its descendants; this cell is said to have undergone determination. It may be said to have *firm biases*—a determined cell gives rise to a clone of cells with similar determination (Schneiderman, 1969, and Postlethwait and Schneiderman, 1970). Some reproducing populations of cells with firm biases persist in the adult organism—for example, the cells that form skin, sperm, red blood corpuscles, and so forth.

Some spectacular examples of the stability of firm biases are found among insects. In larvae of fruitflies, tiny nests of cells derived from the embryo give rise to particular adult organs such as legs and genitalia. These nests—known as *imaginal discs*—differentiate into specific adult structures; a leg disc forms a leg, a genital disc forms genitalia, and so on. Fragments of imaginal discs have been cultured in the abdomens of adult flies

for more than 1,000 cell generations, and in most cases the resulting cells retain the capacity to form normal adult structures (Hadorn, 1968). Although the program for organ differentiation is normally passed from parent to daughter cells through many generations, such a program may occasionally change spontaneously. For example, cells derived from a leg disc sometimes differentiate into wing structures. When such a change in determination occurs, it usually occurs in a small group of cells at the same time. Each of these cells then transmits the new program to its descendants.

Inheritable stability of determination and differentiation is essential for the long-term coordination of a multicellular organism. However, the ability to adapt to changing circumstances is of equal importance. The control systems that provide the organism with flexible responses have been called *transitory biases* (Schneiderman, 1969). Like firm biases, transitory biases result in specific differentiations. Transitory biases persist only as long as the inducing conditions persist and are not passed on to descendant cells after the inducing conditions disappear. Transitory biases include most hormonal effects in animals and plants, enzymatic induction processes in bacteria, and similar processes. For example, the cells of the thyroid secrete thyroid hormone only when circulating thyrotropic hormone (produced by the pituitary gland) is present. Similarly, the gonads and necessary sex organs remain functional and differentiated only as long as gonadotropin hormones (also secreted by the pituitary gland) are present in their vicinity (Turner, 1966).

Another example of a transitory bias is the action of the juvenile hormone of insects. In insects such as the *Cecropia* silkworm, the type of cuticle secreted by the epidermal cells varies with the amount of juvenile hormone in the blood (Schneiderman, 1969). When juvenile hormone is present, the epidermal cells secrete a juvenile or larval cuticle. When juvenile hormone decreases in amount, the epidermal cells cease to make larval cuticle and instead make pupal cuticle. When the level of juvenile hormone drops still further, the epidermal cells secrete an adult cuticle. Juvenile hormone apparently imposes a transitory bias on the epidermal cells, causing them to differentiate in a particular fashion. This bias persists only as long as the hormone is present.

Examples of transitory biases are found in both microorganisms and multicellular organisms. However, firm biases are almost exclusively limited to multicellular organisms and some complex acellular, eucaryotic organisms (such as ciliates). A bacterial cell is finely tuned to its changing environment. Within moments after a change in the environment, the rates and the types of synthetic activities are altered to match the new conditions. Life for a cell of a multicellular organism is very different. Such a cell is always influenced by its past history. What it can do depends on the genetic information that it possesses, on what it has done and where it has been in the past, and upon its present position in relation to its neighbors.

In a multicellular organism, the loss of one bias and the assumption of a different one—whether firm or transitory—appears to require replication of the cell's DNA. As larval epidermal cells transform to pupal epidermal cells in an insect, DNA synthesis takes place. Adult epidermal cells secrete larval cuticle in the presence of juvenile hormone, but this transformation is accompanied by DNA replication. If DNA synthesis is prevented, the epidermal cells are unable to undertake a new synthetic program even

Figure 18.16. The *Cecropia* silkworm.



though the level of juvenile hormone has changed (Schneiderman, 1969). These findings are consistent with the hypothesis that the reprogramming of cells in multicellular organisms requires some sort of “gene cleaning” that can occur only during DNA replication.

Regulation and Regeneration

Systems that can regulate for, or regenerate, lost parts represent an outstanding case of the loss of stability of cellular determination and differentiation for adaptability. After first or second cleavage in sea urchin embryos, one blastomere can regulate for the loss of the other blastomeres and can produce a complete though small embryo. Similarly, the frog egg can regulate for the loss of almost any part of its cytoplasm.

Regulation also occurs when there are too many rather than too few cells. For example, embryos of a pigmented and of an albino mouse can be mixed together. The mixture of cells re-forms into a single blastula, which can be transplanted into the uterus of a host mother mouse, where it survives and completes its development (Mintz, 1967). The resulting newborn mouse does not have duplicate structures, although its tissues contain cells derived from each of the embryos. Apparently, the early embryo can regulate for the presence of an excess number of cells.

Before an animal has reached the end of its period of embryonic development, most of its tissues take on their mature, differentiated functions. Most of the cells and their descendants will maintain their differentiated conditions within the tissues indefinitely, unless altered by metamorphosis or abnormal circumstances such as disease, uncontrolled cancerous growth, nervous system degeneration, endocrine gland malfunction, and so on. If the equilibrium condition is upset by a wound or amputated appendage, for example, the tissues may respond to restore the balance. A mouse cannot regrow an amputated limb or digit, but the wound caused by amputation will heal. Epidermal cells from the margin of the wound migrate until the wound is covered. In this response to injury, the mouse tissues retain their differentiated characteristics as epidermis, bone, muscle, or connective tissue, although some cells may modify their behavior in response to wounding.

In contrast to the mouse—which responds to amputation by healing the wound—a newt responds by making a new limb in a process called regeneration. After a newt’s leg is amputated, the wound is covered, as it is in a

Figure 18.17. Regeneration in the starfish.



mouse, by migration of epidermal cells that remain differentiated. However, the other tissues in the vicinity of the wound lose their characteristic differentiated features. Muscle, bone, and connective tissue cells contribute to the formation of a mound of "dedifferentiated cells" beneath the wound epidermis. In this accumulation of cells, the various cells appear identical and cannot be distinguished from each other. Even the electron microscope reveals no distinctions among these cells (Bryant, 1970). These apparently dedifferentiated cells increase in number both by further additions from surrounding tissue and by cell division. The accumulated cells then differentiate and form a new limb to replace the one that was lost.

Regeneration apparently depends upon the ability of the cells first to become dedifferentiated, then to proliferate, and finally to redifferentiate and re-form the limb. There is some evidence that the presence of nervous tissue is in some way necessary for the processes of dedifferentiation and proliferation. Regeneration can be induced in the normally nonregenerating limbs of frogs, lizards, and opossums by increasing the proportion of nervous tissue in the stump (Bryant, 1970).

Nuclear Transplantation and Cell Hybridization

In recent years, the technique of nuclear transplantation has provided information about the mechanisms of cellular determination and differentiation. If the nucleus of a fully differentiated adult frog cell, such as a skin cell or liver cell, is taken out of its own cytoplasm and placed into the egg cytoplasm, it begins behaving as a zygote nucleus and participates in the development of a normal frog. Such experiments, though not as dramatic as the experiments on plant cells, show that the nuclear changes accompanying determination and differentiation in animal cells are not irreversible. Another significant conclusion is that the cytoplasmic environment in which the nucleus resides plays a major role in determining which genes are to be expressed.

Similar findings have come from experiments on somatic cell hybridization. Cells as different from one another as erythrocytes (nucleated red blood cells), fibroblasts, pigment cells, and oocytes can be combined so that there are two nuclei within a fused cytoplasm. This type of "mating" in tissue culture can be performed with cells from organisms as different as chicks and humans. Under normal conditions, the nucleus of the hen eryth-

rocyte does not synthesize RNA or DNA. However, when such a nucleus is transplanted into the cytoplasm of a human cell, it resumes both types of synthesis. These experiments, like the nuclear transplantation experiments in frogs, indicate that differentiation does not cause irreversible changes in the nucleus and that the nucleus is strongly influenced by molecules in the cytoplasm.

The Importance of Position

What clues enable a cell to "know" where it is within the developing organism so that it will differentiate in the fashion suitable to its location? For instance, in the development of chick limb buds, the initial group of limb bud cells may become either hind limbs or wings, depending on their location in the embryo. A limb bud area transplanted early in development will differentiate in a fashion appropriate to its new location. After a certain period of development, hind limb buds transplanted to the wing region continue to differentiate as hind limbs. It has been suggested that cells "estimate" their position within a group by the use of particular markers or reference points (Wolpert, 1969). After determination has occurred, altered positional influences can no longer affect the differentiation of the cells.

The importance of positional influence is illustrated in certain fruitfly mutations that cause various parts of the antenna to transform into leg structures. When such transformations occur, they are extremely precise in terms of the kind of leg structures that arise from particular regions of the antenna. For example, a transformation of the very end of the antenna leads to the production of the end portion of a leg with a claw. A transformation of a central part of the antenna leads to the production of the corresponding central part of a leg. That is, the nature of the leg structure produced by a group of transformed antenna cells depends upon the position of these cells within the antenna. The leg cells respond appropriately to the same set of influences that provide positional information for the antenna cells (Postlethwait and Schneiderman, 1970).

Little is known about the mechanisms through which positional information is specified—the landmarks that cells may use to locate themselves. Among the hypotheses being considered are responses to particular chemical substances produced by neighboring cells or responses to varying gradients of the concentration of such substances across the cell (Wolpert, 1970).

Cellular Communication

There is evidence that adjacent cells can communicate directly with each other, at least during certain critical periods of development. As a fertilized egg becomes partitioned into an increasing number of blastomeres during cleavage, the individual cells continue to remain electrically coupled (Ito and Loewenstein, 1969). If microelectrodes are implanted in adjacent cells, small ions will carry electrical charges directly from one cell to another without passing through the external medium. Such intercellular communication during development might aid in determining a cell's position within a group.

In some well-documented instances, communication between cells involves far more than electrical coupling and ion movements. During oogenesis in some insects, the egg cell has open channels of cytoplasmic communication with surrounding nurse cells (Koch, et al., 1967). Electron

micrographs show many organelles in the communication channels, suggesting that free exchange of ribosomes, centrioles, mitochondria, and other cellular constituents may take place. In these insects, RNA is synthesized in the nurse cells and passed into the oocyte, which apparently does not synthesize RNA of its own (Bier, 1963). This transfer of RNA was discovered through autoradiographic studies in which radioactive RNA precursors supplied to the cells were first incorporated in the nurse cells, then moved into the cytoplasmic channels, and finally moved into the cytoplasm of the oocyte.

Shaping Up

As discussed earlier in the chapter, plant morphogenesis is accomplished largely by cell division along particular planes or cell elongation along particular axes. In animals—whose cells lack rigid walls and are more mobile—cell movements play a much more important role, but there are some examples of animal morphogenesis involving oriented cell divisions. The formation of the hollow sphere, or blastula, in sea urchin embryos is accomplished by restriction of the planes of division to radial axes, so that daughter cells are added to the surface of the sphere rather than being pushed to the interior or the exterior (Gustafson and Wolpert, 1967).

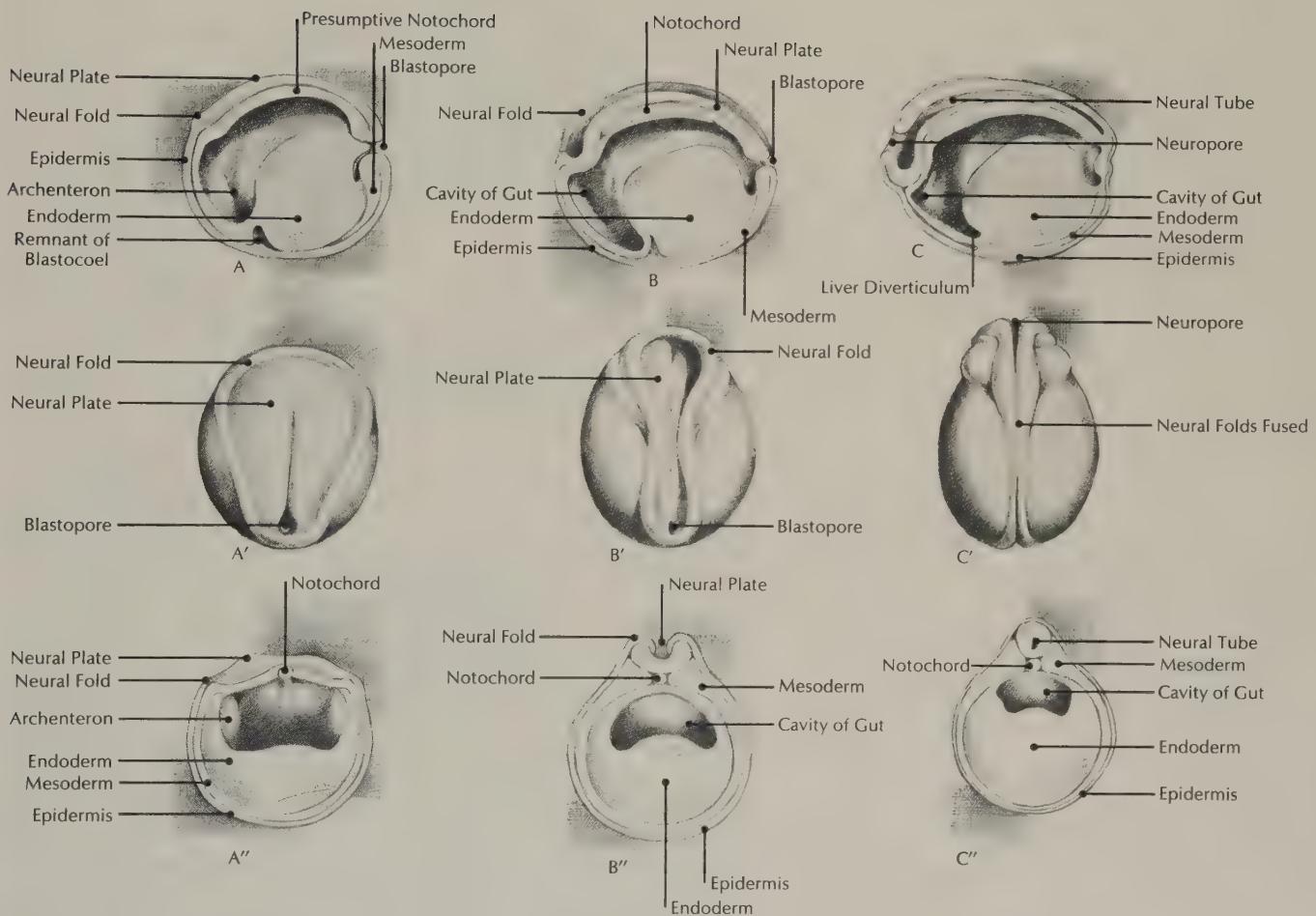
Another example is found in the morphogenesis of insects. Clonally derived patches of cells—that is, cells originating from the same ancestral cell—appear as stripes along the length of the legs (Bryant and Schneiderman, 1969), the wings, and the antennae of adult flies. Such patches would be formed if the planes of cell division were consistently oriented at right angles to the long axis of the appendage, forming an elongated line of daughter cells. Patches of similar shape have been shown in other insects—such as bees and butterflies—that undergo complete metamorphosis during development. It has been suggested that oriented cell division is a major factor in morphogenesis of insects (Postlethwait and Schneiderman, 1970).

On the other hand, there is no evidence that oriented cell divisions are responsible for the elongated shape of a chick limb. Differential rates of cell division in different parts of the limb bud may play a part in shaping the limb (Hornbruch and Wolpert, 1970). After the various regions such as upper leg, lower leg, and foot have become determined in the limb, each assumes a different rate of cell division, ultimately producing a properly proportioned limb. Although differential division rates can account for the greater length of the foreleg region as compared to the foot region, it cannot account for the detailed contours of the limb.

Another mechanism involved in animal morphogenesis is controlled cell death. For example, the foot region of a chick's hind limb first develops as a flattened, palette-shape structure. The individual toes become separated from one another by the death of intervening cells (Saunders and Fallon, 1966). A similar mechanism has been discovered in the development of the claws on fly feet (Whitten, 1969). In such cases, individual cells are sacrificed for the good of the entire organism, emphasizing that the multicellular organism—not the individual cells of which it is composed—is the basic unit of multicellular life.

Gastrulation in the sea urchin begins with reorganization of the spherical blastula into a cone shape. The base of this cone then indents into the blastocoel to form the primitive gut. It appears that this indentation is caused by a change in shape of the individual cells that remain attached to

Figure 18.18. Neural formations in a frog embryo. The A series shows very early embryos; B series shows midterm embryos; C series shows late-term embryos with the neural tube almost completely closed. The top series shows the right side of embryos; the middle series shows whole embryos in dorsal view; and the bottom series shows the anterior halves of transversely cut embryos. (See Figure 18.14.)



neighboring cells in the sheet. The changes in cell shape may be due to rearrangements of the internal skeletal elements of the cells such as microfilaments. It has also been suggested that the changes in cell shapes result from changes in the degree of adhesion that cells have with their neighbors (Gustafson and Wolpert, 1967). Whatever the mechanism, many important morphogenetic events depend upon changes in cell shape and the resulting movements of sheets of cells.

Examples of individual cells moving to create shapes are less common, but one outstanding example is the migration of neural crest cells in vertebrate embryos (Weston, 1970). After gastrulation, a line of cells along the dorsal side of the embryo begins to divide rapidly, producing a thick *neural plate*, which folds into the embryo to form the *neural groove* (Figure 18.18). The folds along the edges of the neural groove become higher, curve

inward over the groove, and eventually fuse, transforming the neural groove into the *neural tube*. This tube later forms the brain and the central canal of the spinal cord. A number of cells from the tips of the neural folds, the *neural crest cells*, migrate away from the neural tube and in new and diverse locations play a profound role in the morphogenesis of a variety of organs and tissues. For example, neural crest cells form spinal ganglia, sympathetic ganglia, head cartilage, pigment cells, and Schwann cells. These cells do not move in random directions away from the neural tube but leave in two well-defined streams—one headed dorsally toward the epidermis and the other ventrally toward the mesenchyme. The mechanism of migration and the positional clues that guide the migrating cells to their destinations in the embryo are as yet unknown.

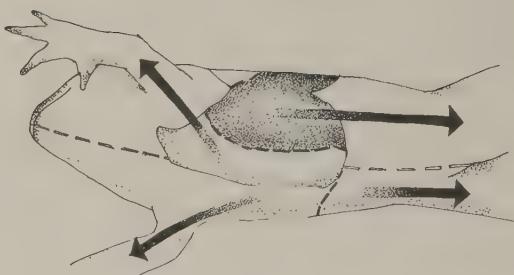
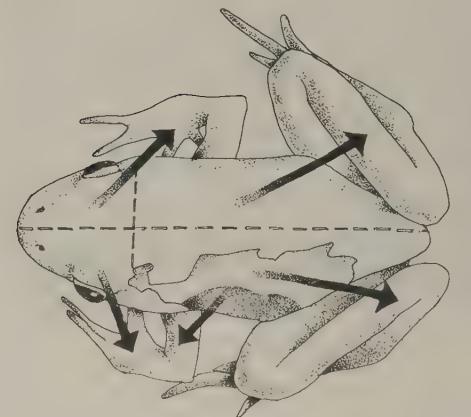
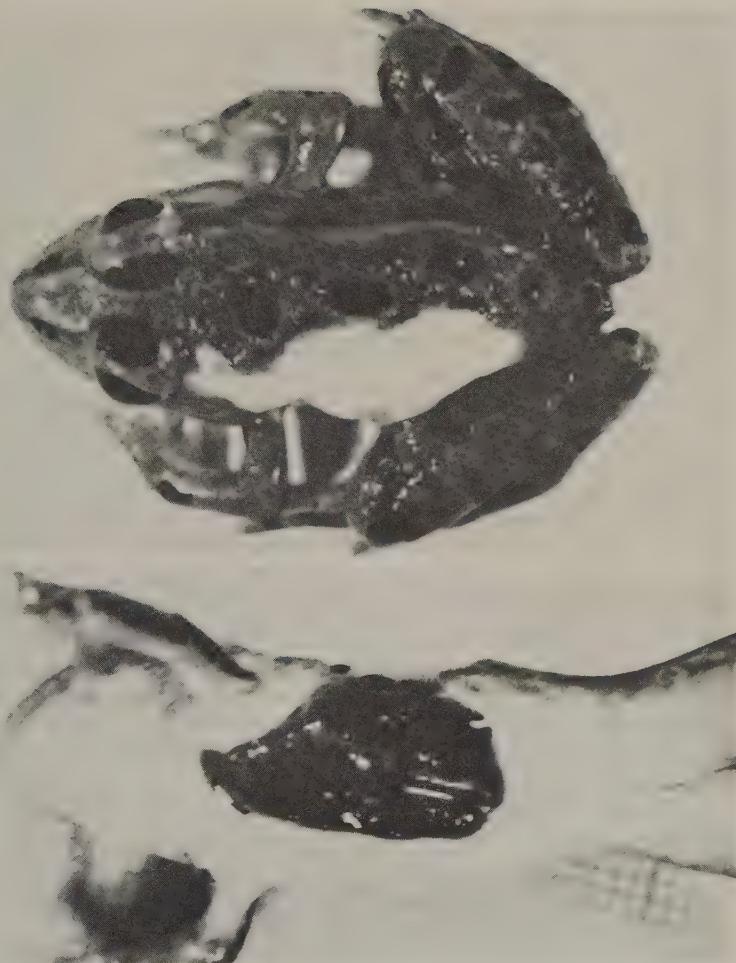
The individual neurons of the nervous system do not actively migrate, but they do send out extremely long cytoplasmic processes, the axons, into the peripheral tissues. These axons make contact with muscles or sense organs and establish electrochemical communication with them. The factors that guide axons to appropriate target cells at great distances from the cell bodies in the spinal cord are not known. It has been suggested that the target tissues attract nerve axons by production of specific chemicals, but attempts to verify this theory by experiment have been unsuccessful so far (Hughes, 1968). In early development, the organization of the nervous system is quite plastic, for nerves that would innervate (form synaptic connections with) only a small portion of a leg can be made to innervate the whole of a grafted extra leg. Functional organization of nerve connections within the central nervous system occurs after the peripheral connections have been made and is made in a way appropriate to the peripheral connections. Thus, the sensory and motor nerves of an extra leg become coordinated into the nervous system in a way that permits the leg to move in synchrony with the adjacent normal leg (Hughes, 1968).

Further evidence of the functional plasticity of the nervous system comes from experiments in which patches of skin have been interchanged between the belly and the back of tadpoles (Jacobson and Baker, 1969). Just after metamorphosis, a stimulus to the back skin on the belly causes the frog to make wiping motions at the proper location on the belly. A short time later, however, the frog will respond to a similar stimulus by inappropriately attempting to wipe the point on the back where the skin should be located. Apparently, the nerves transmit some kind of information from the transplanted skin to the central nervous system, indicating that the skin is back skin regardless of its real location.

The cellular slime molds are of particular interest because the development of their fruiting bodies represents an extreme example of morphogenesis through cell migration. Under certain conditions such as scarcity of food, the amoeboid cells living as separate individuals migrate toward one another and form a multicellular creature with a characteristic shape. The coordination of the individual cells into a multicellular body is achieved by a chemical attractant called acrasin, which is secreted into the environment by some cells. This chemical overcomes the antagonism that keeps the individual cells apart, and aggregation is then possible (Trinkaus, 1969).

Cells from tissues of multicellular organisms exhibit repulsive tendencies similar to those of the amoeboid cells of the slime molds prior to aggregation. When pieces of embryonic tissues such as heart or cartilage are cul-

Figure 18.19. Photographs and diagrams illustrating the interchange of skin from the back to the belly of the frog.



tured on a flat surface, cells leave the tissues and spread out to form a single layer over the surface. Under normal circumstances, they do not crawl over each other. In fact, time-lapse movies show that two cells closely approaching each other stop moving and, if space is available, reverse the direction of their movement so as to move away from each other. Careful examination of the movies shows that the advancing cytoplasmic front of each cell consists of a thin cytoplasmic sheet that is constantly undulating. When this so-called *ruffled membrane* contacts another cell, it stops undulating and the cell becomes temporarily paralyzed, a phenomenon called *contact inhibition*. Soon a ruffled membrane forms on some other part of the cell, and the cell moves away in that direction. Chemicals such as acrasin may suppress the wandering tendencies of cells. The coordinating function of acrasin might also be served by other mechanisms, such as intercellular communication through cell junctions or the secretion of a solid, extracellular matrix that inhibits movement of cells.

Metamorphosis and Maturation

For many animals, the end of embryonic life is the end of the most rapid phase of growth and change. However, for a large number of vertebrates

Figure 18.20. Side of wax cells cut away to expose young honeybees developing inside.



and invertebrates there is an additional phase of rapid change, known as *metamorphosis*, when a juvenile form of the organism is transformed into an adult with a very different mode of life. Organisms such as flies and frogs, which undergo complete metamorphosis prior to sexual maturity, get the best of two worlds by being adapted to one habitat as larvae and to another as adults. In insects such as flies and moths, the changes that take place at metamorphosis are so extreme that the organisms construct many specialized parts of the adult from cells that were set aside in the embryo and never became functional parts of the juvenile organism. These cells are the *imaginal discs* discussed earlier, which live a virtually parasitic existence in the larva of the insect. At the end of larval life, in response to hormonal changes, many of the larval cells die, whereas the imaginal discs grow to form the pupa and then the adult (Schneiderman, 1969).

In all animals, including those that do not undergo metamorphosis, growth is the most conspicuous developmental phenomenon in the early postembryonic period. This growth appears to be stimulated in vertebrates, as in plants, by hormones and continues until sexual maturity. At the time of sexual maturity, other hormones are secreted, and these promote maturation and inhibit overall growth.

Aging

The phenomena of aging, or senescence, are part of the normal development of an animal. Senescence is the gradual deterioration of function and structure at both cellular and organismic levels. One school of thought is that senescence is primarily caused by accumulation of errors in the genetic information of cells through spontaneous mutations or errors in DNA replication during cell division. Vertebrate cells develop abnormal chromosome numbers relatively rapidly in culture, and it appears that such cells are capable of only a limited number of normal divisions in culture—about 50 in human fibroblasts (Hayflick, 1968). On the other hand, insect imaginal discs proliferated in culture for more than 1,000 cell divisions still exhibit normal differentiation (Hadorn, 1965).

Another possibility is that deterioration of the coordinating systems of the body is responsible for a wide variety of other malfunctions involved in senescence. Age pigments accumulate in neurons, and these and other accumulated waste products might cause general and cumulative deterioration of the nervous system. The human circulatory system also seems particularly susceptible to deterioration with age, and its malfunction could certainly contribute to the senescence of other tissues. Various tissues and organs probably senesce at different rates, and some organized tissues could possibly remain alive indefinitely in an appropriate environment.

At present, the processes of senescence are an inevitable part of the functioning of the mature organism. A great increase in lifespan could be accomplished only by prolonging the period of active growth. In this period, new cells are produced more rapidly than old ones die.

PROBLEMS OF DEVELOPMENTAL BIOLOGY

The problems of developmental biology are many and the answers are few. Fortunately, a number of powerful experimental techniques are available that promise to provide more answers.

The technique of somatic cell hybridization not only holds promise for the elucidation of genetic control mechanisms of development but may

make possible the preparation of genetic maps of human chromosomes. With refined surgical instruments such as ultraviolet microbeams and laser beams, biologists can perform fine operations on such tiny structures as chromosomes and mitochondria. By removing microscopic and submicroscopic components of cells, biologists will be better equipped to determine their roles in developmental processes. Drugs that inhibit various parts of the replication-transcription-translation processes and DNA-RNA hybridization techniques are providing new information about the control of gene action and protein synthesis in development (Gall and Pardue, 1969). Improved techniques for culturing cells and tissues and the use of radioactive isotopes as markers are leading to many discoveries about developmental mechanisms (Weston, 1967). Computer modeling of developmental processes is providing a convenient method of testing hypotheses about complex developmental interactions (Waddington and Cowe, 1969, and Ede and Law, 1969).

Because understanding of developmental processes is of major importance to medical progress, there is an active interplay between the researches of developmental biologists and the investigations of medical researchers. Many studies are aimed toward an understanding of malfunctions that cause normal cells to embark upon the uncontrolled division of cancerous tissue. Despite a great deal of interest and many important discoveries, the field of developmental biology has yet to make the major breakthrough to an understanding of major principles that has appeared imminent for the past few years. If that breakthrough is made, it will be of major importance for the general understanding of multicellular organisms and of human medicine.

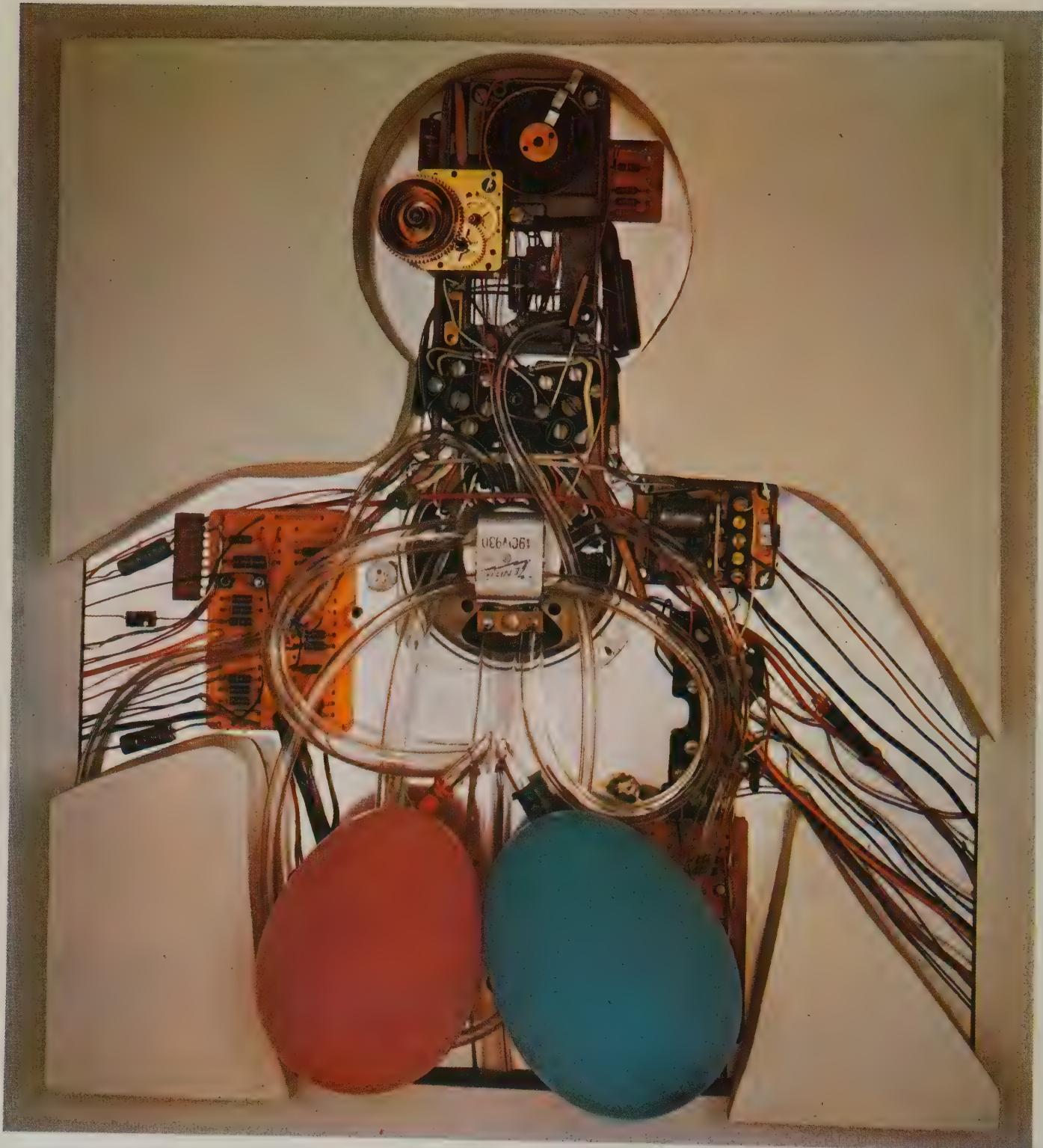
FURTHER READING

For more specific information about plant development, see books by James Bonner (1966), Esau (1965), Laetsch and Cleland (1967), Leopold (1964), and Steward (1968). A great many books are available as introductions to animal development, including those by Balinsky (1970), Ebert and Sussex (1970), and Waddington (1966).

Articles useful as introductions to general or specialized topics include those by Butler and Downs (1960), Edwards (1966), Frieden (1963), Gurdon (1968), Konigsberg (1964), Moscona (1961), Overbeek (1968), Steward (1963), and Wessells and Rutter (1969).

19

Physiology



All organisms must cope with essentially the same challenges. Each organism must obtain from the environment the materials needed to build the structures of its body, to repair those structures, and to reproduce. To build and maintain an ordered structure and to carry out the physical and chemical processes of life, every organism must obtain energy. Within the organism, materials must be transported from place to place and made available where needed. Waste products of metabolism must be eliminated selectively from the body. Heat must also be selectively retained or dissipated to keep the internal environment within the rather narrow temperature limits that permit efficient biochemical reactions. Thus, every living thing—from the simplest prokaryotic cell to the most complex multicellular organism—maintains *homeostasis*, or balance, by continually adjusting physiological functions for the maintenance of optimal internal conditions.

NUTRITION

The general term “nutrition” refers to the intake by an organism of the materials and energy needed to support life. Every organism must obtain carbon, oxygen, hydrogen, nitrogen, and smaller amounts of other elements in order to synthesize the macromolecules that make up its body.

Nutritional Requirements

Organisms that are able to utilize simple inorganic substances and synthesize all organic molecules needed by their bodies are called *autotrophs*. Organisms that are unable to perform all necessary syntheses and therefore must obtain some organic molecules from other organisms are called *heterotrophs*. There are two types of autotrophs. Photosynthetic autotrophs are able to trap the radiant energy of sunlight and to use this energy to synthesize carbohydrates (Chapter 5). Chemosynthetic autotrophs are bacteria that oxidize various inorganic substances such as sulfur, ammonia, nitrite, iron compounds, and hydrogen and use that energy to synthesize carbohydrates. All other organisms (heterotrophs) can use energy only after it has been trapped by autotrophs and stored in the form of chemical bonds in carbohydrates. The basic nutritional requirement for heterotrophs, then, is carbohydrate as an energy source. Most heterotrophs also require oxygen to burn carbohydrates to carbon dioxide and water—the most efficient method of using the energy in carbohydrates. In addition, most heterotrophs also must obtain certain other ready-made organic molecules. For example, rats and humans cannot synthesize 8 of the 20 amino acids that occur in proteins of all organisms. They must be obtained in the diet. Certain inorganic ions are required by all living things. The diverse but vital functions served by these ions are listed in Table 19.1.

Ingestion and Digestion

The simplest way for a cell to obtain nutrients is to depend upon diffusion to bring nutrients from the environment. The rate of diffusion depends upon the surface area of the cell and upon the difference in concentrations between outside and inside. As the size of a cell increases, the need for nutrients increases roughly in proportion to the volume of the cell (and therefore to the cube of its radius). The surface area of a simple spherical cell increases only in proportion to the square of its radius. The larger an organism is, therefore, the more complexly folded its surface must be in order to

Figure 19.1a. These free-living planarian flatworms have a single opening to the digestive tract, which serves for both ingestion and egestion. This opening is located at the midpoint of the body at the end of a muscular tubular pharynx. To feed, the animal crawls on top of the food, extends the pharynx, and pumps fluids and small bits of food into the digestive tract.



provide sufficient surface area for rapid diffusion of nutrients. Such an organism would also be limited to solutions that contain a relatively high

Table 19.1
Inorganic Ions Required by Living Things

ION*	Some Principal Functions
Na ⁺	Chief cation in extracellular fluids of most animals; carrier of current in action potentials in most nerves and muscles
Cl ⁻	Chief anion in extracellular fluids of most organisms
Mg ⁺⁺	Important in maintaining stability of intercellular substances and cell membranes; integral part of the chlorophyll molecule; required for muscle contractility; cofactor for many enzymatic reactions
Ca ⁺⁺	Involved in stability of intercellular substances and cell membranes; component of bones and teeth; key factor in initiation of muscle contraction
K ⁺	Chief cation in intracellular fluids of most organisms; carrier of outward current in action potentials in nerves and muscles
Fe ⁺⁺ , Fe ⁺⁺⁺	Integral part of heme, component of hemoglobin and cytochromes that transport electrons in oxidative metabolism
Co ⁺⁺	Part of vitamin B ₁₂ , cyanocobalamin
Cu ⁺⁺ , Cu ⁺⁺⁺	Part of hemocyanin molecule that transports oxygen in the blood of some invertebrates; cofactor in mitochondrial electron transport systems
Zn ⁺⁺	Integral part of several enzymes
I ⁻	Used in amino acids of some structural proteins found in invertebrates; part of thyroxin molecule, the vertebrate thyroid hormone
Mn ⁺⁺	Cofactor for some enzymes
PO ₄ ⁻⁻⁻	Part of ATP molecule used in energy storage and release; component of bones and teeth; component of nucleic acids
S†	Occurs in several amino acids; S—S bonds contribute to formation of tertiary structure of proteins

*Other inorganic ions may be required in trace amounts by some plants and animals.

†S is obtained by organisms either as SO₄⁻⁻ or in amino acids; some bacteria can use molecular sulfur or H₂S.

concentration of dissolved nutrients; in very dilute solutions, the rate of diffusion becomes too low to supply the needs of an organism.

Because organic substances are relatively scarce in most environments, heterotrophs must often extract food particles from large volumes of the surrounding fluid or actively pursue and capture their food. Large food par-

Figure 19.1b (left). The coelenterate *Hydra* represents a polyp form that feeds upon small fresh-water zooplankton. The feeding response is elicited by the chemical and tactile stimuli of the prey, which is then captured by nematocyst batteries and pulled into the gastrovascular cavity.

Figure 19.1c (right). This marine jellyfish feeds on small crustacea and fish that it catches with tentacles equipped with nematocyst batteries.



ticles are taken into some sort of internal digestive system, where they are physically and chemically broken down to units that can be absorbed.

Unicellular organisms, using the processes of phagocytosis and pinocytosis (Chapter 10), bring large molecules or small particles of food into the cell by enfolding the food particle in a membrane pocket that is taken into the cytoplasm as a vacuole. Digestive enzymes are secreted into the vacuoles, and the small organic molecules produced by digestion are moved across the vacuole membrane into the cytoplasm by diffusion or active transport.

In the simplest heterotrophic cells, pinocytosis or phagocytosis can occur at any point on the cell surface. In more complex protozoa, a single part of the surface may be specialized for phagocytosis, and cilia are often present to move food particles toward this primitive mouth. Fungi secrete enzymes that break large organic molecules into smaller units, which are then brought into the cell by diffusion or active transport.

Coelenterates such as *Hydra*, jellyfish, or sea anemones feed on small crustacea or fish, which they catch with tentacles equipped with stinging cells (nematocysts) that inject a paralyzing poison into their prey. When the prey is immobilized, the tentacles bring it to the mouth, the mouth opens, and the food is stuffed into the digestive cavity. This cavity is a closed pocket lined by cells that secrete digestive enzymes and that absorb nutrients by phagocytosis and active transport. Any undigested residue is ejected back through the mouth.

In all higher animals beginning with the roundworm, the digestive tract is a continuous tube with a mouth at one end and an anus at the other. Food is progressively digested as it moves through the tube, or gut, and processing is not interrupted by each new ingestion. Movement of food through the tube is accomplished by rhythmic contractions, or peristalsis, of muscles in the gut walls. In more complex organisms, the length of the digestive system is increased by coiling or folding of the tube; the surface area available for absorption of small organic molecules is increased by foldings or projections of the tube lining; and the tube is differentiated into specialized passageways and cavities within which various parts of the digestive process occur.

The basic chemical process of digestion is one of hydrolysis, breaking down macromolecules to simpler organic molecules such as amino acids, glucose, glycerol, and fatty acids. These smaller molecules are then absorbed across the lining of the digestive tract. Hydrolytic enzymes are released into the digestive tract from specialized secretory cells and organs.

Figure 19.2 (below). Selected features of the digestive tracts of some representative invertebrate animals. From top to bottom are an earthworm, with buccal cavity, crop, gizzard, and intestine; a crayfish, with cardiac and pyloric stomach and intestine; and a mosquito, with a large ventral diverticulum to store the blood meal.

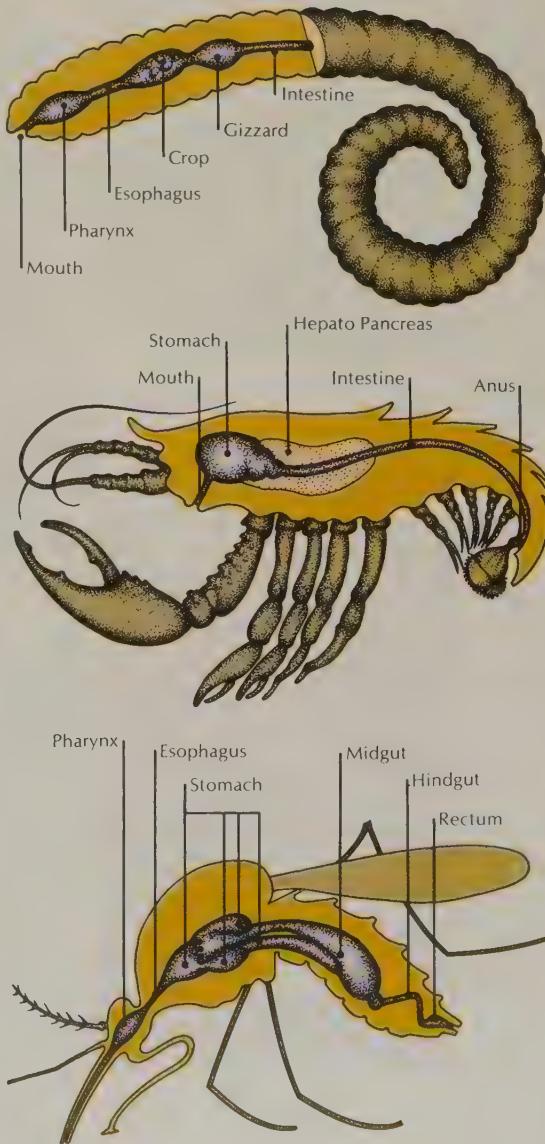
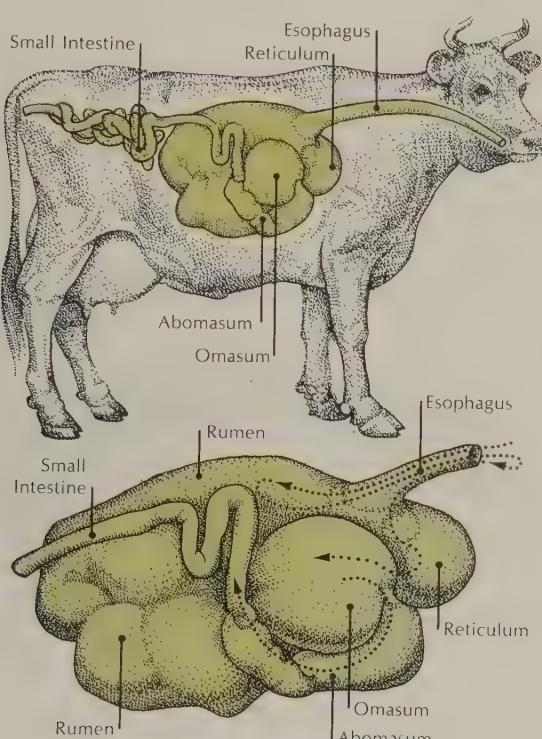


Figure 19.3 (above). Digestive tract of the cow. The cow stomach is divided into four compartments: rumen, reticulum, omasum, and abomasum. In the first two compartments, bacterial and protozoan action break down cellulose to simple sugars; the omasum functions as a water conservation device, whereas the conventional digestive activity of the stomach takes place in the abomasum.

Figure 19.4 (below). Cross section of the gastric mucosa.



For these chemical reactions to occur, large chunks of food must first be physically broken down into small pieces, allowing access to the digestive enzymes.

The mouth region is specialized for the intake of food into the digestive tract. In many animals, specialized structures such as teeth or beaks assist in the physical disintegration of food. For animals that feed on green plants, the preliminary cutting and crushing of food is particularly important, because the tough cellulose walls of plant cells are extremely resistant to the action of digestive enzymes. Some complex animals use their muscular tongues to bring food into the mouth and to maneuver it within the mouth cavity during chewing and swallowing. In mammals the food mixture is lubricated with *saliva*, which is secreted within the mouth and mixed with the food during chewing and which contains an enzyme that hydrolyzes starches into disaccharides.

Animals that feed intermittently may store food in the *crop*, from which food can be released more slowly as needed into the digestive system. In birds food is crushed internally in the *gizzard*, a muscular sac that contains coarse sand or small stones and has a hard lining. In mammals food moves from the mouth into the gullet, or *esophagus*, which is a tube serving primarily to transport food to the stomach. Muscle contractions move food along the tube, and lubricating fluids are secreted from the glands in the tube walls.

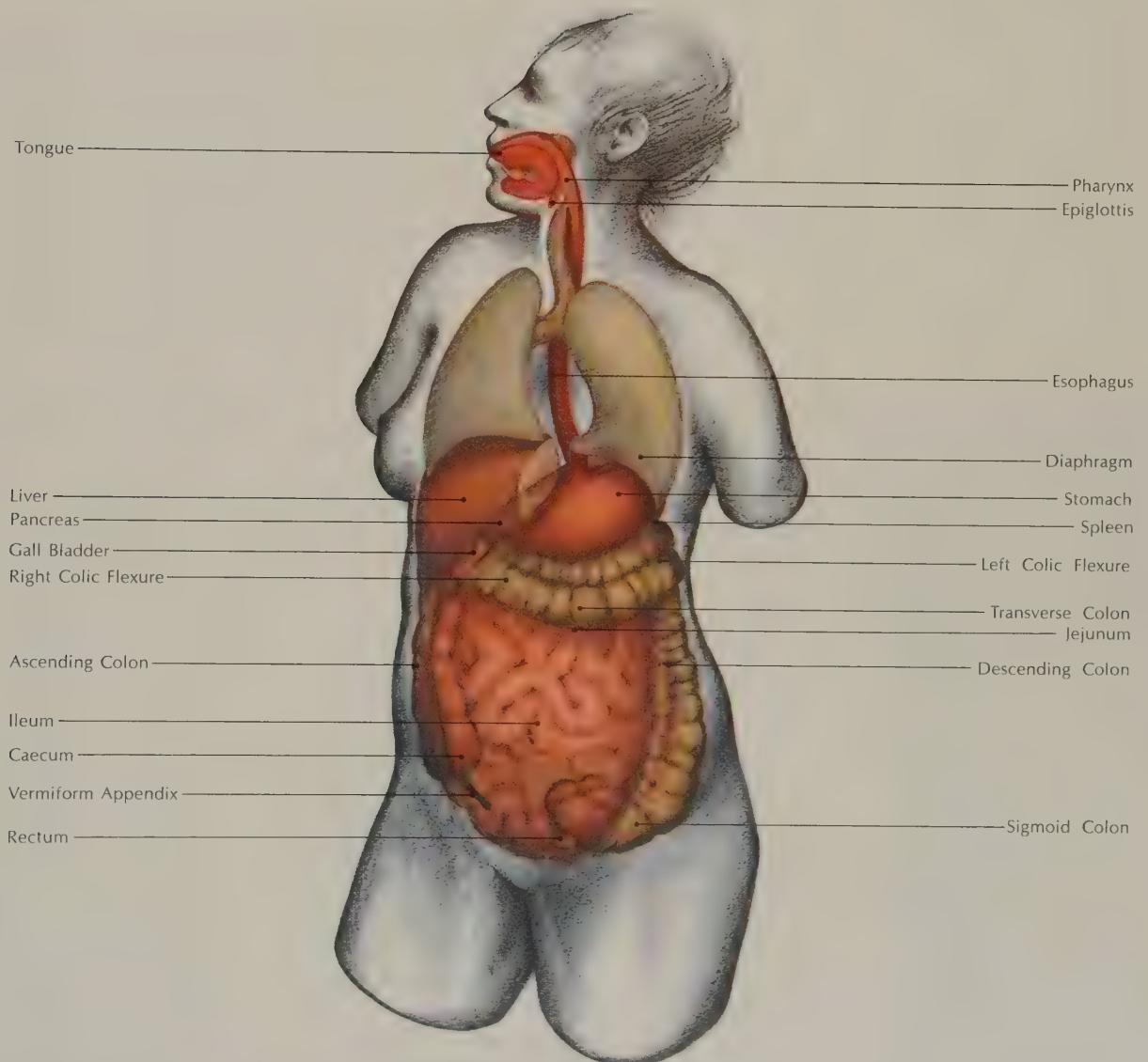
In some grass-eating mammals, such as the cow, the food moves from the esophagus into a large organ called the *rumen*. Within the rumen lives a population of bacteria and protozoa that are capable of breaking down cellulose to simple sugars. In addition, they synthesize many of the vitamins needed by the cow. Food is stored and fermented in the rumen for some time, being regurgitated and rechewed from time to time before it is passed along to the stomach.

The stomach of a mammal is a muscular, baglike organ that can be closed at both ends by *sphincters*, or rings of muscle. Muscle contractions in the stomach wall churn and squeeze the food, thoroughly mixing it with substances secreted by cells and glands in the stomach lining. The epithelial lining of the stomach, or *gastric mucosa*, includes several kinds of cells. The outer parts of folds in the lining are covered mostly with *mucous cells*, which secrete a viscous fluid that coats the stomach lining. This fluid protects the stomach lining from the actions of digestive enzymes and dilutes the food mixture. *Parietal cells*, which secrete hydrochloric acid, and *chief cells*, which secrete the protein-hydrolyzing enzyme *pepsin*, are abundant between folds of the lining. After the food mixture is thoroughly mixed with mucous fluid, acid, pepsin, and other enzymes, the posterior sphincter opens, the stomach contracts, and the food is forced into the small intestine.

Within the long, coiled tube of the small intestine, digestion is completed and the resulting small organic molecules are absorbed into cells that line the tube. The surface of the small intestine is covered with minute, fingerlike projections, or *villi*. The cell surfaces lining the villi are themselves covered with submicroscopic projections, or *microvilli*. These structures produce a great surface area—about 2,000 square feet in the human small intestine—which ensures a complete absorption of nutrients.

The short segment of small intestine closest to the stomach is known as the *duodenum*. Here secretions from the pancreas and liver enter the gut. Pancreatic juice contains enzymes that break down the most common

Figure 19.5. A diagram of the human digestive system.



types of biological macromolecules. Pancreatic amylase completes the hydrolysis of starch (begun by salivary amylase) into disaccharides, while other sugar-digesting enzymes act on other sugar chains. Ribonuclease and deoxyribonuclease catalyze the hydrolysis of RNA and DNA into short nucleotide chains. Pancreatic lipase breaks down fats into fatty acids and glycerol. The proteolytic (protein-digesting) enzymes of the pancreas have been intensively studied. Several enzymes with different specificities ensure complete digestion of proteins to amino acids. Trypsin and chymotrypsin cleave long protein chains into short peptides, while aminopeptidase and the carboxypeptidases liberate amino acids from the amino and carboxyl ends of the peptides, respectively. These proteolytic enzymes are synthesized by the pancreas in an inactive form so that they do not destroy themselves or other pancreatic proteins. Upon arrival in the duodenum,

these enzymes are activated by residual proteolytic enzymes, which cleave away susceptible sections of the inactive precursor enzyme protein chains, thus unblocking their active sites. In addition to enzymes, pancreatic juice contains sodium bicarbonate, which neutralizes the acid passed down from the stomach and makes the contents of the intestine slightly alkaline. Pancreatic secretion is largely controlled by the hormone secretin.

Another secretion aiding in digestion is produced by the liver in the form of bile. Bile travels from the liver into the gall bladder, where it is stored until it is needed. When food enters the duodenum, bile flows into the duodenum through the bile duct. Bile contains *bile salts*, sodium salts of certain complex organic acids, which function as detergents, breaking up insoluble droplets of fats and making the fat molecules accessible to the action of pancreatic lipase. One of the many functions of the liver is the breakdown of hemoglobin from red blood cells. Heme from hemoglobin is degraded into products that are included in bile as *bile pigments*. Considerable amounts of bile pigments pass out with excrement, giving it a characteristic brown color.

Nutrients are absorbed from the intestine by transport across cell membranes lining the intestine, a process followed by diffusion into the circulatory system. The products of fat digestion—glycerol and fatty acids—cross the membrane passively and diffuse into the *central lacteal*, a lymphatic vessel in each villus. The absorbed nutrients are carried away from the intestine by a capillary network that feeds into the hepatic portal vein. This vein transports dissolved nutrients to another capillary network in the liver. The liver removes excess nutrients for storage and releases nutrients from storage if the blood level of some substance falls too low. In these ways, the liver helps to maintain optimum levels of glucose and amino acids in the blood throughout periods of fasting and glutony.

Undigested residue moves from the small intestine into the *caecum*, a pouch at the beginning of the large intestine. In many herbivorous animals, such as rabbits, the caecum plays a role similar to that of the rumen. Bacteria living in the caecum of the rabbit convert cellulose to sugar. In carnivorous and omnivorous mammals, the caecum is relatively small or is absent. In man, the short caecum terminates in the appendix. The major part of the large intestine, the *colon*, is a corrugated tube with a smooth lining that has virtually no villi. The colon serves primarily to remove excess water from undigested material. It houses large numbers of bacteria, principally the species *Escherichia coli*, which are also found in lesser numbers elsewhere in the gut. These bacteria synthesize significant amounts of some vitamins, which are subsequently absorbed by the colon and utilized by the body. At the end of the large intestine is a short section, the *rectum*, where undigested waste material is stored until eliminated from the body through the anus.

Absorption in Plants

Fungi and other organisms specializing in absorption of nutrients are restricted to environments that provide a relatively high concentration of nutrient substances. Fungi secrete digestive enzymes that hydrolyze macromolecules outside the organism, breaking them down to simpler organic molecules that can cross cell membranes by diffusion and active transport.

Aquatic plants obtain mineral salts and water by diffusion across the cell membranes, aided in some cases by active transport of certain ions. Land

Figure 19.6. Enlarged photograph of a growing root tip. Note the abundance of root hairs along the margin of the root in the region of cell elongation. These root hairs provide increased surface area for the diffusion and active transport of essential nutrients and water. (Courtesy Carolina Biological Supply Company)

plants have roots specialized to absorb mineral salts and water from the soil. Like villi of the animal intestine, plant root hairs provide a greatly increased surface area across which diffusion and active transport can take place. Most plant root cells are extremely efficient at pumping potassium ions into the organism and pumping sodium ions out. Active transport of other ions has also been demonstrated. The active accumulation of ions produces an osmotic force that drives water into root cells. Because the cells have relatively rigid walls, they do not expand greatly, but a high pressure is built up inside the cells. As water is drawn from inner cells up through the xylem, the pressure in inner cells of the root decreases. High pressure in outer cells forces more water into the xylem, and more water is drawn osmotically from the soil.

GAS EXCHANGE

Small aquatic organisms readily obtain oxygen and carbon dioxide by diffusion from water. In the light, photosynthetic organisms produce more oxygen than respiration concurrently uses up, and the plant must obtain CO₂ from the environment. In the dark, no photosynthesis occurs; and these organisms must acquire oxygen as do all nonphotosynthetic organisms. In order to capture sunlight, the multicellular photosynthetic organism must have cells spread out in relatively thin layers or sheets. Because most of the cells thus have large surface areas in contact with the surrounding water, most aquatic plants are able to obtain the needed gases by simple diffusion directly into the photosynthetic cells and thus require no specialized respiratory systems.

In aquatic organisms that move about to find food, more compact structures for gas exchange appear to be advantageous. In the simplest forms, these structures consist of extensions or indentations of the surface. Most larger aquatic animals possess structures that serve to increase the surface area available for diffusion of oxygen into the organism. With increasing size, however, a means must be provided for delivering dissolved O₂ to cells that may be distant from the site of gas exchange. Therefore, structures specialized for gas exchange bring the dissolved gases in close contact with the animal's blood or body fluid.

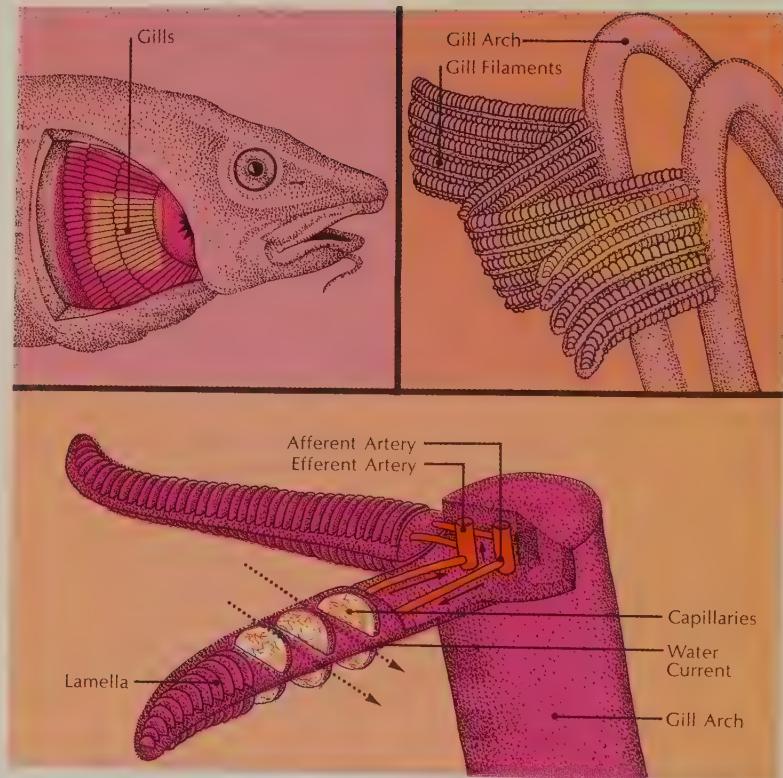
In more complex aquatic organisms, extensively convoluted surface regions, or *gills*, are common. Gills are highly specialized to maximize the movement of gases while minimizing the movement of water into cells (Figure 19.7). In most aquatic animals, special mechanisms move a steady stream of water past the gills. Fish generally have sets of muscles in the floor of the mouth and in gill covers that constantly pump water into the mouth and out over the gills. Sharks and mackerel, however, lack the ability to make these breathing movements. They must constantly swim with their mouths open to pass water over their gills and suffocate if prevented from swimming. The flow of blood within fish gills is in a direction opposite to that in which water flows over the gills. This *countercurrent* system causes the blood leaving the gills to be exposed to oxygen-rich water entering the gills and results in an efficient transfer of oxygen into the organism. Amphibians carry on a substantial fraction of their gas exchange through their moist skins, although they also have lungs or gills.

In one way, obtaining oxygen from air is an easier task than from water. A given volume of air contains 40 times as much oxygen as does the same volume of water. Furthermore, oxygen diffuses 300,000 times more rapidly



Figure 19.7 (right). Structural diagram of a typical teleost (bony fish) gill. The lamella construction of each gill filament serves to increase the surface area and thus the efficiency of gas exchange.

Figure 19.8 (left). Tracheal tube respiratory system as diagrammed in a generalized insect. This system operates by simple diffusion of gases through a complex tube system throughout the body of the organism. The tubes open on the body surface to allow exchange with the external atmosphere.



in air than it does in water. However, gills, which function so well in water, cannot obtain oxygen from the air. Most gills are made up of highly branched extensions of the organism's outer surface and require the support of water.

Land animals possess internal respiratory systems that are braced against collapse. The concentration of water vapor within the cavity can remain high, thereby reducing water loss. Small land organisms such as snails and some crustaceans have simple lungs. Insects breathe through a system of tracheae, thin tubes that open on the body surface and ramify extensively throughout every tissue, bringing the air supply close to the cells and minimizing the role of the body fluid in the transport of gases.

Most large animals possess lungs, which are alternately emptied and filled with fresh air by active movements, or ventilation. Oxygen enters the respiratory system of a land animal in the gaseous state. It then is dissolved in a thin film of water on the lining of the respiratory tract and diffuses across cell membranes in solution.

Air enters the mammalian respiratory system through the nostrils and the nasal chamber, passes through the throat and the windpipe, or trachea, and enters the branching tubes, or bronchi and bronchioles (Figure 19.9). The walls of the trachea, bronchi, and bronchioles are relatively rigid to prevent collapse, but they can be somewhat expanded or contracted to control the flow of air. After branching repeatedly, the tubes, or alveolar ducts, end in millions of microscopic pouches, the alveoli that make up the lungs. The thin epithelial lining of these pouches, where gas exchange takes place, has a total area of about 750 square feet in man (Comroe, 1966). Each alveolus

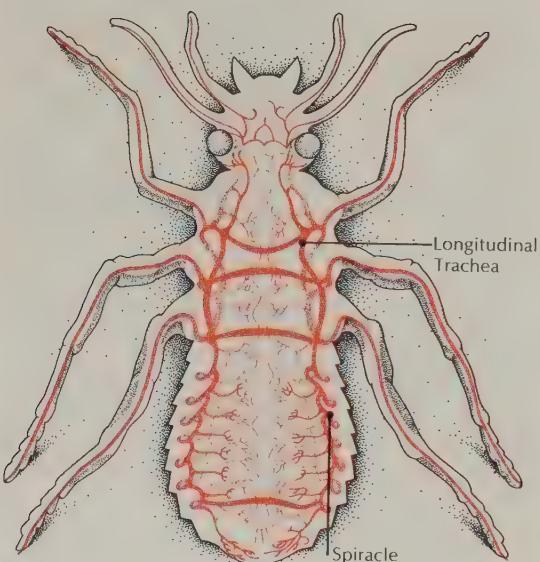


Figure 19.9. The human respiratory system. General view (top); bronchioles terminating in the grapelike clusters of alveoli (lower left); alveoli surrounded by a capillary network (lower right).

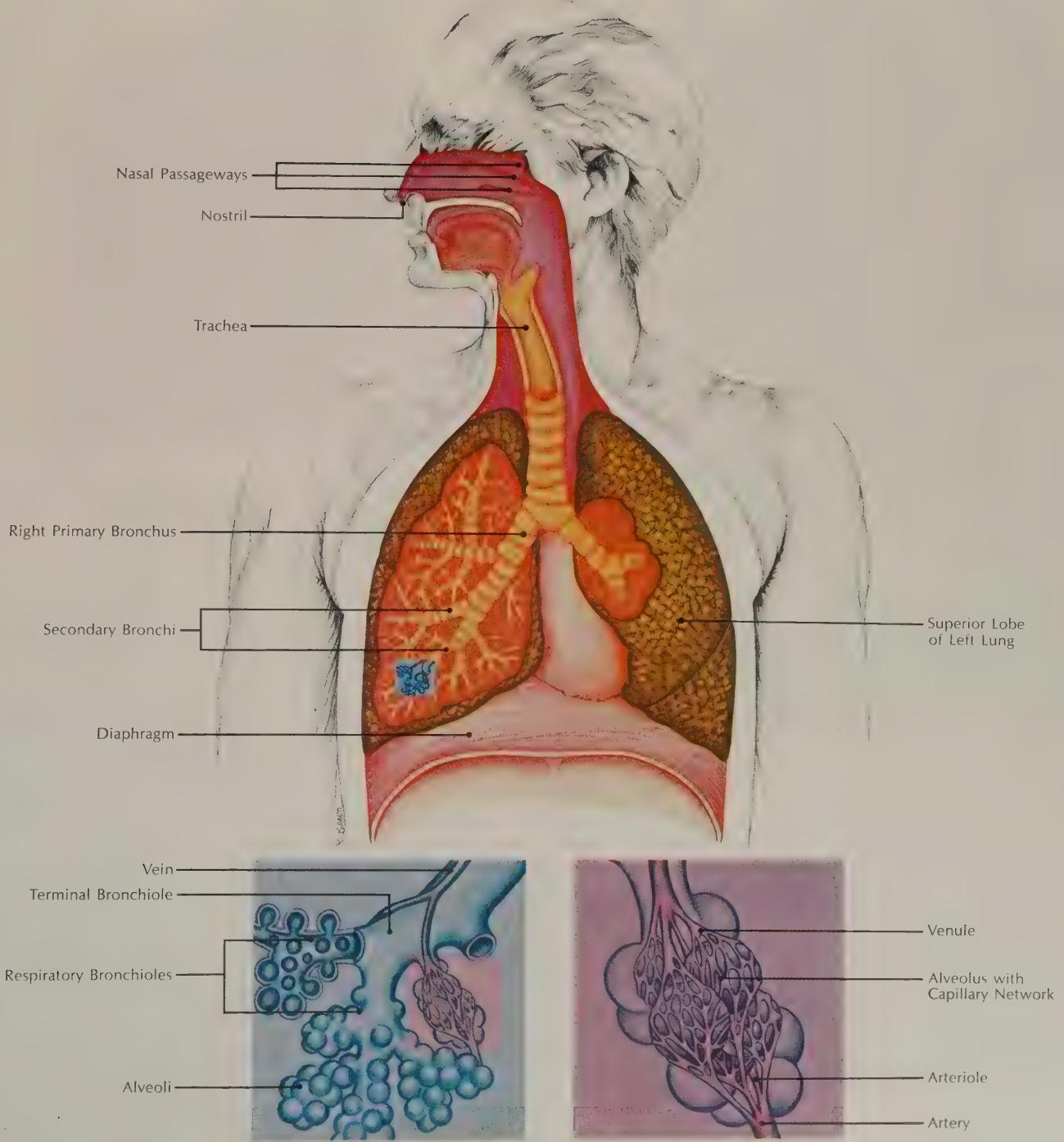
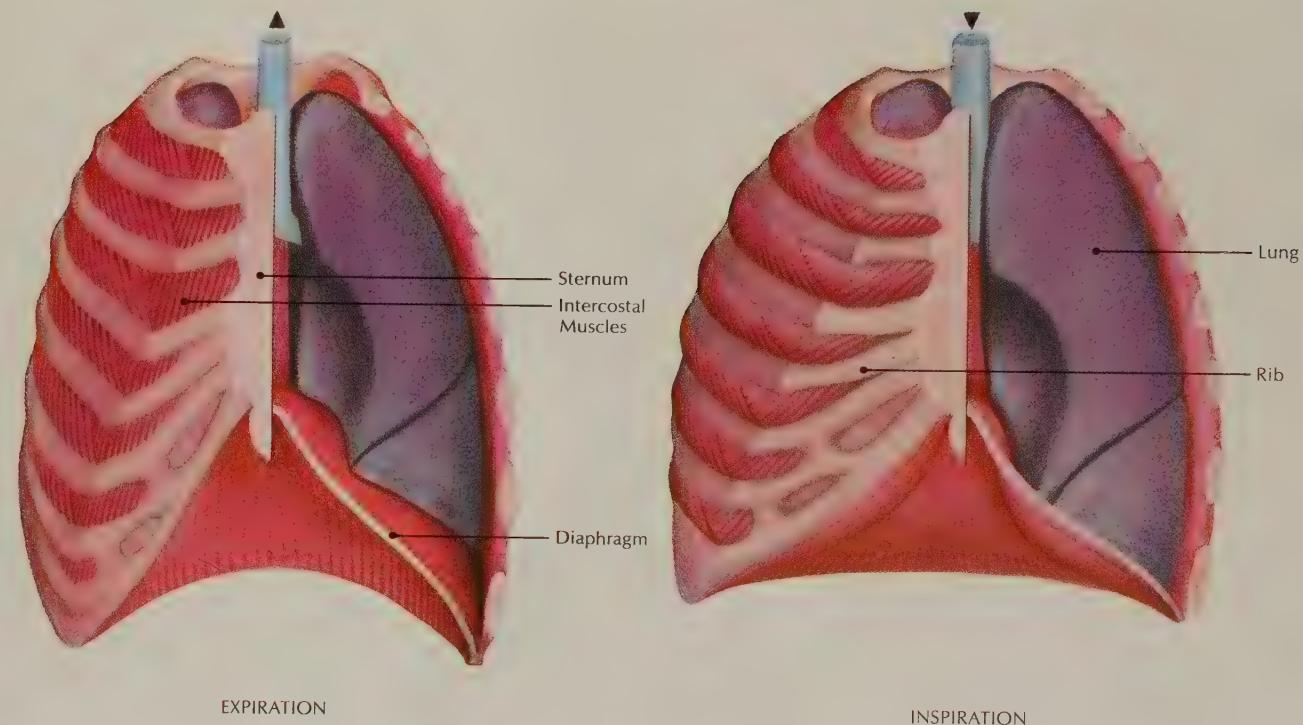


Figure 19.10. Position changes in the diaphragm and rib cage during expiration and inspiration.

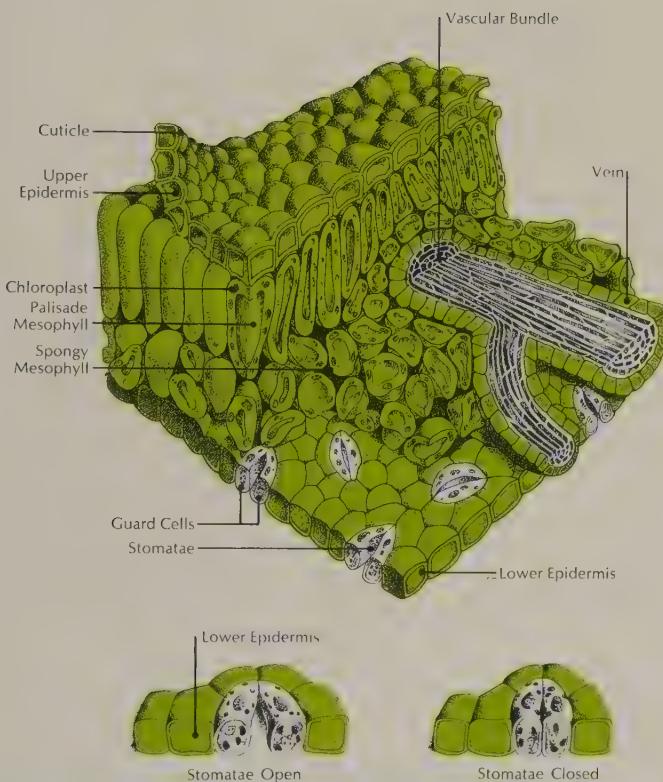


is invested with a capillary network. Blood gives up CO_2 and takes on O_2 as it passes through these capillaries in close contact with the alveolar epithelium.

The lungs occupy most of the chest cavity and are protected by the somewhat flexible rib cage. The floor of the chest cavity is a sheet of muscle called the *diaphragm*. Inhalation is caused by expansion of the rib cage and contraction of the diaphragm. When the diaphragm contracts, it flattens and thus enlarges the volume of the chest cavity. Atmospheric pressure forces air into the lungs so that they expand to fill the enlarged cavity. Exhalation occurs when the diaphragm relaxes and bulges upward, reducing the volume of the chest cavity and forcing air out of the lungs.

The rate of breathing and the extent to which the lungs are refilled on each breath are under partial voluntary control but normally are controlled involuntarily by a respiratory center at the base of the brain. This respiratory center is affected, among other things, by the concentration of CO_2 in the blood.

Like the land animal, the land plant has a surface that is relatively impermeable to water (preventing excessive water loss through evaporation). Therefore, land plants are unable to absorb oxygen or carbon dioxide over their surface, as do aquatic plants. Instead, gases are absorbed at the surfaces of intercellular spaces within the leaves. The site of gas exchange is close to the site of use and release so that the problem of transport is minimized. Gases enter the spaces through microscopic slits in the undersurface of the leaf, the *stomata*. *Guard cells*, which flank the stomata, regulate the flow of gases by opening or closing the slit. The control mechanism of



the guard cells is not yet fully understood, but it appears that a decrease in CO_2 concentration within these cells caused by active photosynthesis somehow increases the turgidity of the cells and opens the stomata. If excessive water loss causes wilting, the guard cells become flaccid and the stomata close.

The rate-control mechanisms of the lungs and stomata (as well as a small ring of muscle that opens and closes the tracheae of insects) hold the movement of gases into the respiratory system to the minimum needed for gas supply, thus minimizing the loss of water to the atmosphere.

INTERNAL TRANSPORT

Once nutrient materials have entered the organism, they must be transported to the sites where they can be used in chemical reactions. The respiratory system obtains oxygen from the environment, and this oxygen must be efficiently distributed. Finally, waste products must be transported to the excretory system for elimination. These functions are served by circulatory systems.

Circulatory Systems

In unicellular organisms and small multicellular organisms, diffusion carries materials from parts of the organism where they enter or are formed to parts where they are consumed or expelled. In algae and sponges, where there is relatively little cell specialization, the body organization is such that most cells are exposed to the external sea water or to water circulated through passages in the body. Each cell is able to exchange materials with

Figure 19.11a (left). Diagrammatic representation of a leaf section. Note the four distinct tissue layers.

Figure 19.11b (upper right). Stomata-guard cell complexes on the underside of a leaf.

Figure 19.11c (lower right). Photomicrograph of a leaf cross section. Gas exchange takes place through small pores, called stomata, located on the underside of the leaf.

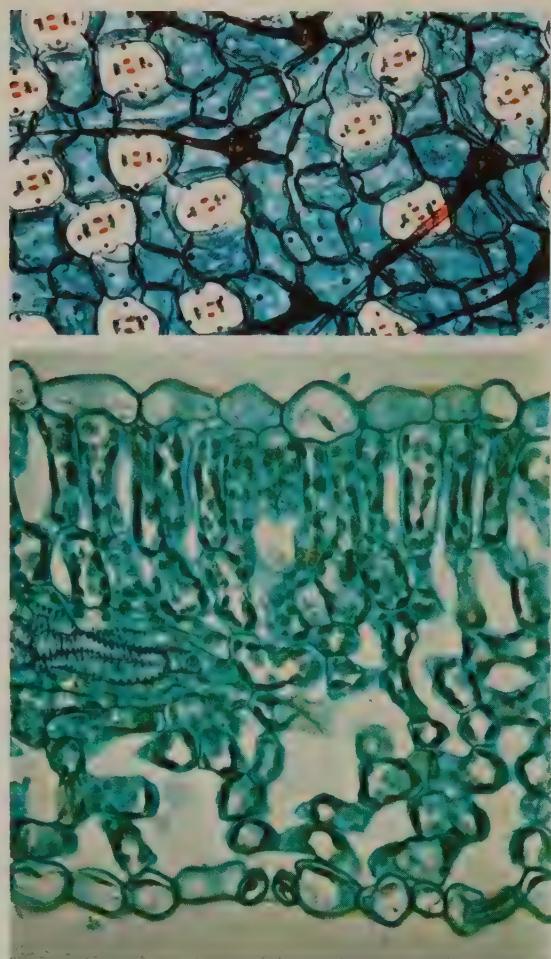
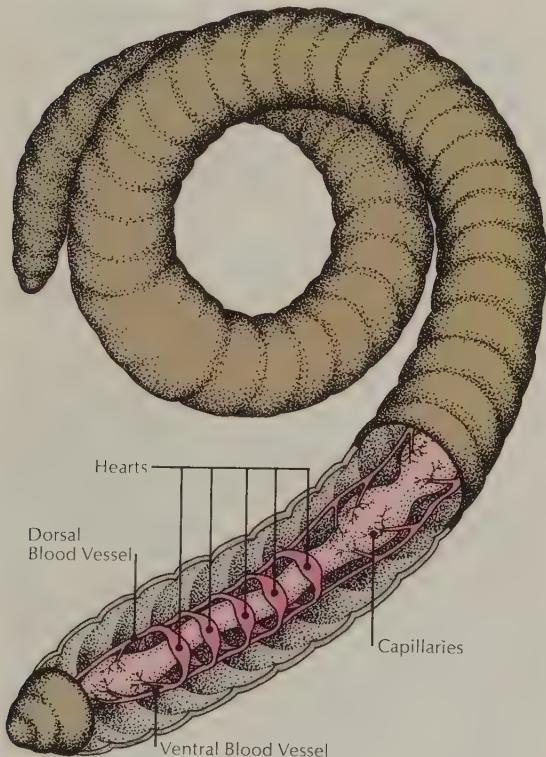


Figure 19.12. The closed circulatory system of the earthworm, representing the annelid phylum. A simple tubular heart forces the blood forward along the dorsal vessel and backward along the ventral vessel.



water or to exchange materials with nearby cells that are in contact with water. The flat body structure of algae allows every cell to be near the external environment. In simple animals, water is circulated through internal passages by cilia.

An unusual transport system exists in the coelenterates. Some of the cells in the epithelial layer that lines the body cavity are amoeboid. After engulfing food particles by phagocytosis, these cells move through the organism and distribute the digested nutrients to other cells.

In flatworms, food is taken into the gut and mechanically broken down to small particles. The particles are taken in by cells lining the gut and are digested. Because of the flattened body structure and the small size of flatworms, every body cell is within a few cell widths of some part of the branched gut. Thus, diffusion can carry nutrients to all cells. Animals above the flatworms generally have some sort of specialized circulatory system with one or more pumps to move fluid throughout the system. In molluscs and arthropods, a heart pumps the internal fluid through tubes or vessels that empty into various parts of the body. The fluid bathes the tissues of the body and collects in cavities called blood sinuses, which communicate with a chamber surrounding the heart. When the heart relaxes, blood from the surrounding chamber is sucked into the heart through openings in its walls called *ostia*. When the heart contracts, the ostia close, and blood is forced out through the vessels. This arrangement is called an *open circulatory system* because the blood is not enclosed in vessels during its entire circuit through the body.

The circulatory system of annelids is a closed network of vessels. A simple tubular heart produces waves of muscular contraction that force blood forward along a dorsal vessel and backward along a ventral vessel (Figure 19.12). The movements of blood are somewhat irregular, and fluid may flow either way in various parts of the network.

Echinoderms have well-developed tubular systems extending through the body, but none of these appears to carry nutrients and waste products. The water-vascular system is filled with sea water pushed into the tubes by cilia; the water pressure inside this system helps in movement of body parts. Nutrients are transported within the body by three mechanisms: amoeboid cells, bathing of tissues with sea water in internal cavities, and branching of the gut and digestive system into most parts of the body.

Vertebrates, like annelids, have a closed circulatory system. A heart pumps into large vessels called *arteries*, which then carry the blood to various parts of the body. The arteries branch repeatedly, finally becoming tiny, thin-wall vessels called *capillaries*. As the blood moves through the capillaries, materials such as oxygen and carbon dioxide are exchanged with neighboring cells. The blood then moves into larger, thicker-wall *veins*, which carry it back to the heart.

Blood

The blood of vertebrates is composed of *plasma*, a fluid in which many proteins, ions, nutrients, and waste materials are dissolved. Floating in the plasma are *red blood cells*, several types of *white blood cells*, and a kind of cell fragment called *thrombocytes*, or *platelets* (Figure 19.13).

Materials move between blood and cells by diffusion. Some of these substances are simply dissolved in the blood plasma. However, the red blood cells of vertebrates play a special role in oxygen transport. These cells contain the protein *hemoglobin*, which gives blood its red color.

Figure 19.13a (upper left). Red blood cells, which function in the transportation and distribution of oxygen in mammals. Note the absence of nuclei.

Figure 19.13b (lower left). Blood platelets. These structures are not usually whole cells but membrane-covered cell fragments, often without nuclei. Platelets are involved in the blood-clotting mechanism.

Hemoglobin combines reversibly with oxygen so that mammalian blood is capable of transporting about 60 times more oxygen than would dissolve in plasma. When the oxygen concentration in cells around blood is high (as it is in the capillaries of the lungs or gills), hemoglobin combines readily with oxygen. In body parts where the oxygen concentration is low, hemoglobin readily gives up oxygen and allows it to diffuse out of the blood into the body tissues.

Hemoglobin contains four subunits, each of which consists of a protein chain, globin, and heme (a complex polycyclic ring structure containing iron). Each subunit can bind one oxygen molecule. The four subunits interact in a cooperative or allosteric way so that after one subunit binds an oxygen molecule, the other subunits bind additional oxygen molecules more readily. Hemoglobin can thus perform efficiently in its role of binding oxygen in the lungs and releasing it to the body tissues.

Carbon dioxide, which is produced as a waste product of metabolic respiration, is carried by the blood from the body cells to the lungs or gills, where it is excreted. In the blood, dissolved CO_2 gas is in equilibrium with carbonic acid and bicarbonate ion: $\text{CO}_2 + \text{H}_2\text{O} \rightleftharpoons \text{H}_2\text{CO}_3 \rightleftharpoons \text{H}^+ + \text{HCO}_3^-$. Because the hydration of CO_2 (and the dehydration of carbonic acid) is a relatively slow reaction, red blood cells contain the enzyme carbonic anhydrase to catalyze this reaction. This catalysis speeds up the reaction so that bicarbonate can be converted to gaseous CO_2 for exchange during the rapid flow of blood through the capillaries of the lungs.

Dissolved proteins and the platelets are involved in blood clotting, a complex series of reactions that occur in case of injury to the circulatory system. The end result of these reactions is the formation of a clot, which

Figure 19.14 (right). Hemoglobin molecule. The structure of the molecule has been deduced primarily from x-ray diffraction studies. The molecule consists of four closely associated polypeptide chains. Each molecule contains two alpha and two beta chains, each of which binds an oxygen-carrying heme group.

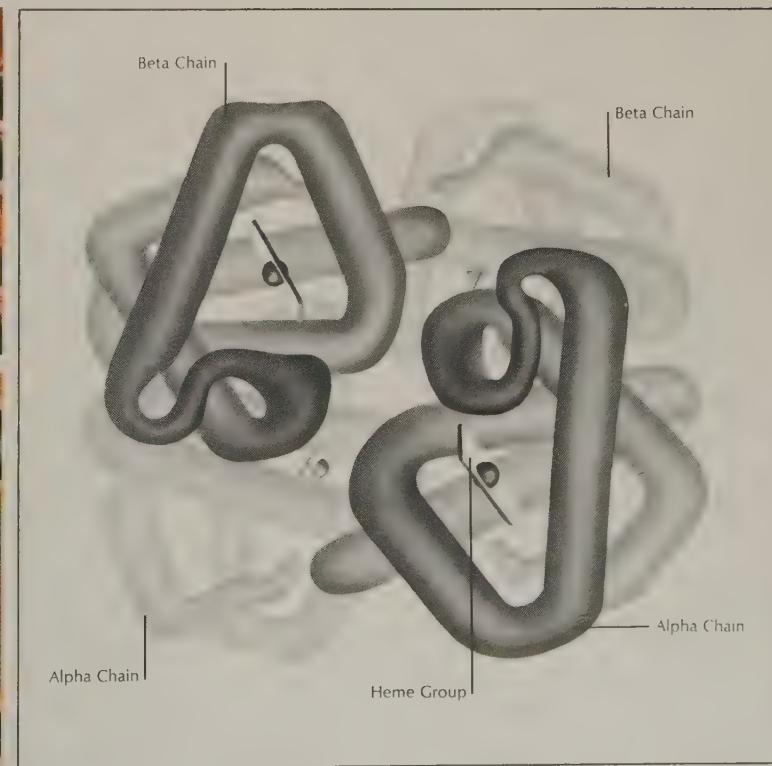
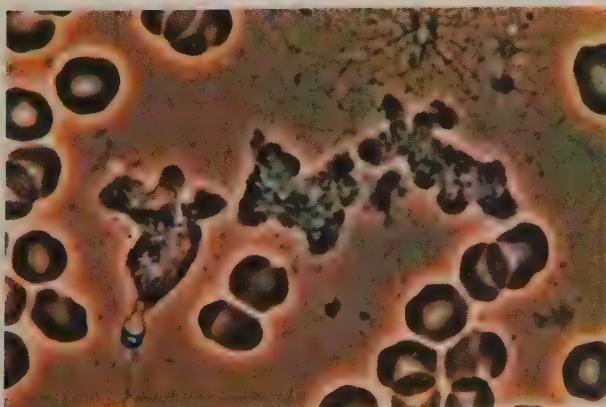
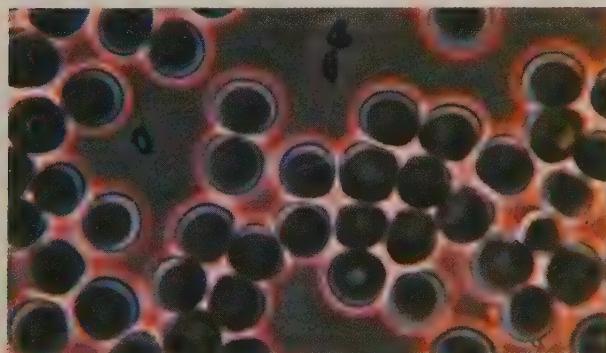


Figure 19.15 (above). The main reactions of the clotting process. Thrombokinase, an enzymatically active substance, is emitted from ruptured platelets and interacts with both prothrombin (inactive) and calcium ions in the blood to produce active thrombin. Thrombin interacts with fibrinogen to form fibrin, an insoluble coagulated protein. Fibrin forms a meshwork of fibers that traps the cellular components of blood, thus forming a clot.

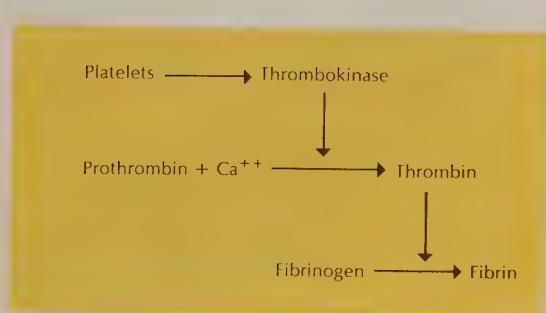


Figure 19.16 (below). The neurogenic heart of a typical arthropod. Note the large central nerve ganglion. Control of heartbeat in these organisms is extrinsic; if nerves to the heart are cut, it stops beating.

temporarily seals off the injured area until the damage is repaired. An outline of the processes involved in blood clotting is presented in Figure 19.15.

The Heart

Among invertebrates, the form and function of the heart seems to be more directly correlated with habitat and style of life than phylogenetic level. Hearts range from none at all in some small animals to simple spontaneously pulsating blood vessels in annelids and relatively sedentary echinoderms. Molluscs have multichamber hearts with valves to direct the flow of blood.

The muscles that make up animal hearts (except for the hearts of arthropods) are *myogenic*, that is, they contract spontaneously. Nerves from the central nervous system may enter myogenic hearts and affect the strength or frequency of the beat by excitation or inhibition, but if the nerves are cut, a myogenic heart will continue to beat.

The hearts of almost all adult arthropods, on the other hand, are able to contract only on command from the nervous system. Such *neurogenic* hearts generally have a nest of nerve cells, or *ganglion*, that completely control the rate of beat. If the nerve fibers running from these nerve cells to the heart muscle are cut, the heart stops.

The heart of a fish is a two-chamber pump, with a thin-wall atrium, which takes in blood from the veins and delivers it to the thicker-wall, more powerful ventricle, which pumps blood under high pressure into the arteries. The arteries carry blood to the gills, where it gains oxygen and gives up CO_2 in a capillary network. Blood then moves into other arteries, which carry it to capillary networks in all parts of the body. Capillaries drain into veins, and blood returns to the heart. The difficulty with this system is that a single pump must provide sufficient pressure to force blood through the resistance of two capillary networks.

The amphibian heart has two atria, which lead into a single ventricle. One atrium receives blood from the lungs; the other receives blood from the general body circulation. The two atria beat simultaneously, and oxygenated and deoxygenated blood are mixed in the single ventricle.

The reptile heart has two atria and two ventricles, although in most species the two ventricles are not completely separate. In the hearts of birds and mammals, oxygenated blood is completely separated from deoxygenated blood. The path of the blood through the two separate circulatory systems of an animal with a four-chamber heart is illustrated in Figure 19.17.

The vertebrate heart is made up of a specialized striated muscle that contracts spontaneously. The mammalian heartbeat is initiated and coordinated in the right atrium by the sinoatrial node, a patch of muscle cells that are specialized for electrical conduction rather than contraction. This pacemaker initiates electrical activity that spreads through the cardiac muscle fibers of the atria to another specialized region, the atrioventricular (AV) node, located in the wall between the two atria just above the ventricles. The AV node passes the wave of excitation to a bundle of similar conducting tissue that ramifies throughout the ventricles.

Because the heart muscle is constantly working, it requires a plentiful supply of oxygen to carry out its energy-releasing metabolism. The heart muscle contains its own system of blood vessels. If these vessels become blocked and a portion of the heart muscle becomes short of oxygen, the heart fails to function. One condition that can lead to blockage of the coro-

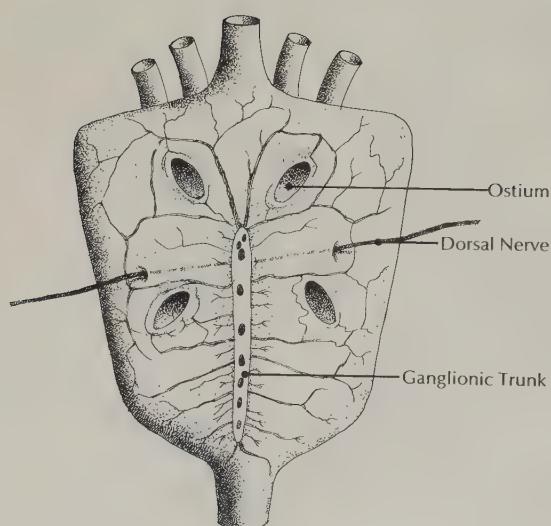
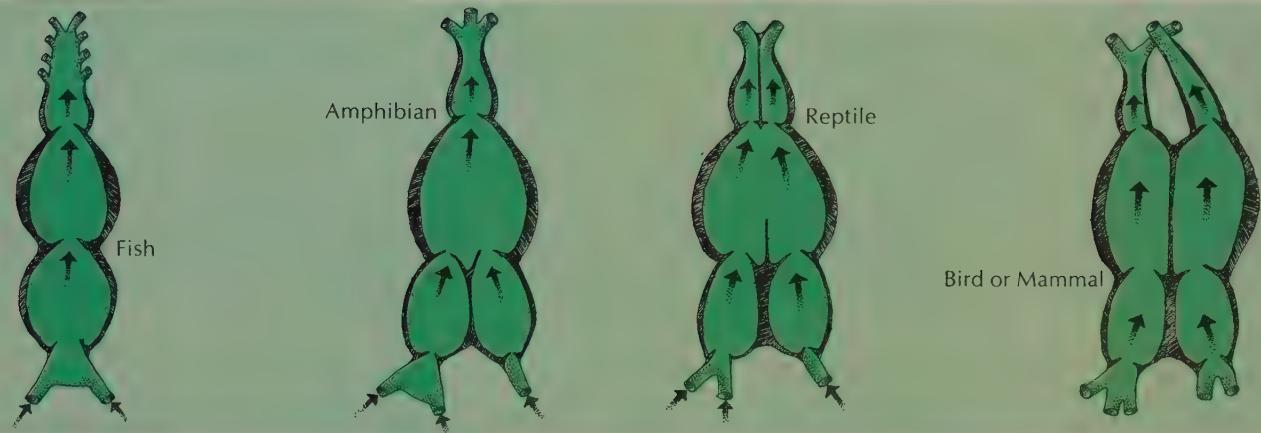


Figure 19.17. Comparative anatomy and evolution of the vertebrate heart. The four-chamber heart of birds and mammals is actually the result of two subdivided atria and ventricles.



nary circulation is hardening of the arteries, a disease in which there is deposition of the steroid cholesterol in the walls of the arteries. A blood clot traveling in the bloodstream also may interfere with the coronary circulation and cause heart failure. A clot lodged in the heart is called a coronary thrombosis and is a common cause of heart attacks.

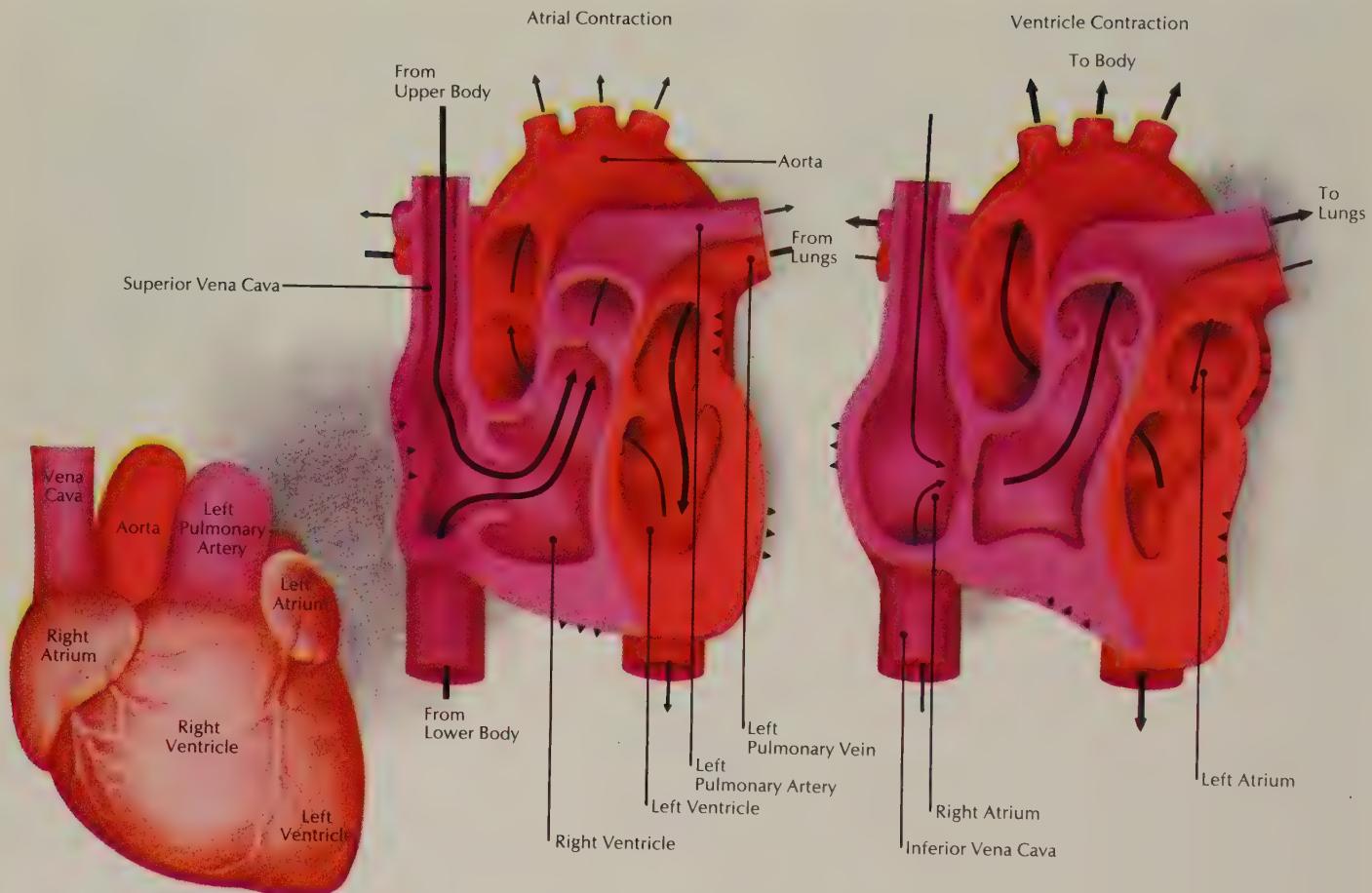
Circulation in Mammals

Blood arriving at the heart from the body tissues is high in CO_2 and lower in oxygen. It enters the right atrium, which pumps blood into the right ventricle. The right ventricle sends blood to the lungs via the pulmonary artery. After the blood exchanges its CO_2 for O_2 in the capillaries of the lungs, it returns to the left atrium of the heart via the pulmonary vein. The left atrium pumps blood into the left ventricle, which pumps blood out to all parts of the body through a large artery, the aorta. The heart, like all pumps, must have valves in order to prevent backward flow. There are four valves in the hearts of birds and mammals (Figure 19.18).

The arteries have relatively thick walls of muscular tissue, which hold blood under pressure as it moves away from the heart. In a human, the contraction of the ventricle pushes blood into the aorta under a pressure equivalent to about 120 millimeters of mercury. As blood moves away from the heart, the arteries branch to form numerous smaller arteries and eventually lead into the capillary system. Capillary walls consist of a single layer of epithelial cells, permitting ready diffusion of substances from blood to tissue cells and vice versa. The number of capillaries in the body is enormous (a few thousand in a single cubic millimeter of skeletal muscle tissue, for example), and every cell of the body is within a cell or two of a capillary.

Because of the increasing frictional resistance of the arterial system as blood moves away from the heart into an ever-increasing number of smaller vessels, the blood pressure drops to about 35 millimeters of mercury (mm Hg) at the arterial end of a capillary. The concentration of proteins in the plasma produces an osmotic pressure of about 25 mm Hg, which tends to move water into the blood vessel. Thus, near the arterial end of a capillary, there is a net pressure of about 10 mm Hg pushing water out of the capillary into the surrounding cells. At the venous end of the capillary, blood pressure drops to about 15 mm Hg pushing outward, whereas the osmotic pressure remains the same. At the venous end of the capillary,

Figure 19.18. External and internal anatomy of the human heart. Deoxygenated blood from the body flows through the right side of the heart and becomes oxygenated in the lungs. It then returns to the left side of the heart and is pumped to the body. Triangles denote contraction.



there is a net pressure of about 10 mm Hg pushing water from the cells into the capillary, and the water content of the blood is restored. This movement of water in and out of blood assists in the exchange of dissolved materials with the cells. The balance of pressures is delicate, and any disturbance of blood pressure or of the concentration of proteins in blood plasma can cause bloating or tissue dehydration.

Veins have thinner walls and are less elastic than arteries. A layer of fibrous tissue and a thin layer of muscle surround the epithelial cells that line veins. Veins lack sufficient elasticity to keep blood under pressure. Blood arrives in veins from capillaries under very low pressure, and its movement back through veins to the heart is largely dependent on contraction of skeletal muscles. As the muscles contract, they squeeze blood through the veins that pass through muscle tissue. Valves along the veins keep blood from moving back toward the capillaries. The flow of blood through the circulatory system is controlled both by the rate at which the heart beats and by the relaxation or constriction of muscles in artery walls.

Nerves leading from special centers in the brain can speed up or slow down heartbeat. These centers are activated by various sensory inputs, including receptors that detect unusual stretching of arteries (leading to a

Figure 19.19. The human cardiovascular system. Only the major vessels and organs associated with the circulation system are outlined.

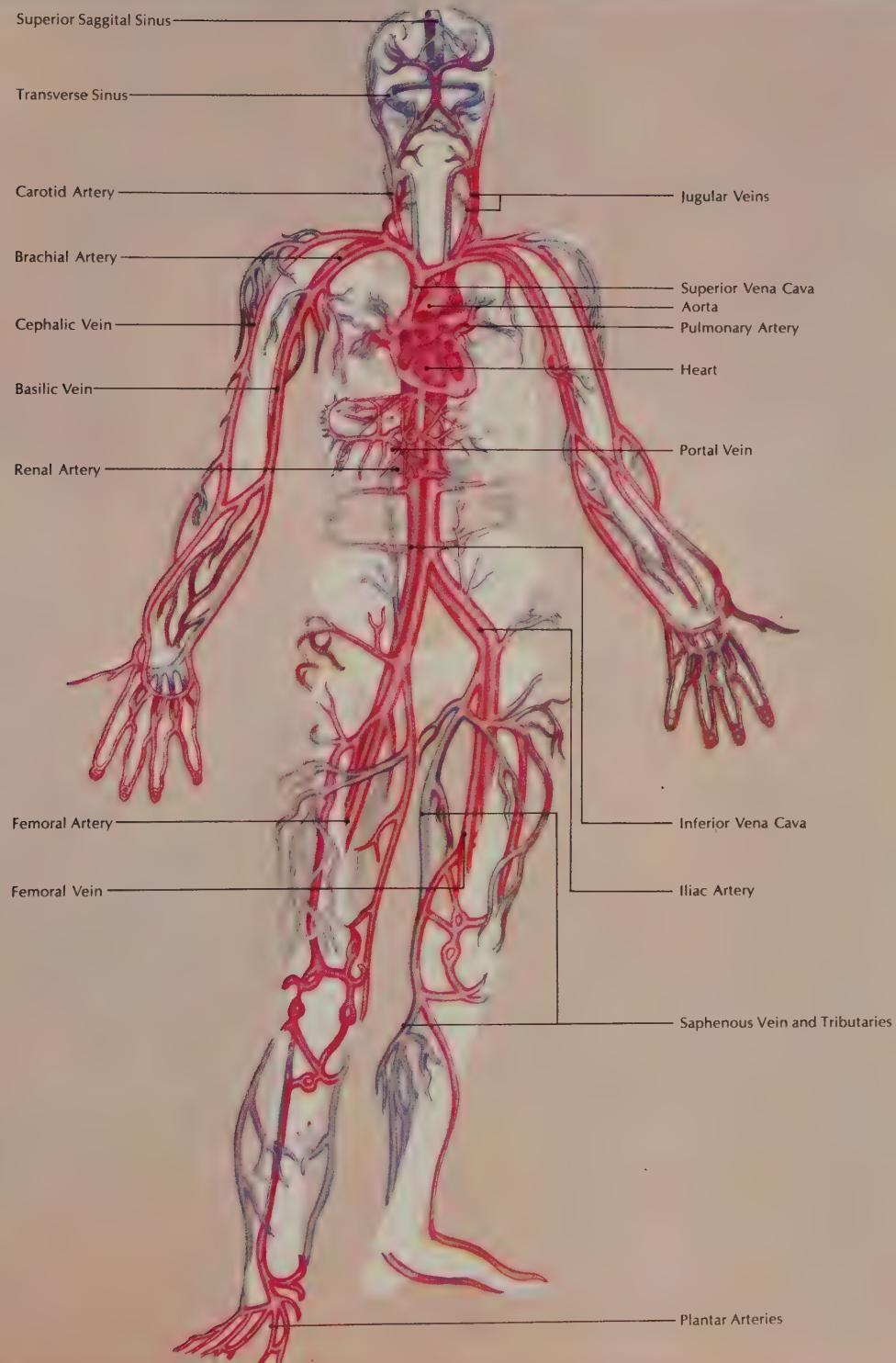
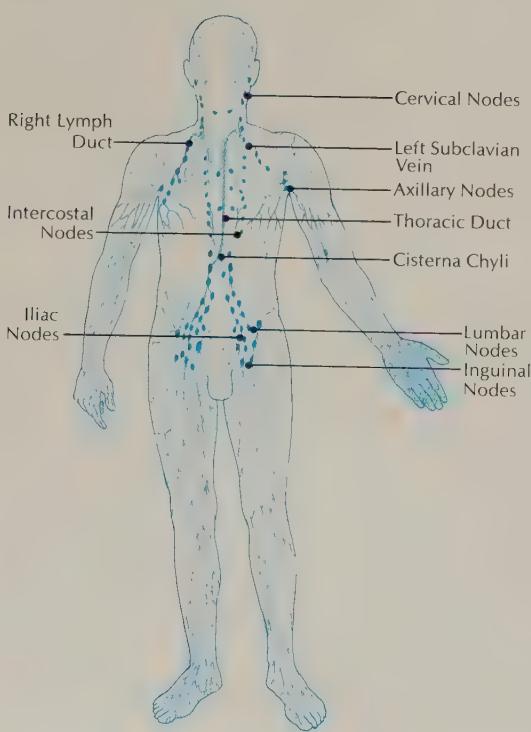


Figure 19.20 (left). The human lymphatic system. Lymphatic vessels function as an auxiliary branch of the main cardiovascular system by returning tissue fluid back to the main bloodstream. Lymph nodes located along the vessels trap foreign matter, including invading bacteria. (From "The Lymphatic System" by H. S. Mayerson. © 1963 by Scientific American, Inc.)

Figure 19.21 (right). The vascular tissue of higher plants is a complex of various cell types. Tracheids, vessels, ray cells, fibers, and parenchyma cells all interact both structurally and physiologically as xylem tissue. Sieve

tubes, companion cells, ray cells, phloem fibers, and parenchyma cells interact as phloem. Xylem conducts water and dissolved mineral salts and forms supportive tissue (wood). Phloem conducts synthesized products from the leaf canopy to the lower portions of stems and roots.



slowing of heartbeat) or of veins (leading to a speeding of heartbeat) as well as receptors that react to changes in CO_2 or O_2 concentrations of blood. The constriction or dilation of arteries also is directed by nervous impulses. In this way, the flow of blood can be directed toward or away from different parts of the body in response to various conditions.

In vertebrates the lymphatic system, an independent transport system, is involved in movement of tissue fluids outside blood vessels. Some of the fluid that leaves arterial capillaries, together with fluids produced by tissue cells themselves, diffuses into small tubes called lymph capillaries. The tissue fluid, or *lymph*, that enters these vessels moves through the lymph capillaries into larger lymph vessels and eventually into compact, ovoid organs called *lymph nodes*. Foreign particles or microorganisms that wander into tissues are likely to be carried into lymph nodes, for walls of blood capillaries are quite resistant to the passage of such intruders. The small white blood cells known as lymphocytes are found in lymph as well as blood, and the lymphatic system is important to the body's immune responses (Chapter 22). The lymph nodes are drained by other lymph vessels that come together to form two large ducts that empty the filtered lymph into large veins.

Internal Transport in Higher Plants

Fluid movement in higher plants occurs in specialized cells of the vascular system (Chapter 11). Plants do not have fluid-filled spaces between tissues, nor do they have vessels with multicellular walls—both features that play important roles in internal transport in animals. Nutrients and water are conducted in xylem tissue from the roots to body cells through hollow con-

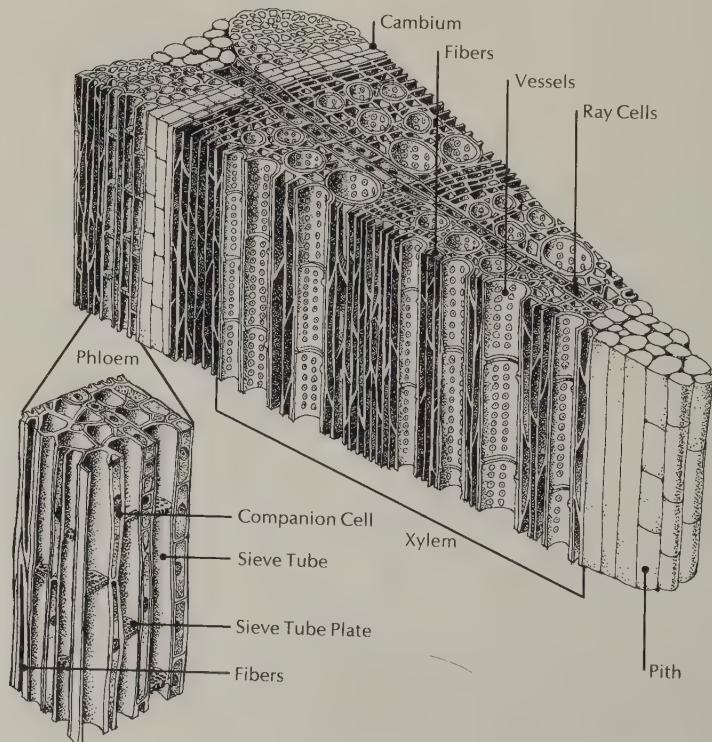


Figure 19.22a (above). Transpiration in the leaf.

Figure 19.22b (below). Water guttation in the strawberry plant. When the humidity is too high to permit sufficient water loss by evaporation, water under pressure is forced out at the leaf ends. This process, in which water droplets are formed, is called guttation. (Courtesy Carolina Biological Supply Company)

ducting tubes formed by the disappearance of nuclei and cytoplasm from tracheid cells. In many species, tracheids become joined end-to-end to form long vessel elements. The thick cellulose walls of tracheids and vessel elements give strength and rigidity to the plant body and prevent collapse of conducting tissues during periods of desiccation. Organic molecules such as carbohydrates and hormones are transported through the plant body in thin-wall, living cells of the phloem tissue. These living sieve cells may be joined end-to-end to form sieve tubes. The sieve cells retain their cytoplasm and probably their nuclei at maturity, and nutrients are moved from cell to cell through the cytoplasm.

The upward movement of fluids in xylem tissue is related to the loss of water (transpiration) from leaves. Water forms fine columns within the tracheids and vessels, running without interruption up from the roots into the veins of leaves. Hydrogen bonding between water molecules holds this column together, causing it to behave much like a fine wire. The column of water is pulled upward as water is lost through transpiration from the upper end of the column. Experiments show that a column of water inside a thin, airtight tube can withstand a pull of 300 pounds per square inch without breaking—a pull that would be sufficient to lift water to the top of even the highest tree.

Phloem cells are drastically altered by almost any method of observation. Thus, far less is known about the mechanism by which sugars and other materials are moved through the phloem. Transport in the phloem, unlike that in the xylem, depends upon the activities of living cells. The rate of movement through the phloem is far slower than that through the xylem, and sugars move from regions of high concentration toward regions of lower concentration. Radioactive tracers show that different substances move at different rates through the phloem. These studies also show that materials moving from a leaf to the stem divide in the phloem tissue of the stem, with some of the solution moving upward through the stem phloem and most of it moving downward. The upward and downward movements through the phloem occur in separate bundles of vascular tissue. It appears that the upper leaves of a plant supply nutrients chiefly to the apex; the lower leaves supply nutrients chiefly to the roots; and the intermediate leaves supply nutrients in both directions.

Among the mechanisms suggested to account for transport in the phloem are diffusion, cytoplasmic streaming, flow under gravitational pull, osmotic movement across cell membranes, and some form of active transport across sieve cell membranes. None of these hypotheses has proven entirely satisfactory for the explanation of all known facts about phloem transport.

The vascular systems of plants are capable of moving large volumes of fluids, but the rate of movement is much slower than that in animals. Because plants do not expend energy in muscular movements and generally do not require a rapid supply of nutrients to support sudden changes in activity, the slow movements of fluids through the xylem and phloem are sufficient to meet the needs of the organism.

EXCRETION

To maintain proper internal conditions, an organism must not only obtain nutrients but must regulate its internal composition and get rid of waste products. The principal wastes to be handled are carbon dioxide (the major

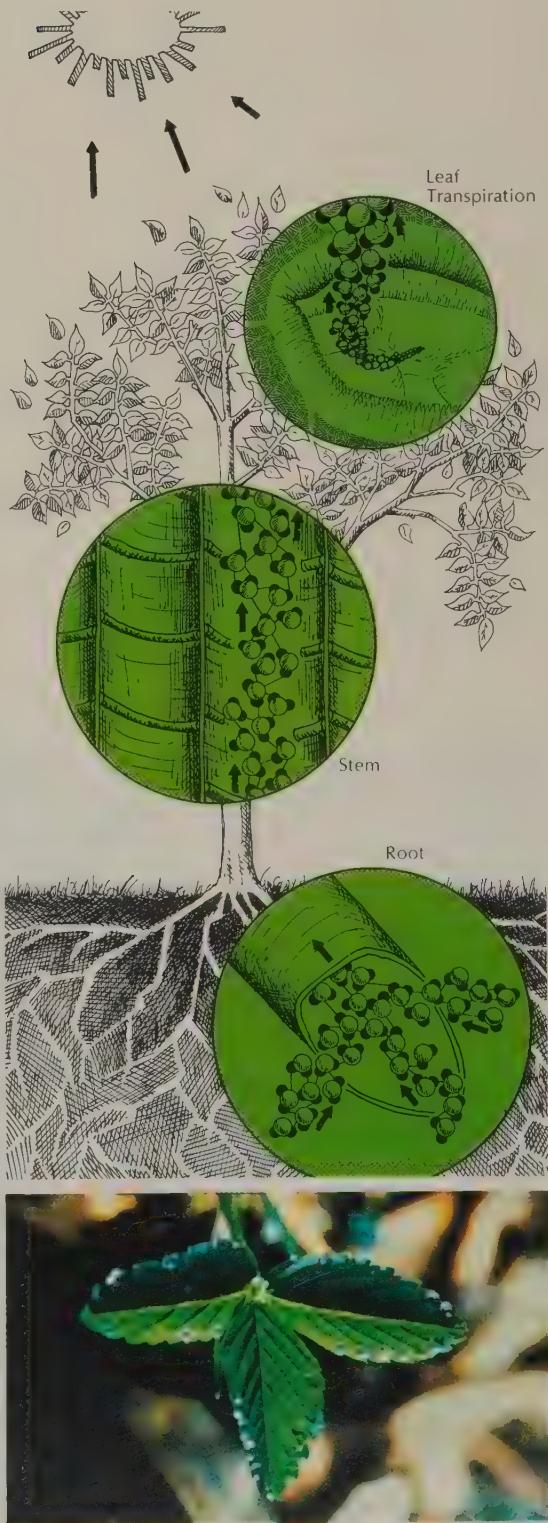


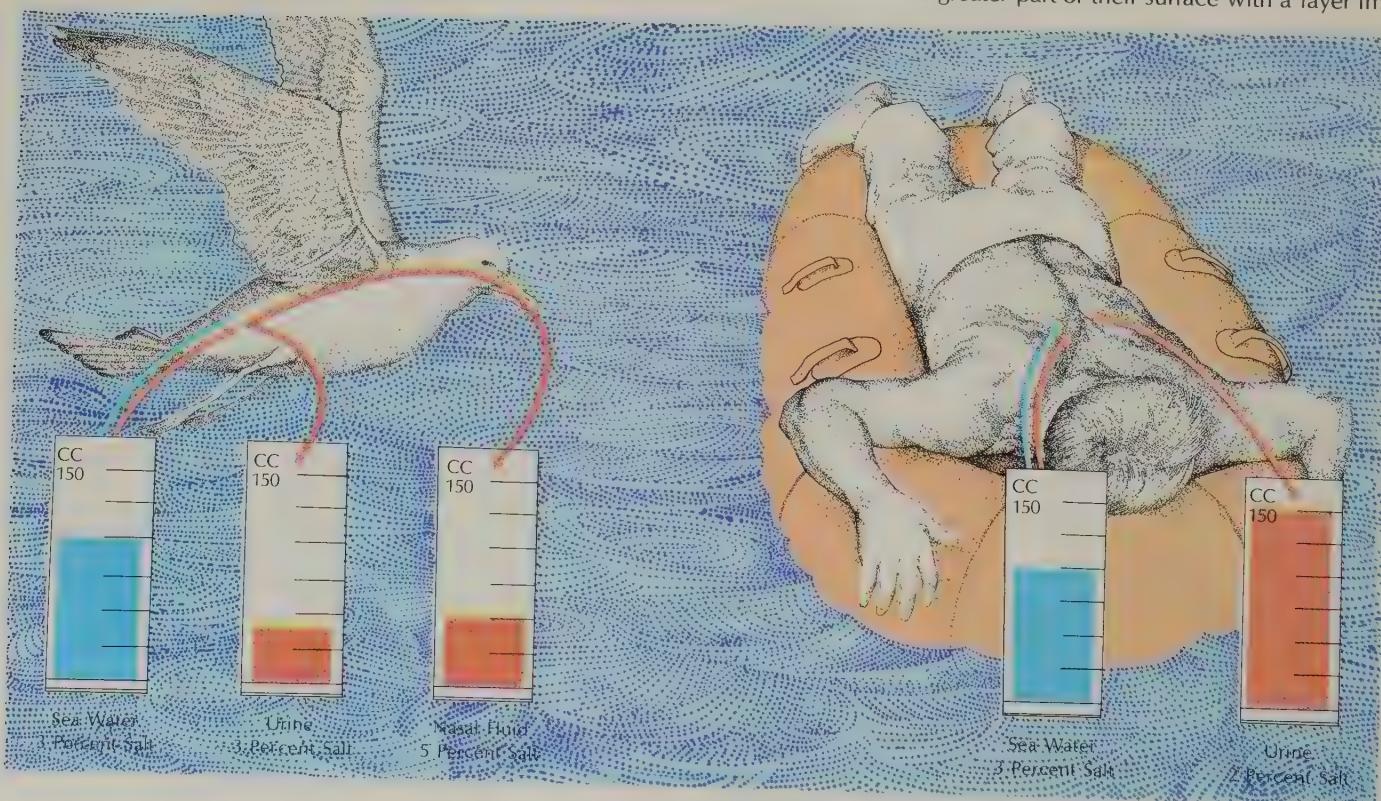
Figure 19.23. Salt excretion in birds and men. Sea birds such as the gull are capable of a limited sea-water intake due to active secretion of salt by special glands located in the beak. Thus, excess salts can be actively transported out of the body fluids. Man, however, cannot drink sea water. In eliminating the salt, he will lose more water than he has drunk.
 (From "Salt Glands" by K. Schmidt-Nielsen. © 1959 by Scientific American, Inc.)

waste product of cellular respiration), salts, and nitrogenous wastes of protein metabolism. A proper water balance must also be maintained. The functions of maintaining salt and water balance and disposing of nitrogenous wastes are performed by excretory systems.

Ion and Water Balance

Most marine invertebrates are essentially *isosmotic* with sea water—that is, their cells neither shrink nor swell. Because their ionic composition is different from sea water, they must have active transport mechanisms to control their composition. Most marine vertebrates have an osmotic concentration of body fluids far lower than that of sea water. In part, the water exchange is accomplished by making the major portion of the body surface impermeable to water. However, a certain amount of sea water is always swallowed with food, and tissues specialized for gas exchange must come in close contact with sea water. Therefore, there is an osmotic flow of water out of the organism and a danger of dehydration. Organisms that live under these conditions have mechanisms for actively secreting salts and conserving water. For example, higher marine fish excrete small quantities of very concentrated urine and actively transport salt out through the surfaces of the gills.

For organisms living in fresh water, the problem is reversed. Osmotic forces cause water to move into the organism. The fresh-water plants have rigid cell walls that permit the build-up of internal pressure sufficient to counteract the inward movement of water. Animals that live in this environment are covered over the greater part of their surface with a layer im-



permeable to water. Fresh-water fish and amphibians have kidneys that are specialized for the reabsorption of ions. They excrete large volumes of very dilute urine. Some fresh-water fish also have active transport mechanisms in their gills to take ions out of the water and pump them into their bodies.

Land animals must obtain water by drinking and eating. Because excess salts are frequently included in their food, their excretory systems are specialized for elimination of salts and retention of water. In many land animals, salts are also eliminated through the body surface by sweating.

The kidneys of some desert mammals are so efficient that these animals can survive without drinking water. Water loss from the body is minimized by remaining in closed, humid burrows during the day, by excreting urine and feces with very little water content, and by minimizing loss of water through the skin. (Most of these animals have few sweat glands.) With this rigid program of water conservation, these animals are able to meet their water needs from water obtained during metabolic respiration of carbohydrates. When such animals are limited to a diet of high-protein foods such as soybeans, large amounts of nitrogenous wastes are produced in metabolism. Even with concentrated urine, so much water must be used to get rid of these wastes that the animals become dehydrated without a source of drinking water. However, unlike most other mammals, these desert dwellers can satisfy their thirst with sea water. As shipwrecked sailors have discovered to their dismay, sea water is a hopeless means of meeting water needs. The concentration of salts in sea water is greater than that in human urine, and thus there is a net loss of water from the body in getting rid of the salts. The urine of animals such as the kangaroo rat is more concentrated than sea water, and thus the animal can eliminate the excess salts and still retain some water in its body.

Nitrogenous Waste

Much of the food that an animal takes in consists of proteins that make up the bodies of its prey or of plants. In the digestive process, proteins are hydrolyzed into amino acids, which are absorbed into the cells. Some of these amino acids are reused in synthesizing proteins needed by the organism, but the greater part of this supply is further broken down by the liver as a source of chemical energy. The first step in the metabolism of an amino acid is its conversion into an organic acid by the removal of amino groups. The amino groups are converted into ammonia during this process of deamination.

Ammonia is toxic to the organism if it accumulates in high concentration. In aquatic organisms, most of the ammonia diffuses out of the body in much the same fashion as does carbon dioxide. However, disposal of ammonia would be a major problem for land organisms because ammonia does not diffuse readily into the air as does carbon dioxide. A water solution of ammonia would have to be excreted, and, because only very dilute ammonia solutions can be tolerated, this method of disposal would result in a great deal of water loss from the organism. In adult amphibians and mammals, ammonia is combined with carbon dioxide to form urea, a substance that can be tolerated by the organism in concentrations substantially higher than that of ammonia. Even with urea, a substantial amount of water must be used in excretion unless the excretory system is capable of reclaiming water from the urea solution after it is isolated from the general internal fluids in a special excretory system. In egg-laying land animals such as

insects, reptiles, and birds, ammonia is converted to *uric acid*, a substance that precipitates out of solution to form a solid and thus is kept out of the internal fluids. The solid or nearly solid uric acid can be expelled from the body with relatively little loss of water.

Excretory Systems

Plants have relatively simple excretory functions. Both the oxygen produced as a waste product of photosynthesis and the carbon dioxide produced as a waste product of respiration serve as a nutrient for the other metabolic function. Excess amounts of either gas are readily diffused into the atmosphere through the same exchange surfaces that bring these materials into the plant body. Stomata regulate this exchange in order to prevent excessive water loss. Membranes of the root epidermal cells selectively move ions in and out of the organism, regulating ion concentrations inside the plant. Because the plant does not take in significant amounts of organic materials, it has relatively few nitrogenous waste products. In some woody plants, nitrogenous compounds such as lignin and alkaloids are deposited in the empty tubes of old xylem tissue, forming the dark and solid heartwood of the stem. It is not clear whether this process serves primarily as a means of disposing of these materials or as a means of strengthening the structure of the stem.

In a simple unicellular organism, waste materials diffuse out of the body. An internal environment with substances present in concentrations different from those in the external environment is maintained through active transport and selective permeability of the cell membrane. Protozoa use active transport to concentrate waste products in vacuoles, which move to the cell membrane and discharge their contents to the exterior.

Most invertebrate excretory systems consist of simple tubes leading from various parts of the body to the exterior. Waste products and water are diffused or actively transported into these tubes by cells that line them, and cilia within the tubes move the solution out of the body. As the fluid moves along the tubes, cells absorb materials from or secrete substances into it, thus closely regulating the composition of the fluid that is finally expelled and the internal composition of the body.

In vertebrates, the kidney adjusts the concentrations of various ions. In addition, the kidney helps to regulate blood concentration of glucose and excretes nitrogenous wastes such as urea, products of hemoglobin breakdown, and creatinine formed as a waste product of muscular activity. Useless materials that find their way into the organism across the intestinal or respiratory epithelia are also excreted by the kidney. These functions are accomplished by a combination of three processes: *ultrafiltration* of blood, *reabsorption* from the filtrate of materials required by the organism, and *specific secretion* of certain materials directly into the filtrate.

The kidney is essentially a collection of many thousands of similar small units called *nephrons*. Each nephron consists of a network of blood capillaries and a *renal tubule*. The wall of the renal tubule is made up of a single layer of epithelial cells resting on a basement membrane. In the other part of the kidney, the *renal cortex*, the tubule ends in a cup-shape structure, *Bowman's capsule*, which surrounds a network of capillaries. A narrow, twisting portion of the tubule (the proximal convoluted tubule) extends from the capsule to a straighter *loop of Henle*, which loops into the inner portion, or *medulla*, of the kidney and returns to the cortex, where it leads

Figure 19.24a. The flatworm excretory system consists of a long collecting duct along each side of the body. Each of these ducts has input from numerous cul-de-sac flame cells.

Figure 19.24b. Excretion in *Hydra* is accomplished by each individual cell through either passive or active transport. The simple organizational level of this organism does not require sophisticated excretory organs.

Figure 19.24c. The excretory system of a mosquito consists of a group of excretory tubules that collect wastes from the body fluids and empty it into the gut. The malpighian tubules are characteristic of most arthropods.

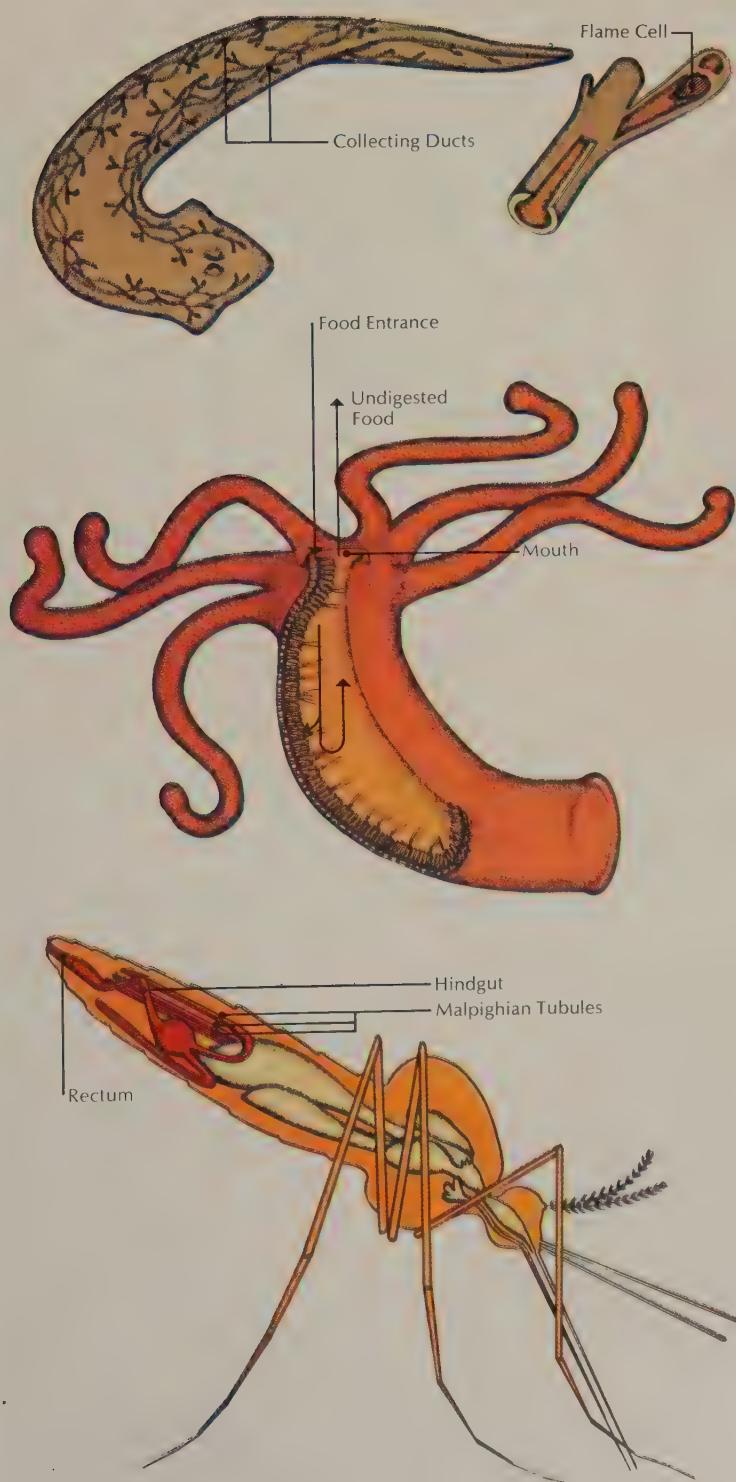
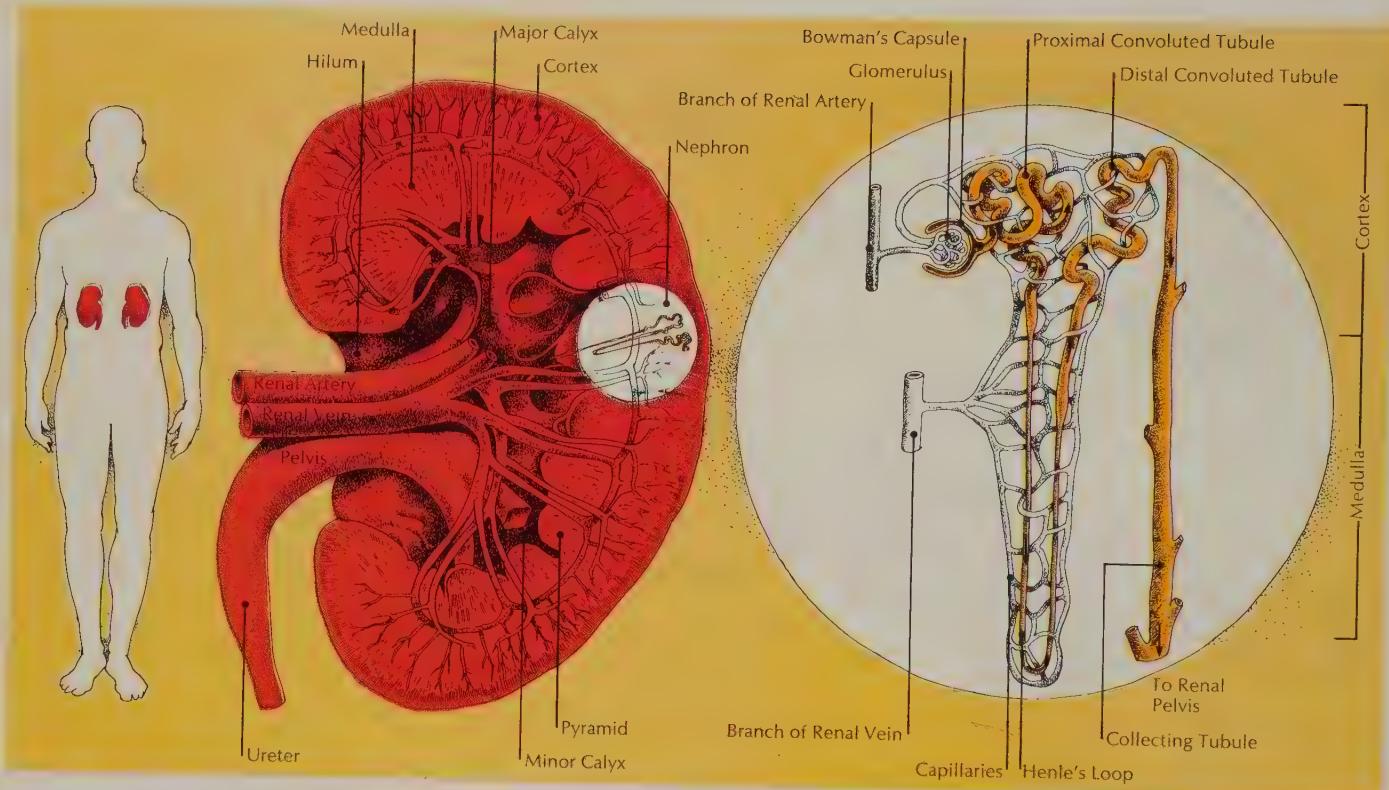


Figure 19.25. The major organ of the human excretory system is the kidney. Three processes, including filtration, reabsorption, and secretion, enable this organ to remove wastes from the blood and simultaneously conserve the useful components of the blood.



into another narrow, twisted segment of tubule (the distal convoluted tubule). This tubule joins with other tubules to form a larger *collecting tubule*, which leads back through the medulla to the *renal pelvis* in the center of the kidney.

The cluster of capillaries within the cup of Bowman's capsule is called the *glomerulus*. Blood flows into the glomerulus from a branch of the arterial system serving the kidney, and blood flows out of the glomerular capillaries into a smaller arteriole that leads to a second capillary network surrounding the proximal and distal convoluted tubules. From this network, blood collects into the veins leading from the kidney back toward the heart.

Blood filtration takes place in the glomerulus. Because the exit from the glomerular capillaries is smaller than the entrance, considerable blood pressure is built up in these capillaries. Under this high pressure, about one-fifth of the fluid portion of blood is forced through the capillary walls into Bowman's capsule, leaving only blood cells, plasma proteins, and fluid within the capillaries. The ultrafiltrate in the kidney tubule at this stage then contains the same concentration of small molecules as blood, including nutrients and salts in addition to waste products. (The human kidneys filter the blood at such a rapid rate that a volume of plasma equivalent to the total contents of the circulatory system passes through the Bowman's capsules about every 25 minutes.)

The filtrate moves through the renal tubule, where it again comes into proximity with the blood from which it was filtered. Cells lining the proximal and distal convoluted tubules are specialized for carrying on active transport of particular substances. They pump glucose, amino acids, and

Figure 19.26. Diagrammatic representation of osmotic pressure involved in urine formation.

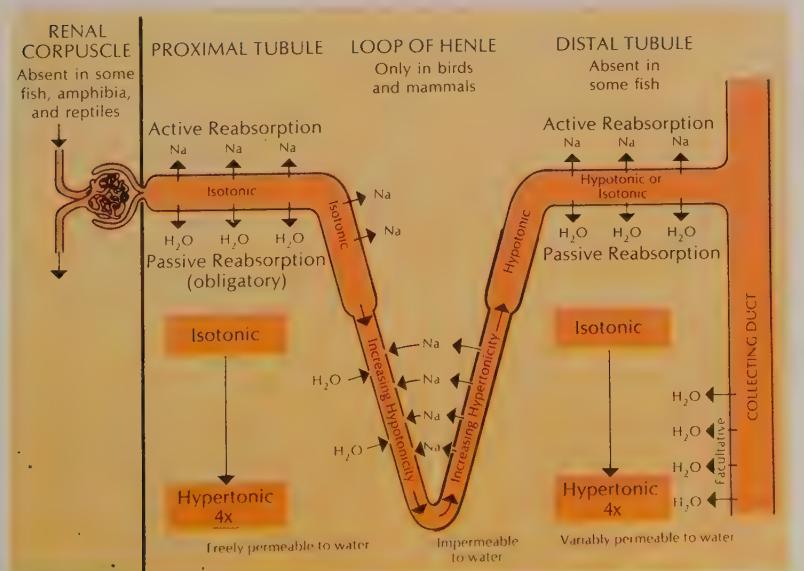
some ions out of the tubule fluid, and these materials then diffuse back into the blood. The high concentration of dissolved materials in blood creates an osmotic pressure that forces water out of the tubule and back into blood.

Excluding water, only substances that are actively transported by the tubule cells are removed from the filtrate. This method of operation makes the nephron a fail-safe system because all waste products and foreign materials not recognized by the tubule cells remain in the filtrate and are passed out in urine. A few substances that are not removed from the blood in Bowman's capsule—hydrogen ions, for example—are actively transported by the tubule cells into tubule fluid.

Each region of the tubule processes a different group of substances. Glucose and amino acids are returned to blood in the twisted region near the capsule, whereas ions move back into the blood in all parts of the tubule except the collecting duct. Under normal conditions, all glucose is returned to the blood, whereas the amount of ions left in the tubule fluid is variable and depends upon the physiological needs of the organism. Sufficient amounts of each ion are actively transported back to the blood to restore the normal homeostatic concentration of each substance in the circulatory fluid. The urine that leaves the collecting duct represents about 1 percent of the volume of fluid that entered the Bowman's capsule.

Most vertebrates excrete urine that is osmotically less concentrated than blood, but mammals and birds produce urine of higher concentration than blood plasma. This concentration is made possible by the structure called Henle's loop, which is present only in the kidneys of these two groups. In the loop, active transport moves sodium ions from tubule fluid into intercellular fluids of the renal medulla. This movement temporarily makes the tubule fluid less concentrated, but it serves to create a strong osmotic pressure that moves water out of the collecting ducts and into the intercellular fluids as the ducts pass through the medulla. This feat of concentration of urine in the collecting duct is accomplished by a countercurrent exchange system that operates on the same principle as that in the gills of fishes.

Urine passes into the ureters—bilateral, thick-wall, muscular tubes that



convey fluid from the kidney to the urinary bladder. The bladder serves as a storage vessel for urine. Its epithelial lining is composed of five or six layers of bulging, pear-shape cells. As urine accumulates and distends the bladder, these cells slide past one another and spread to form a thinner membrane of greater surface area. Urine passes from the bladder to the exterior of the organism through the *urethra*, which can be opened and closed by muscles that are under voluntary control.

Urine formation is subject to controls that tend to adjust the urine volume and concentration to counteract changes in the internal environment. These controls are based on two types of information—blood pressure and osmotic concentration of body fluids. When arterial pressure increases, the pressure in the glomerulus rises, forcing more blood through the filter and resulting in an increase in the volume of urine. A corresponding decrease in blood volume reduces the blood pressure and the system returns to normal. The osmotic concentration of body fluids is monitored by sensory receptors in the brain. When the receptors are exposed to an increased concentration of solutes in the fluid that bathes them, they tend to shrink. Osmotic shrinkage excites these receptors and starts a chain of events in the brain that results in the release of a hormone from the posterior pituitary gland. This antidiuretic hormone (ADH) increases the permeability to water of cells lining the collecting tubules in the kidney. The result is an increase in the amount of water returned to blood and the excretion of a smaller volume of more concentrated urine. An increase in the amount of water returned to blood decreases the osmotic concentration of body fluids, again tending to restore normal conditions. Two common substances affect these control mechanisms. Caffeine, found in coffee, stimulates the arteries to contract, raising the blood pressure and producing an increased flow of urine. Alcohol inhibits a component of the system that maintains a steady level of antidiuretic hormone. When the secretion of ADH is cut down, less water is removed from the urine and the volume of urine increases.

TEMPERATURE REGULATION

Enzymes regulate the chemical reactions that make up the life processes of all organisms, but enzymes can function only over a limited range of temperatures. Therefore, in order to survive, organisms must somehow control their internal temperature, either by finding an environment with a suitable temperature range or making some physiological compromise with the environment. The body heat of living organisms comes from the oxidation of foods. Some of the energy obtained in this way is stored in the chemical bonds of ATP, and the rest is released as heat. In one way, heat produced in metabolism is wasted energy because it cannot be used to synthesize new molecules for growth and maintenance. On the other hand, if used well, this heat can maintain the body temperature at a level above that of the environment.

All plants and animals, except birds and mammals, have little control over their body temperatures. Cold-blooded animals, or *poikilotherms*, cannot regulate their body temperatures. Plants and cold-blooded animals that live in climates that are cold for a part of the year have two alternatives—they may live for only one season or go dormant in some fashion during the cold season. Bacteria and fungi can form spores, which are very resistant to cold, and certain seed plants (called annuals) form seeds and die at the end of a growing season. When the environment becomes favorable

again, the spores or seeds germinate, producing a new generation. Many plants (perennials) and cold-blooded animals go through a period of inactivity during cold weather. Their metabolic processes slow down, and they can live in a state of dormancy for several months. Nonregulating organisms adapted to cold climates often have higher metabolic rates (as indicated by their oxygen consumption) than similar animals from warmer environments.

Birds and mammals are called *homeotherms*, or warm-blooded, because they can regulate their body temperatures. In these animals, a portion of the brain, the hypothalamus, acts as a thermostat. Alterations in the external temperature are sensed in various ways. There are heat and cold receptors at various points on the body surface. The brain also responds to changes in the temperature of blood and sets various processes in motion to return the body temperature to normal.

If the body temperature rises, several mechanisms are used to dissipate heat. Blood vessels in the skin expand, increasing the amount of blood in a position to be cooled. Sweat glands secrete liquid, which spreads over the skin and evaporates, a physical process that takes up heat. Animals that are covered with fur have few sweat glands; they use evaporation of water from their tongues as a cooling mechanism. Panting moves air over the tongue and speeds evaporation.

If the body temperature falls, the thermoregulatory system tries to minimize heat loss by constricting the blood vessels in the skin. Extra heat is also produced by muscle movements such as shivering and increased activity. If a warm-blooded animal is unprotected in cold environmental temperatures for a prolonged period, it dies because of loss of body heat. Thus, there are many physiological, anatomical, and behavioral adaptations that allow plants and animals to live under all sorts of extreme conditions.

Although the millions of species of organisms represent a diversity of body structures, there is a surprising degree of similarity in the functions performed by organisms. At the biochemical or molecular level, the basic structural components and activities are similar throughout most of the spectrum of living things. Even at the level of tissues and organs, every organism possesses systems specialized to carry out a few vital functions. Although evolution has produced many different mechanisms to perform these functions, the basic similarities are in many ways more striking than the differences.

FURTHER READING

For more detailed discussions of animal physiology, see books by Barrington (1968), D'Amour (1961), Griffin (1962), Hoar (1966), Krogh (1959), Larimer (1968), Prosser and Brown (1961), Scheer (1963), and Knut Schmidt-Nielsen (1964a, 1964b). Further details of plant physiology will be found in books by Galston (1964), Meyer, et al. (1960), Ray (1963), Salisbury and Parke (1970), and Steward (1959, 1963, 1964).

Detailed discussions of human physiology will be found in books by Best and Taylor (1961), Guyton (1961), Ruch and Fulton (1960), and Winton and Bayliss (1962).

Among *Scientific American* articles relating to topics of this chapter are those by Adolph (1967), Bartholomew and Hudson (1961), Benzinger (1961), Bogert (1959), Chapman and Mitchell (1965), Clements (1962), Comroe (1966), Fertig and Edmonds (1969), Hock (1970), Irving (1966), Kylstra (1968), Mayerson (1963), Neurath (1964), Knut Schmidt-Nielsen (1959a, 1959b), Knut Schmidt-Nielsen and Bodil Schmidt-Nielsen (1953), Scholander (1957, 1963), Wiggers (1957), Winter and Lowenstein (1969), J. E. Wood (1968), and Zweifach (1959).

20

Plant Hormones



In 1902 British physiologists William M. Bayliss and Ernest H. Starling isolated secretin, a chemical manufactured in the lining of the small intestine when food is present. Secretin moves into the bloodstream and travels throughout the body without apparent effect until it reaches the pancreas. When it reaches the cells of the pancreas, however, that gland begins to manufacture and release the enzymes that assist in digestion. Two years later, Bayliss and Starling concluded that such chemical messengers play many important roles in animal physiology. They coined the term "hormone" (the Greek *'ormōn* means stimulating) to describe substances that are manufactured in one part of the body and distributed to other parts of the body, where they stimulate changes in certain cells, tissues, or organs.

Long before the work of Bayliss and Starling, however, animal and plant physiologists independently had suggested that such chemical messengers exist and play important roles in development and physiology.

PLANT GROWTH SUBSTANCES

Auxins

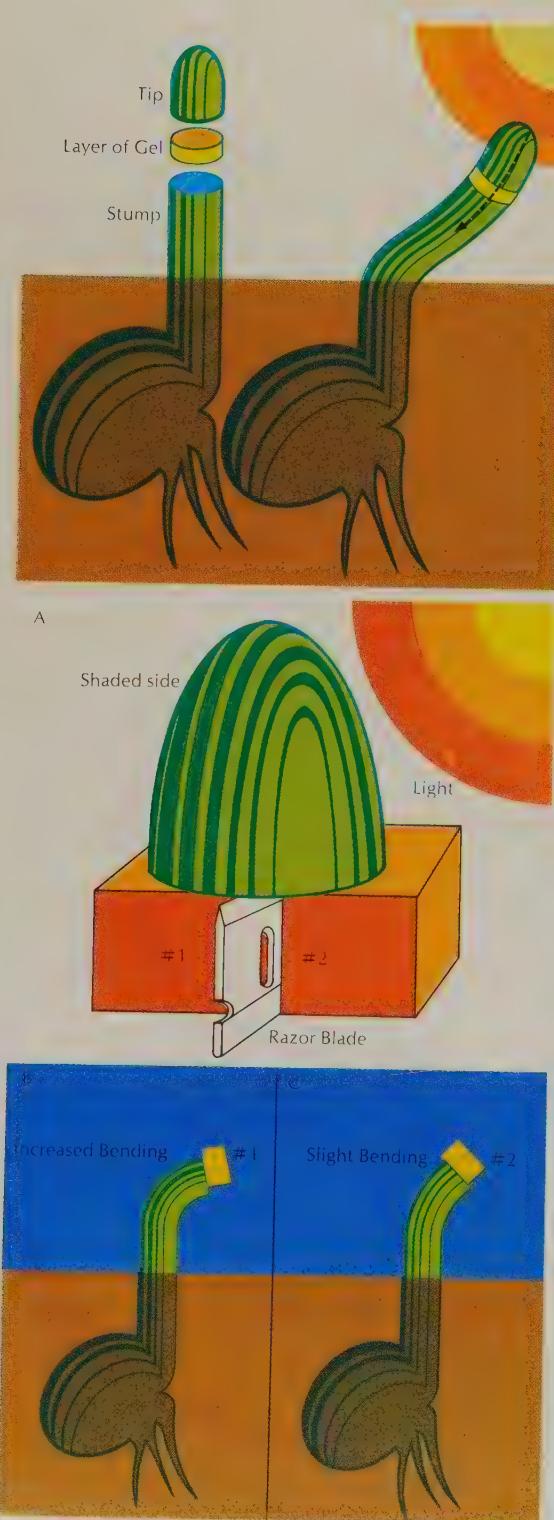
The first suggestion of chemical messengers in plants was made by Charles and Francis Darwin (1880) in their study of phototropism. Working with plants of the grass family, they found that a seedling illuminated from one side curves toward the light as it grows unless its extreme tip is covered with an opaque cap. Because the curved zone is well below the tip, they deduced that an "influence" moves from the tip to the parts below. A clue toward the nature of this influence was obtained in 1911 by a Danish botanist, Peter Boysen-Jensen, who demonstrated that a seedling curves toward the light if the tip is cut off and stuck back on again with gelatin. This experiment strongly suggested that the influence is a chemical substance that can diffuse across the cut through the gelatin. In 1919 a Hungarian researcher, Arpad Paál, showed that the influence accelerates ordinary, noncurving stem growth in the part of the seedling beneath the tip. By this time, it was clear that the influence is a growth-promoting substance that can diffuse through gelatin (Figure 20.1).

In their experiments, Boysen-Jensen and Paál used the *coleoptile*, or first shoot, of the oat (*Avena sativa*). The oat coleoptile, which has become the standard subject for similar studies, consists of a hollow sheath, six to seven cells thick, inside which the first few leaves are tightly rolled up. Two vascular bundles run up the sides, giving the coleoptile an elliptical cross section. In many ways, the coleoptile is like a first leaf sheath without the blade. Both the outer and inner surfaces of the sheath are covered with layers of epidermal cells punctuated by stomata. The coleoptile tip is a dome-like or conical structure, solid for a length of about 0.3 millimeter from the top. The uppermost ends of the vascular bundles lie in the lower part of this solid tip. In most experiments, the "tips" removed from coleoptiles consist of considerably more than the minute solid caps.

After Paál's work, several attempts were made to extract the postulated growth substance from ground-up coleoptile tips or other plant substances. P. Stark in 1921 developed a clever technique for detection of the substance. The material to be tested for growth substance is mixed with melted agar, cooled, and cut into small blocks. Coleoptiles are decapitated and the leaves inside are partially pulled out, leaving a small piece of leaf as

Figure 20.1 (above). Boysen-Jensen's experiment demonstrated that an oat seedling coleoptile will still curve toward the light if its tip is cut off and a gelatin layer is placed between the tip and the stump. This evidence strongly suggests that the chemical substance influencing growth can diffuse across the cut through the gelatin.

Figure 20.2 (below). In Went's experiment, an excised coleoptile tip that had been exposed to a unilateral source of light was placed on two agar blocks separated by a razor blade (A). Growth substance from the tip was secreted into each of the blocks, which were then placed in contact with decapitated test plants (B and C). The block that had received growth substance from the shaded side of the tip caused the test plant to curve over twice the amount (16°) than did the plant with growth substance from the lighted side (6°). This experiment showed that light has an inhibiting effect on the amount of growth substance released by the tip or redistributed by the tip tissues.



separated by a razor blade (A). Growth substance from the tip was secreted into each of the blocks, which were then placed in contact with decapitated test plants (B and C). The block that had received growth substance from the shaded side of the tip caused the test plant to curve over twice the amount (16°) than did the plant with growth substance from the lighted side (6°). This experiment showed that light has an inhibiting effect on the amount of growth substance released by the tip or redistributed by the tip tissues.

support. Each agar block is applied to one side of a decapitated coleoptile, resting against the remaining piece of leaf. If the agar contains growth substance, the side of the coleoptile that it touches should elongate more than the other side, causing the coleoptile to curve away from the agar block. Unfortunately, none of Stark's coleoptiles curved.

Stark's technique was used by another researcher, however, with greater success. Elizabeth Seubert caused coleoptiles to curve by using materials derived from malt extract and human saliva. If the technique is valid as a means of detecting growth substance, then the substance exists in organisms other than plants. Later, it was detected in several kinds of fungi.

Frits W. Went in 1928 successfully extracted growth substance by letting the living tips secrete it. He placed intact coleoptile tips on agar blocks for a few hours. The agar blocks then produced curved coleoptiles in Stark's test for growth substance. Went demonstrated that the angle of curvature of the coleoptile, measured after the agar block has been applied for a standard time, is proportional to the number of coleoptile tips that had been placed on the agar block and to the time they remained there. Thus, Stark's technique was modified to provide a quantitative measure of the amount of growth substance.

Of Went's many experiments with the plant growth substance, one was of particular importance. Russian botanist Nikolai Cholodny suggested that both phototropism and geotropism in plant shoots and roots are caused by unequal distribution of growth substance (Chapter 18). The effects of light or gravity cause more growth substance to accumulate in one side of the root or shoot tip than in the other. As the substance moves away from the tip, more growth is stimulated on one side of the root or shoot than on the other side, thus causing curvature. Cholodny, working mostly with roots, obtained some indirect evidence to support his hypothesis, but Went was able to confirm it directly. Went illuminated a coleoptile from one side and then cut off its tip. He placed the tip on a line between two small agar blocks separated by a razor blade, so that the previously lighted side of the tip was over one block and the previously shaded side was over the other block. The two blocks were then applied to decapitated test plants in darkness. The block that had received growth substance from the shaded side of the tip produced a curvature of 16° , whereas the block with growth substance from the lighted side of the tip produced a curvature of 6° . In other words, the shaded side of the tip produced more than twice as much growth substance as the lighted side (Figure 20.2). In the same laboratory, H. E. Dolk used similar techniques to demonstrate the mechanism of geotropism. In this case, the tips were laid horizontally and growth substance was collected from their upper and lower sides. The block in contact with the lower side produced about twice as much curvature as that in contact with the upper side.

With a good assay method now available, attempts soon were made to isolate and identify the growth substance. The amount in coleoptile tips, however, was far too small for chemical analysis. Experimenters in Holland found rich sources of growth substance in human urine and in yeast. American researchers obtained large quantities of growth substance from cultures of the fungus *Rhizopus stolonifer* maintained under certain growth conditions. The growth substance was extracted and analyzed from all three different sources, and in each case it was identified as indole-3-acetic acid, or IAA (Thimann, 1935a). This substance was already known to bio-

Figure 20.3. Three of the most active synthetic auxins—NAA(II), 2,4-D(III), and TCBA(IV)—are shown along with the structural formulae of some of the IAA precursors and derivatives shown in Table 20.1.

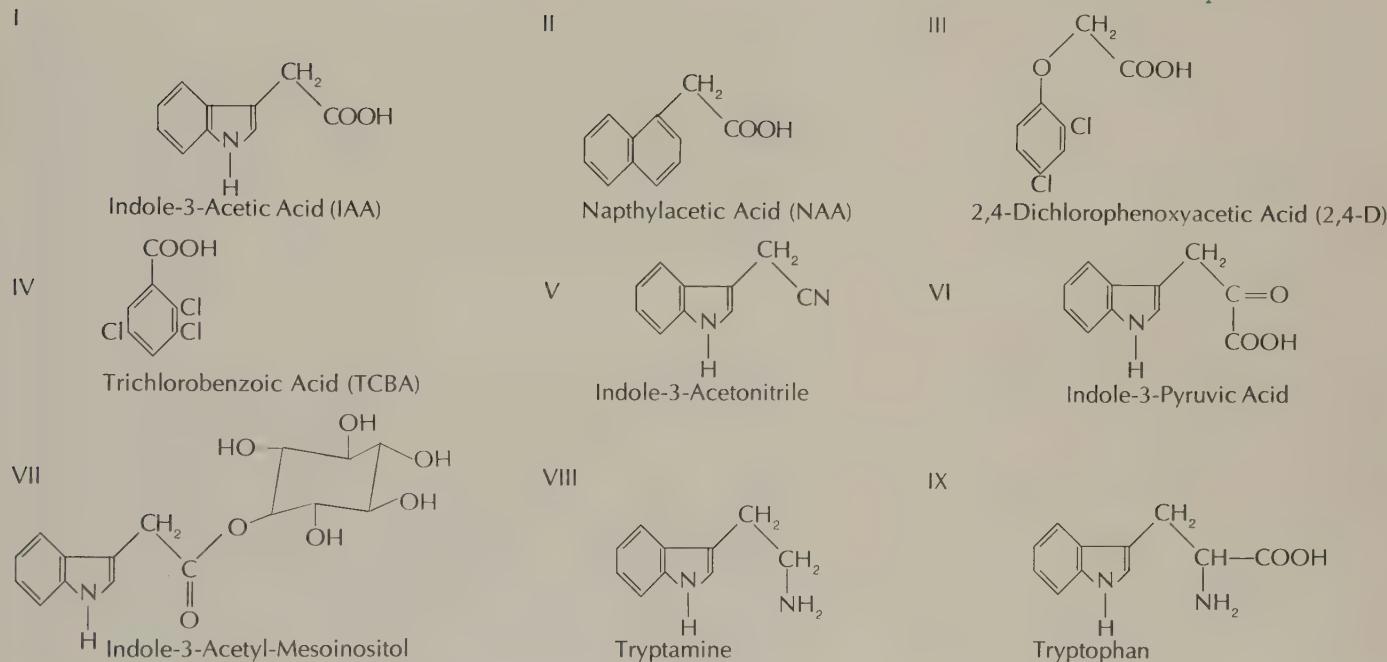


Table 20.1
 Naturally Occurring Derivatives and Precursors of Indole-3-Acetic Acid (IAA)

DERIVATIVE	Plant Source	Process of Conversion to IAA	System Responsible
Indole-3-acetonitrile (V)	cabbage, Brussels sprouts	hydrolysis	nitrilase enzyme (crucifers, cereal leaves)
Indole-3-acetaldehyde	several etiolated seedlings	oxidation or dehydrogenation	aldehyde dehydrogenase (milk, bacteria, and so on)
Ethyl indole-3-acetate	apples (may be artifact of using ethanol)	hydrolysis	esterase
Indole-3-pyruvic acid (VI)	corn seeds (certain cultivars)	oxidative decarboxylation requires 1/2 O ₂ & evolves CO ₂	spontaneous in warm alkaline solution
N-(3-indolyl) aspartic acid	pea seedlings treated with IAA	hydrolysis to IAA and aspartic acid	heating with alkali
Indole-3-acetyl-mesoinositol and its arabinoside (VII)	corn (certain varieties, each occurring in two modifications)	hydrolysis	spontaneous but hastened by acid or alkali
Tryptamine (VIII)	leaves	oxidative deamination to indole-3-acetaldehyde (q.v.)	monoamine oxidase
Tryptophan (IX)	all plant proteins	oxidative deamination, or transamination to indole-3-pyruvic acid (q.v.) followed by oxidation	not certain that conversion occurs in higher plants free from bacteria
Gluco-brassicin	cabbage family	hydrolysis liberating indole-3-acetonitrile (q.v.), with sulfate and glucose	myrosinase

Figure 20.4 (right). Structural formulae for the gibberellins GA_3 and GA_7 .

Figure 20.5 (left). Some dwarf varieties of corn will become almost indistinguishable from naturally occurring tall forms after treatment with gibberellins.

chemists, although it had not been suspected to play a role in plant growth.

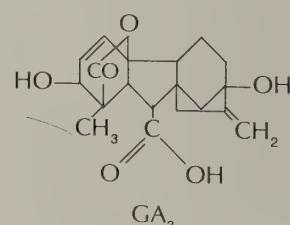
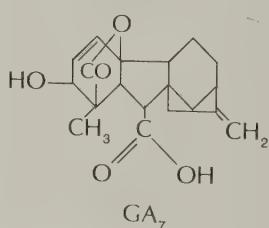
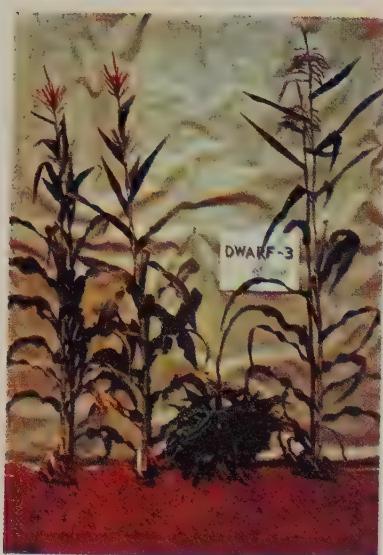
In subsequent years, a number of closely related growth compounds have been identified in plant extracts. All seem to owe their activity as growth substances, or auxins, to the ability of the plant to convert them into indole-3-acetic acid (Table 20.1). Many additional auxins have been synthesized. These molecules are similar in general structure to IAA (I), but considerable variation is possible. Three of the most active synthetic auxins—NAA (II), 2,4-D (III), and TCBA (IV)—are shown in Figure 20.3 along with structural formulae of the major natural auxins listed in Table 20.1.

Gibberellins

While the auxins were being identified, E. Kurosawa, a Japanese agricultural officer working in Taiwan, was studying a disease that causes rice plants to turn yellow and to grow excessively tall. From diseased plants, he isolated the fungus *Gibberella fujikuroi* and found that healthy rice plants develop the disease symptoms when treated with a medium in which the fungus has grown, although the fungus itself is not transmitted to the plants by this treatment. Apparently, the symptoms are caused by some substance that the fungus secretes into the medium. Chemical work in Japan led to the isolation from the fungus of an extract capable of producing the symptoms. This extract was named gibberellin A, after the fungus. Because of World War II, the Japanese research did not come to the attention of Western biologists for several years. In 1956 John MacMillan in England isolated from the gibberellin A extract a pure compound, which he called gibberellic acid. This compound not only produces the disease symptoms in rice but causes excessive stem elongation in a wide variety of other plants.

Subsequent chemical studies have led to the identification of a whole family of closely related compounds, the gibberellins, which have similar biological effects and varying degrees of activity. On the whole, GA_7 has the highest activity—more than triple that of the original compound, which is now called GA_3 (Figure 20.4). Several of the gibberellins have only very slight activity.

At first, biologists assumed that they had discovered an interesting product of fungal metabolism—a substance that acts as a “plant drug,” with effects on plants as unnatural as those of caffeine or opium on man. However, two kinds of experiments first performed about 1956 revealed a very different picture of the role of gibberellins. First, it was shown that gibberellins have their greatest elongating effect on the stems of dwarf plants. Dwarf varieties of peas (such as Little Marvel) grow as tall as the naturally tall varieties (such as Telephone) after treatment with gibberellins. Some dwarf varieties of corn (though not all) become almost indistinguishable from the naturally tall forms after treatment with gibberellins (Figure 20.5). Such experiments suggest that the dwarf varieties are



naturally deficient in gibberellin. Botanists began to suspect that gibberellins, like auxins, are natural plant hormones.

The second kind of experiment provided proof that gibberellins do exist normally in plants. They were found first in the seeds of a very long and straggly desert gourd, *Echinocystis*, and later in many other seeds and in bamboo shoots. Chemically, these natural plant gibberellins are members of the same group of compounds as the substances produced by *Gibberella fujikuroi*. In fact, more than one-third of the 20 or so gibberellins that have been isolated from the fungus subsequently have been found in plants. Thus, the gibberellins represent a second class of plant hormones, chemically and biologically distinct from the auxins.

Cytokinins

The discovery of a third class of plant hormones was made possible by scientific advances that followed discovery of the auxins. The long-sought goal of getting bits of plant tissue to grow in the test tube was achieved through the addition of minute amounts of IAA or synthetic auxins to the nutrient medium. This plant-tissue culture was first accomplished in 1939 in France by R. J. Gautheret with willow cambium tissue and by Pierre Nobécourt with carrot tissue and in the United States by P. R. White with tobacco tumor tissue. Many kinds of plant cells and tissues, including fruits and flowers, subsequently have been grown in culture, and there have been some striking applications—for example, the propagation of orchids has been revolutionized.

The path that led to discovery of cytokinins, the third class of plant hormones, started from the study of the tissue-culture technique itself and from the study of the effects of various additions to the medium. Cultures of tobacco pith cells normally produced masses of callus, or parenchyma tissue.

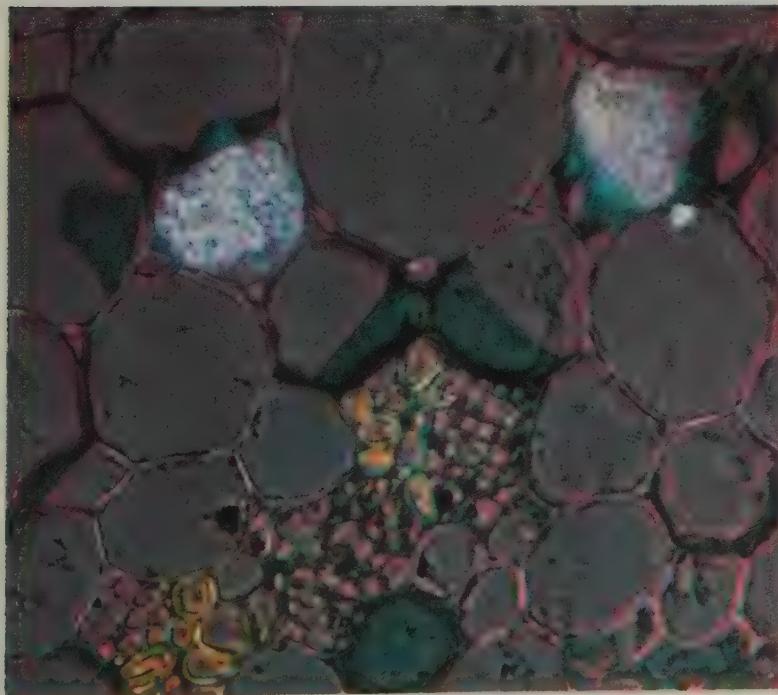
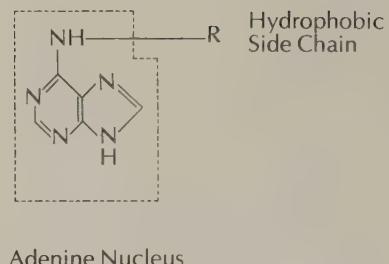


Figure 20.6 (left). Tobacco pith cells. Cultures of these cells were used in experiments that led to the discovery of the bud-forming substance, kinetin.

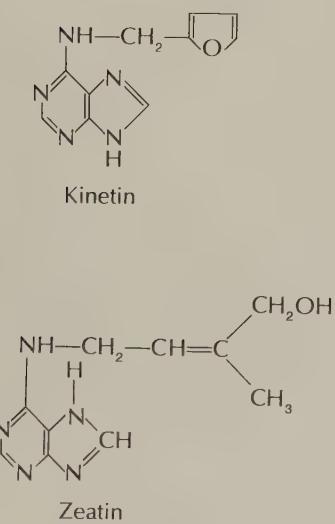
Figure 20.7(right). The structural formula of cytokinin.



Adenine Nucleus

Figure 20.8 (above). The structural formula of kinetin.

Figure 20.9 (below). The structural formula of zeatin.



Addition of adenosine and its phosphate to the medium caused an increase in bud formation. Further experimentation revealed that yeast extract is more effective, and yeast nucleic acid is better still. Different samples of yeast nucleic acid extract were found to vary in bud-forming effectiveness, so Folke Skoog and his colleagues at the University of Wisconsin examined some of these samples chemically. The bud-forming activity was traced to a simple constituent, the previously unknown compound 6-furfylaminopurine, or kinetin (Figure 20.8).

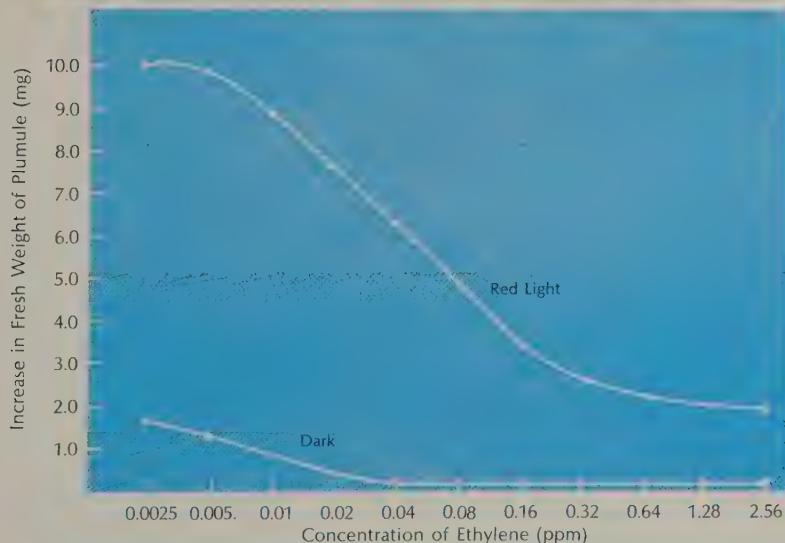
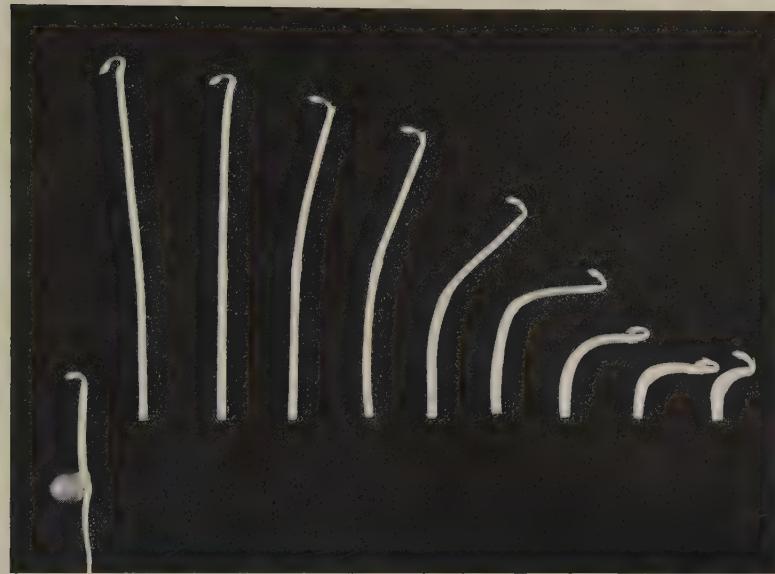
As in the case of gibberellin, kinetin was at first a substance extracted from other organisms and shown to affect the growth of plants. Evidence of a kinetinlike substance in plants was soon obtained, for numerous plant extracts were shown to produce effects on tissue cultures. Particularly effective extracts were obtained from unripe corn and young fruits in general. Preliminary purification studies pointed to a purine—probably an adenine derivative—as the active factor, and researchers in many laboratories set out to isolate and identify the active compound. The search culminated in 1964 with the isolation of zeatin from unripe corn. This compound has a hydroxymethyl side chain instead of the furfuryl group of kinetin (Figure 20.9). Later, a bacterium that causes development of multiple buds in plants was found to produce a compound identical to zeatin except for the absence of the OH group. The family of similar compounds is now called the cytokinins.

Other Plant Hormones

There are probably other plant hormones. There certainly are other compounds that control plant growth and development. A hormone, however, is defined as a chemical messenger—a substance formed in one part of an organism and transported to another part where it acts. Some substances are growth regulators, but they are not readily transported and therefore are not called hormones. Probably in this group lie the many phenolic compounds in plants, which stimulate or inhibit the destruction of auxin by oxidizing enzymes. Phenols with two adjacent OH groups inhibit this process and thus protect the auxin, whereas phenols with only one such group accelerate the destruction. Scores of phenols have been found in plants—the blue, purple, red, and some yellow pigments of flowers, fruits, and autumn leaves belong to this group. Abscisic acid, recently isolated from cotton bolls and from dormant buds (it was at first called dormin), appears to be a widespread inhibiting agent; it is not a phenol but is related to the terpenes and carotenoids. It seems to have not only a general growth-inhibiting effect but also inhibits transpiration; because it is produced in wilting leaves, it helps to protect against drying conditions.

The gas ethylene (C_2H_4) is placed in a special category. In 1901 the plant-damaging effects of coal gas were traced to its ethylene content. Orange growers long had ripened stored oranges by heating them. Biologists demonstrated that the ripening is due to ethylene from the oil heaters used in heating the oranges. One of the damaging effects of ethylene on plants is a stimulation of leaf fall. In the presence of ethylene, the petioles of the leaf blades begin to curve downward on the stem in a characteristic way. In the 1930s, it was noticed that tomato plants suffer such a drooping of the leaves when kept in a closed space with ripe bananas. Development of gas chromatography techniques, which can detect less than one part of ethylene in a billion parts of air, has made possible the proof that all fruits give off ethylene during ripening and that ripening begins when the ethylene content

Figure 20.10a (above). Photograph of etiolated pea seedlings showing the effects of various concentrations of ethylene on their growth during the 48-hour treatment period. The seedling at the far left shows the size of the plants at the beginning of the experiment. The seedling second from the left shows the amount of growth attained during the 48 hours by the untreated control. Remaining seedlings, from left to right, were treated with 10, 20, 40, 80, 160, 220, 640, and 1,280 parts per billion (10^{-9}) of ethylene in a flowing stream of air.



reaches about one part per million of the air in fruit tissues. Pears and cherimoyas have proven to be the most active producers of ethylene during ripening; and citrus fruits the least active.

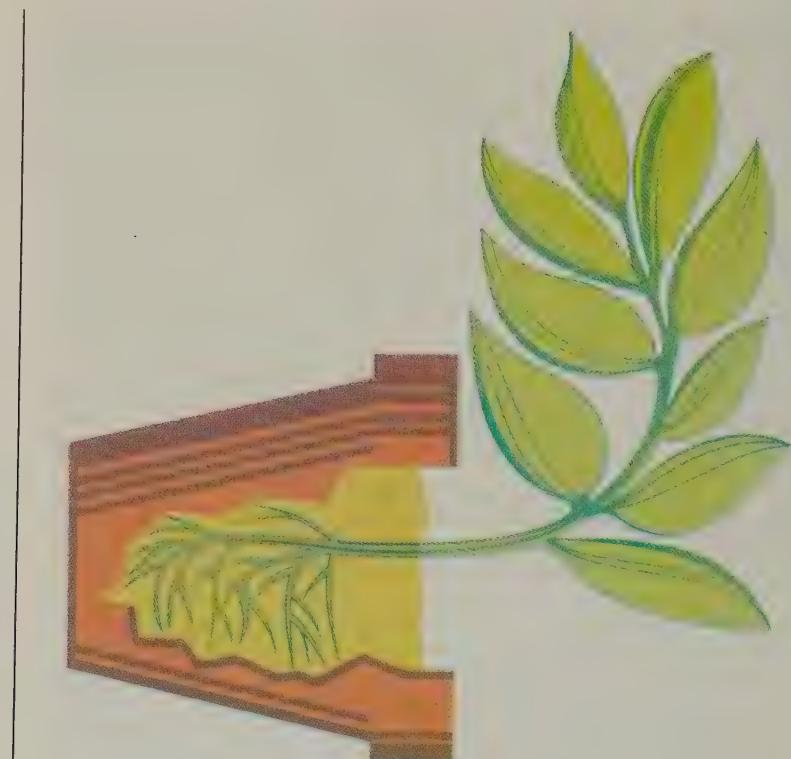
Ethylene is a ripening substance, but is it a hormone? Application of auxin to many tissues stimulates production of ethylene. Because roots are particularly active ethylene producers under auxin stimulation, some biologists believe that the inhibitory effects of ethylene on root and bud growth are actually caused by increased auxin production. Therefore, ethylene may be regarded as a sort of gaseous hormone.

HORMONAL CONTROL OF ELONGATION PROCESSES

Phototropism in coleoptiles provides the clearest example of hormonal control of elongation processes. Light diverts the transport of auxin across

Figure 20.10b (below). Graphs illustrating the effects of various concentrations of ethylene (for 48 hours) on growth (increased fresh weight) of the plumules of 4-day-old pea seedlings in dark and in red light (30 ergs/cm⁻²/sec).

Figure 20.11. The interaction of gravity and auxin distribution is illustrated when a growing plant is laid horizontally. Auxin coming from the tip is diverted downward across the stem, so that the lower side receives up to 3 times the amount of the upper side. As a result, cell proliferation in the lower side is greater and the shoot curves upward until it regains a vertical position.



the plant and concentrates the growth hormone on the shaded side of the coleoptile tip. Similar effects can be demonstrated with the growing apices of bean, radish, or lupine seedlings. More recently, IAA labeled with C^{14} has been applied to intact tips of seedlings. Light diverts the radioactivity to the shaded side. Thus, there is no doubt that light from one side modifies the distribution of auxin between the two sides of the seedling tip. The side with more auxin (the shaded side) grows more, and therefore the plant curves toward the light.

Gravity acts in a comparable way. When a seedling is laid horizontally, auxin coming from the tip is diverted downward across the tip, so that the lower side receives about two or three times as much as the upper side. In the shoot, the lower side grows more, and the shoot curves upward until it regains the vertical position. In the root, the extra auxin inhibits elongation (perhaps through production of an inhibiting level of ethylene), so the root curves downward (Figure 20.11).

These tropisms show that extremely small changes in the auxin concentration cause significant changes in growth rate. Instead of the normal 50:50 auxin ratio between the two sides of a growing seedling, light or gravity produces a ratio of 67:33 or 75:25, and tropisms result. Because the shoot of a young seedling must find its way to the light and the root must reach the moist lower layers of the soil within the short time that the endosperm can provide food for growth, these tropisms are a matter of life or death for a young plant.

The control of straight growth, or simple elongation, is more complex because both auxin and gibberellin act in the same way. Both hormones

Figure 20.12 (above). A cabbage plant treated with gibberellic acid for some weeks showed amazing growth compared to the control plants (at left) and had to be measured with the aid of a stepladder.

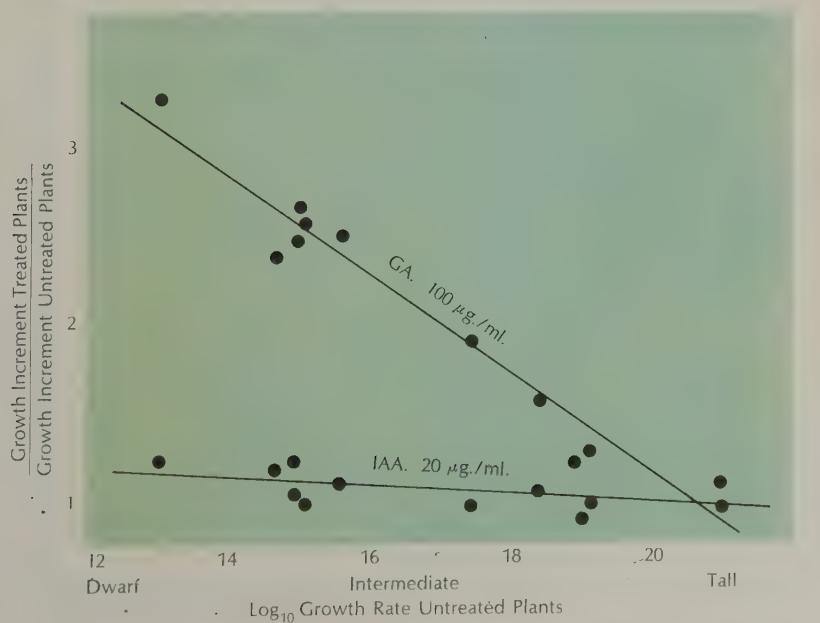
Figure 20.13 (below). Graphs illustrating the relative effects of gibberellic acid (GA) and β -indolyl-1-acetic acid (IAA) in stimulating extensive growth of dwarf, tall, and intermediate varieties of garden peas. Stimulation of GA increases progressively as one passes from tall to dwarf varieties while the effect of IAA is relatively constant.

cause elongation, and their effects are additive. In the oat coleoptile, gibberellin has only a small effect; thus, when auxin is applied symmetrically, the resulting increase in growth is a function of the auxin supplied. In peas, beans, and other experimental plants, however, gibberellin plays the major controlling role. A cabbage plant fed gibberellic acid for some weeks had to be measured with the aid of a stepladder (Figure 20.12). The effect of gibberellin is exerted on the internodes or, in monocotyledonous plants such as corn, mainly on the leaf sheaths. In most cases, leaves become longer, thinner, and yellower.

The control of elongation in roots is even more elusive, for here only the lowest concentrations of auxin promote growth. Auxin concentrations high enough to promote the growth of shoots only inhibit that of roots. The level of auxin produced by the root tip is usually slightly inhibitory, so that careful decapitation (without removing the elongating zone just back of the tip) may cause a temporary acceleration of the growth rate. In seedlings, some auxin also reaches the elongating zone from the shoot above. Isolated roots growing in a nutrient medium may show the promotion effect better than roots in complete plants, but it always is small. Gibberellins neither promote nor inhibit root growth. Thus, if any hormone is an important stimulator of root elongation, it remains to be discovered. Even the inhibiting effect of auxin may be indirect and due to its stimulation of ethylene production.

HORMONAL CONTROL OF CELL DIVISION

Growth essentially is enlargement, and for plants (which can sometimes shrink) the best definition of growth is an irreversible increase in volume. Dry weight may increase—for instance, when an old leaf makes starch by photosynthesis—without an increase in volume, whereas volume may increase reversibly in temporary swelling due to osmotic water intake. Nevertheless, many growth processes involve cell division as well, and because cells do not enlarge indefinitely, continued growth usually depends on



both cell division and cell enlargement. The actions of hormones on cell division are manifold.

Addition of IAA (about one part per million) to a tissue-culture medium makes possible the growth of plant fragments that would otherwise die after a few cell divisions. In the presence of auxin, the cells divide vigorously and repeatedly, making permanent tissue cultures possible. Thus, it is clear that auxin stimulates cell division.

Some tissue cultures show a curious change in auxin requirements after a series of transfers from one auxin-containing medium to another. The tissue changes from firm and solid to crumbly and watery. In its new form, the tissue is able to grow without the addition of auxin to the medium; in fact, its growth is now inhibited by IAA concentrations that formerly were optimal for growth. These *accoutumé*, or adapted, tissues resemble some kinds of tissues derived from plant tumors, which also grow without added auxin. Both tumor and *accoutumé* tissues synthesize auxin, as proved by the fact that more IAA can be extracted from these tissues than they could have accumulated from the nutrient medium. Thus, under some conditions, plant tissues can "learn" to synthesize auxin.

Auxin apparently affects the processes of DNA replication and chromosome doubling during interphase of the cell cycle. Auxin-treated tissues contain many polyploid cells. Cytokinins control synthesis of RNA and cytokinesis, or separation of daughter cells (hence their name). The combination of auxin and cytokinin produces the most actively growing tissue cultures and those with the most normal appearance. The first detectable effect of cytokinin on tobacco pith cells is a drastic increase in the amount of RNA in each cell. Values up to ten times the normal RNA content have been measured. A later effect is multiplication of normal diploid cells, so cytokinin apparently affects mitosis as well as RNA synthesis.

The most striking cell divisions in an intact plant occur in the cambium, where divisions form long new walls in the tangential plane. These divisions and subsequent cell enlargement cause thickening of the stem. At Oxford University in 1933, Robin Snow showed that there is no cambial activity in decapitated sunflower seedlings but that the addition of a small amount of crude auxin extract (from urine) stimulates typical cambial divisions. In later experiments, crystalline IAA has produced strong cambial activity in both herbaceous and woody plants. Cambial activity in trees begins in young twigs in the spring when buds open. It progresses along the branches and down the trunk at a rate of movement close to that determined directly for auxin. Scrapings of the cambium layer taken in the spring are rich in auxin.

Because the cambium normally produces xylem on its inner side and phloem on its outer side, it is not surprising that auxin also stimulates formation of vascular bundles. This formation is very marked in tissue cultures, where many formations are essentially undifferentiated. A local spot of auxin or local insertion of an actively growing bud gives rise to zones of xylem below; if sugar is added, phloem is also formed. In decapitated stems, these zones continue downward until they find their way into existing vascular bundles and join up with them. Oddly enough, however, if that vascular bundle is in connection with an active bud (an auxin source), the newly formed bundle does not fuse with it but is repelled.

A special case of xylem formation occurs when the xylem in a herba-

ceous stem is cut. New xylem cells gradually differentiate just above the cut and form a C-shape strand of xylem, which finally joins into the old xylem below the cut and thus reestablishes the continuity of the conducting tissue. This process occurs only if buds or young leaves are present on the stem above the cut or if auxin is applied above the cut. The number of new xylem strands so formed is proportional to the auxin concentration. As in tissue cultures, if sugar is added, phloem strands form also. Auxin thus acts to heal wounds in the plant body. The actual types of xylem elements formed, however, appear to depend also on cytokinin.

The formation of roots on stems is a very different phenomenon involving cell division. Roots will form spontaneously on stem cuttings of some plants, particularly if developing buds are present. Botanists long ago observed that roots tend to form directly below a bud and on the same side of the stem. Raymond Bouillenne and Frits W. Went demonstrated in 1933 that extracts from rice grains can mimic the effects of the bud. Application of rice grain extract to the upper end of a cutting stimulates root formation at its base. Apparently, a root-forming hormone is produced in the buds and travels downward through the stem. Auxin moves in this fashion and is a growth stimulator, and it was not long before Kenneth V. Thimann and Joseph Koepfli (1935) in the United States demonstrated that pure IAA applied to the apical end of stem cuttings stimulates root production at the base. This treatment is now used widely by nurserymen to stimulate root growth on cuttings used for plant propagation. Commercial preparations containing synthetic auxins are available for this purpose.

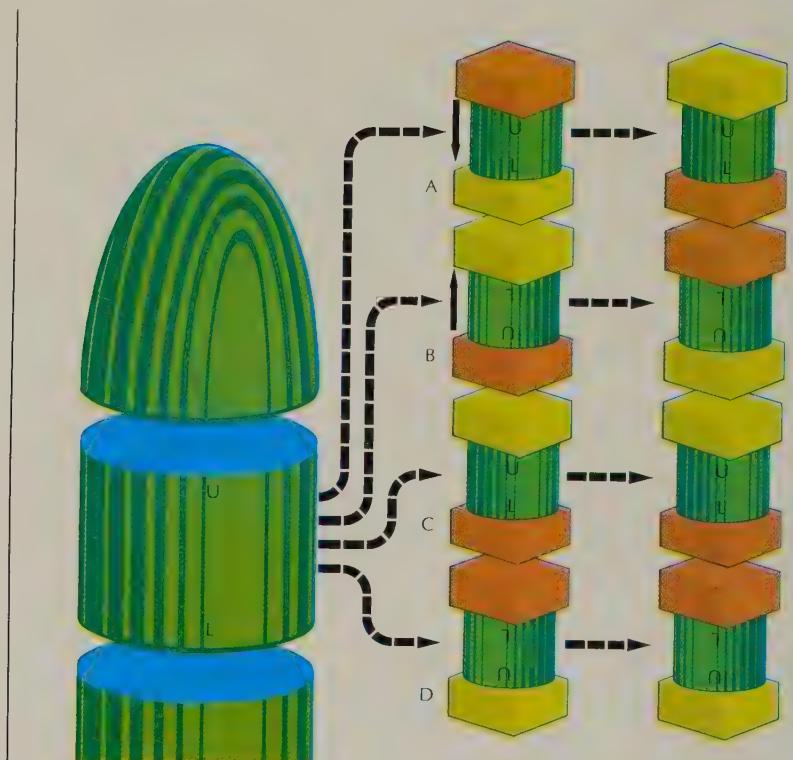
Root development in a stem begins with cell divisions in the layer of cells beneath the epidermis. Other divisions follow rapidly until a conical mass of small cells begins to push outward through the cortex. As this developing root elongates, vascular bundles form behind it and connect with the bundles of the stem. Even on green stems, the root is colorless. It grows out laterally at first and then begins to curve downward, showing a positive geotropism. Thus, differentiation has occurred; the new root shows characteristics typical of root rather than stem tissues. The mechanism that triggers this change in the tissue is completely unknown, except that auxin serves as the initial stimulus to set the changes in motion.

TRANSPORT OF PLANT HORMONES

Throughout the discussion of plant hormone actions, the terms "above" and "below" are prominent. In plants, the directions up and down, as set by gravitation, are very distinct; the properties of the top and bottom—or better, apex and base—of the plant are very different. A cutting can be placed upside down, but roots still form at the basal end of the cutting, although that end is now uppermost. The apex-to-base polarity of the plant is produced by the tendency of auxin to move from plant apices, buds, or young leaves down the stem toward the base. Auxin tends to move from the apex toward the base even when the position of the stem with respect to gravitational forces is altered.

This movement of auxin can be demonstrated readily in a short section cut from an oat coleoptile. An agar block containing auxin is applied to the apical end and a plain agar block to the basal end. After a short time, auxin can be detected in the basal block, and within two or three hours as much as half of the auxin travels through the stem section to the basal block. If the

Figure 20.14. The apex-to-base polarity of auxin movement is illustrated in this series of experiments. A section is cut from a coleoptile (left) and the upper (u) and lower (l) surfaces noted. An agar block containing auxin is applied to the upper surface and a plain agar block to the lower surface. Within 3 hours, the distribution of auxin in both blocks is equal, indicating movement of auxin to the block on the lower surface (both A and B). If the blocks are reversed, and the auxin is applied to the lower surface of the coleoptile, little or no auxin moves through the section into the upper surface agar block (both C and D).



blocks are reversed and the auxin is applied to the basal end, little or no auxin moves through the stem section into the apical agar block. Experiments can detect auxin by testing the blocks on newly decapitated coleoptiles and measuring the angle of curvature produced. Other researchers confirmed these results by using IAA labeled with radioactive carbon and measuring the level of radioactivity in the agar blocks. The rate of auxin transport in most stem tissues at room temperature is 10 to 12 millimeters per hour. At this rate, a given molecule of auxin would travel from the apex to the base of a 50-foot tree in about 2 months.

The strictness of polarity varies from plant to plant. It is generally high in young cereal seedlings, but in the stems of dicotyledons such as bean and sunflower plants, there is sometimes slight base-to-apex movement. If unnaturally large amounts of auxin are applied, the polarity can be overcome, but it requires 100 to 1,000 times the normal auxin concentrations. Auxins can be used as weed killers because high concentrations of synthetic auxins poured on the soil can be taken up by the roots, as is any other dissolved substance (nitrate, for instance), and drawn up to the leaves in the transpiration stream. Tall trees sometimes can be killed in this way. On the way up through the xylem, some auxin diffuses laterally into the living cortex cells, where it becomes subject to polar transport, which conducts it downward again. Thus, a sort of auxin circulation can occur under artificial conditions.

Transport in roots is not so simply polar. Auxin moves from the base of the shoot down into the root, but auxin also is formed in the root tip and transported from there into the elongating zone behind. Thus, there are two

polarities, neither one very strict, and in the intermediate zone there is very little transport in either direction. Because most of the applied auxin becomes either oxidized or "fixed" in the root and disappears, radioactive auxins are being used in current studies of this transport.

Cytokinin and gibberellin are not subject to such polar transport. Gibberellin moves freely in both directions; it can be applied to the base of a stem, or even to the roots, and causes excessive elongation in the growth zone just behind the stem apex. When applied to the terminal bud, it has the same effects. Cytokinin is transported very poorly in living tissue; it has been shown to move down a petiole only to the extent of 2 percent of the amount applied in 24 hours, which is over 100 times less than IAA. Recently, however, cytokinin has been detected in the bleeding sap that exudes when the stem of a healthy plant is cut off; this sap represents water taken in by the roots and squeezed upward in the xylem by osmotic pressure. The sap is known to contain amino acids synthesized in the roots, and the presence of cytokinin as well may help to explain why roots exert so much effect on the growth rate and greenness of shoots. But this explanation is as yet far from certain.

ABSCISSION AND DEFOLIATION

When leaves become old and when fruits become ripe, they fall off, or *abscise*. The process normally depends on special cells that are formed at the base of the petiole where cell divisions begin to occur as the leaf or fruit gets older. Eventually, the soft cementing material that holds these cell walls together begins to hydrolyze and the cells fall apart. As a result, the leaf or fruit soon is held only by the vascular bundle, which breaks off in the slightest wind. All of this process is inhibited by auxin. The *abscission layer* of special cells does not form while the leaf is young and growing because the young leaf secretes a steady stream of auxin (Figure 20.15). Only when that stream wanes to a trickle and then stops does the abscission process begin. Abscission, like elongation of roots, thus is inhibited by auxin under normal conditions. Surprisingly, if massive amounts of auxin are supplied, abscission is promoted. This opposite action is probably due to ethylene, which is formed in many cells under the influence of excess auxin. Thus, concentrated sprays of synthetic auxins are used to thin crops. By this process, some young fruit fall in the spring, and the remaining apples or pears become bigger. Similarly, the army sprays trees in Vietnam from the air to clear the jungle. Both applications use a fine spray of concentrated synthetic auxin solution.

Fruits normally fall in the spring if they have not been fertilized. Without growing seeds, auxin is not produced in the young fruit. It has been shown that the June drop of apples and their fall when ripe in autumn coincide in each case with a minimum in auxin production. The picture is complicated by the fact that gibberellin also promotes abscission somewhat. This hormone seems to reach a maximum at the time of the auxin minimum, so that they work together to promote abscission. Several factors probably interact in this process.

APICAL DOMINANCE AND PLANT INTEGRATION

One aspect of the growth of plants that clearly depends on the interaction between two or more hormones is the influence of buds upon one another.

Figure 20.15. Photograph of a coleus leaf showing the abscission layer at the base of the petiole. Note the small size of the cells comprising the abscission layer. The formation of these cells is dependent upon cessation of auxin flow through the tissues of the leaf petiole.



Figure 20.16. Effects of widespread dissemination of auxins by air on the forests of Vietnam.



Growing buds secrete auxin, which travels down the stem to elicit the formation of roots below. That same auxin inhibits the development of lateral buds on the stem. The growing terminal bud thus prevents other buds from developing (Chapter 18). If the terminal bud is removed and auxin is applied in its place (in amounts comparable with what the bud would have produced), lateral buds remain inhibited. Before auxin was known, such inhibitions were ascribed to the withdrawal of materials for growth by the developing terminal bud, the others thus being starved out. However, auxin applied in such a small concentration that it produces no visible growth of the stem still produces complete inhibition.

The inhibiting action of the terminal bud is incomplete in some plants—larch, gingko (maidenhair), apple, plum, and cherry trees—and as a result they form “short shoots.” The lateral buds open and produce a few leaves or a flower, but the lateral shoots do not elongate more than a millimeter or two. They remain short throughout the season and develop into normal, or “long,” shoots only if the terminal bud is cut off. Again, the effects of the terminal bud are closely mimicked by the application of auxin.

This action of a growth substance to inhibit a typical growth process has caused much speculation. At least nine theories have been proposed at various times to explain it. It now seems probable that the effect is indirect. Under the influence of auxin, the cells in and around the node begin to produce ethylene, which seems to inhibit the small buds arising from each node. Apparently, internode tissue forms very little ethylene.

Auxin is necessary for growth in tissue cultures, but it produces masses of undifferentiated tissue. When kinetin is added to the medium, the tissue produces numerous buds, suggesting that bud growth (as opposed to inhibition) is favored by kinetin. A simple test system has been devised in which a piece of stem with a single node bearing a bud is floated on sugar solution. After a few days the bud develops and elongates, but if auxin has been added to the solution, the bud remains completely inhibited. If kinetin now is added as well, this inhibition is relieved, and the bud grows just as well as in the controls. About two parts of kinetin to one part of auxin are required for such complete reversal, but partial reversal can be obtained with much smaller amounts. Too much kinetin, however, decreases the bud growth again, so that the phenomenon evidently depends on an exact balance between the two hormones.

Cytokinin also can be applied directly to the bud and cause it to grow out. Because cytokinins are poorly transported, the application must be exactly on the bud and not merely nearby. Auxin, on the other hand, can come from the apex many centimeters away. Application of cytokinin directly to the bud can cause outgrowth not only of the lateral bud itself but also of smaller buds at its base, so that a mass of little buds (called a witch’s broom) develops. This mass closely imitates a well-known bacterial plant disease, and it has been shown that cultures of the bacterium on a nutrient medium synthesize a cytokinin. Thus, this particular disease has a rather simple explanation.

The outgrowth of a lateral bud under the influence of cytokinin apparently is due to the formation of a functional vascular bundle leading to the bud. While the bud is inhibited, its vascular connection to the main stem is incomplete, and the units of xylem appear short and not well adapted for conduction. Kinetin causes a connection of normal xylem with long

Figure 20.17. Destruction of the natural auxin IAA.

functional units within about 72 hours. A full understanding of hormone action in this or any other function, however, is a long way off.

THE FORMATION AND DESTRUCTION OF AUXIN

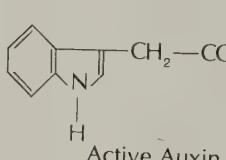
Hormones that are effective in such small amounts must be destroyed rapidly, for their accumulation might cause serious abnormalities. Little is known about the destruction of cytokinin and gibberellin, but the destruction of the natural auxin IAA has been well studied. It is brought about by the enzyme *peroxidase*, so called because it normally causes peroxide (H_2O_2) to oxidize organic compounds such as phenols or ascorbic acid, which occur widely in plants. In oxidizing IAA, however, the peroxidase uses oxygen (O_2) instead of H_2O_2 ; only a trace of H_2O_2 is needed, apparently to keep the enzyme in an active form. The CO_2 of the acid group of IAA is removed and the products rearranged to form a mixture in which 3-methyleneoxindole predominates (Figure 20.17). This compound is totally inactive as a growth hormone and may even have a very weak (and probably unimportant) growth-inhibiting effect.

IAA also can be destroyed by light in the presence of certain activating pigments, such as eosin or riboflavin. Prolonged exposure to bright light is required, and the process is of doubtful biological significance. Ultraviolet light is more effective and can even cause some destruction without an activating pigment, the IAA itself absorbing the ultraviolet light. Among synthetic auxins, 2,4-D is subject to a similar action of bright light in the presence of riboflavin. Its side chain is removed through oxidation, leaving 2,4-dichlorophenol.

The discovery that γ -phenylbutyric acid could suppress the auxin stimulations of growth led to the discovery of chemicals called antiauxins. Since then, various chemicals have been found to be antiauxins. Many of these compounds are synthetic, as naturally occurring ones have not yet been identified chemically.

Strictly speaking, the term "antiauxin" should be used only for those compounds that compete with auxin for the two reaction sites of the sub-

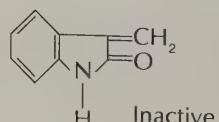
Indole-3-Acetic Acid (IAA)



3-Hydroxymethyloxindole



3-Methyleneoxindole



strate. An auxin has a benzene ring with the para position open and an acid group on the side chain, with specific distance between the two. Antiauxins lack at least one of the requirements of an auxin and so could not fill the two active sites of the substrate.

If only one of the reaction sites is filled, then the product formed is inactive. Antiauxins could fill only one site, thus forming an inactive complex. An excess of auxin could also act as an antiauxin because two molecules could fill up the active sites of one substrate.

THE ROLE OF PLANT HORMONES

Biologists who study animal hormones are accustomed to thinking of the animal body as subject to a multiplicity of hormonal controls—the thyroid hormone controls metabolism, the parathyroid hormone controls calcium deposition, the sex hormones control gonads and secondary sexual characteristics, other hormones control digestive processes, and so on (Chapter 21). These researchers speak of the endocrine system in higher animals and call their science endocrinology. Superimposed on the hormonal system of animals is another control system—faster in action and more localized in its effects—the nervous system.

In contrast, the integration of activity in a higher plant—so far as now is known—is accomplished by a simpler hormonal control system. Relatively few plant hormones are known; these hormones for the most part are not produced in specialized glands. The effects of the plant hormones on growth and metabolism are felt in many parts of the plant rather than being localized in specific target organs as in animals. For these reasons, the study of plant hormones has not become a branch of science as prominent or as independent from general physiology as the science of endocrinology. Nevertheless, hormones are of great importance in plant growth and development, and if more plant hormones are discovered, this field may become a sort of “endocrinology” of plants.

The rather generalized effects of plant hormones suggest a mode of action somewhat different from that of most animal hormones. There is much evidence to suggest that auxin and cytokinin may act through synthesis or modification of specific types of RNA. Cytokinin apparently is incorporated directly into mRNA, which directs protein syntheses in the cell. Gibberellin produces similar effects on preparations of isolated cell nuclei. Thus, the available evidence suggests that plant hormones act directly upon the cellular control mechanisms of the plant cell. This direct effect upon the heart of the cellular mechanism probably accounts for the very general activity of these hormones in plant tissues.

FURTHER READING

Further information about plant hormones will be found in books by Audus (1963), James Bonner (1966), Jensen (1962), Leopold (1955), and Went and Thimann (1937). General books on plant development and physiology listed in Further Readings for Chapters 18 and 19 also contain discussions of plant hormones.

Among the general articles useful as introductions to the study of plant hormones are those by Galston and Davies (1969), Overbeek (1968), and Salisbury (1957). Helgeson (1968) and Letham (1969) deal more specifically with cytokinins. Addicott and Lyon (1969) review research on abscisic acid.

21

Animal Hormones



The idea of animal hormones is not new. Vitalists had recognized that certain substances secreted by glands in various parts of the body have widespread effects throughout the body. For example, it had long been known that castration (removal of the testes) can cause a male to fail to develop normal secondary sexual characteristics. Thus, a mysterious power had long been attributed to the sexual organs. The Austrian physician A. A. Berthold demonstrated that transplantation of a testis into the body cavity of a castrated rooster, or capon, is followed by normal development of male sexual characteristics. Berthold explained this observation in terms of a theory similar to Darwin's theory of pangenesis, suggesting that the sex cells carry particles that travel out to the various parts of the body to direct development. One French physician in 1889 performed a well-publicized series of injections under his own skin of extracts from dog testes. He was 72 years old and claimed that the treatment produced astonishing rejuvenation of his health and sexual prowess.

The control of reproductive functions in the mammalian body provides an excellent example of the complex interweaving of nervous and hormonal controls. This system has been studied extensively because of its importance in animal breeding and in contraception. The control systems affecting development of eggs and sperms are now relatively well understood, although much remains to be learned about the control of the female reproductive system during pregnancy and birth.

MAMMALIAN SEX HORMONES

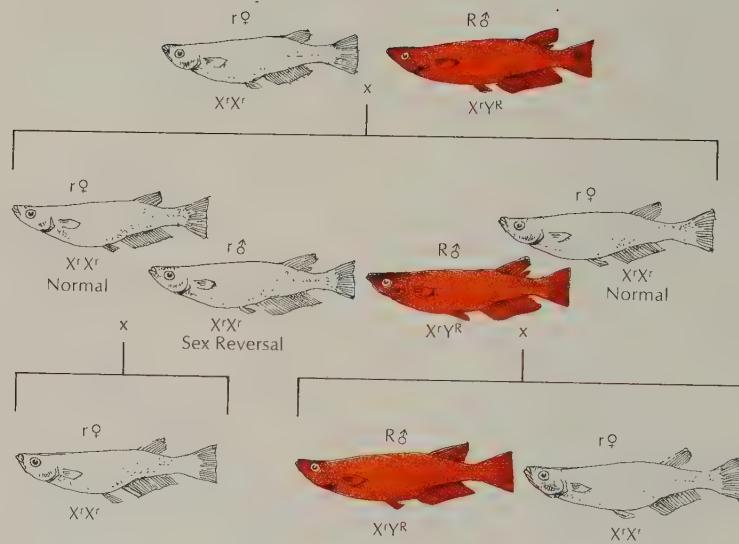
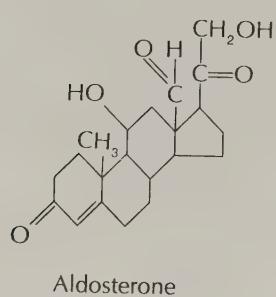
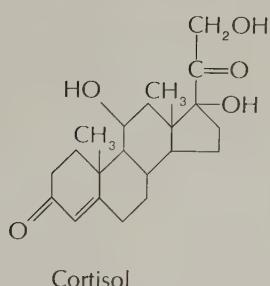
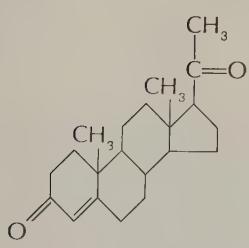
The discovery of sex chromosomes provided one of the first definite proofs that hereditary information is carried by the chromosomes (Chapter 14). It was demonstrated that the cells of a female mammal contain a pair of X chromosomes, whereas those of a male contain one X and one Y chromosome. The genetic inheritance of sex, however, is not the complete story.

The balance of various hormones in the developing embryo—a balance presumably regulated by genetic information on the sex chromosomes—plays the crucial role in controlling the development of tissues and organs toward male or female forms. Complete reversals of sex have been produced in fishes and amphibia by treating developing eggs with various hormones. Such treatments can lead to the development of apparently normal males or females, regardless of the genetic sexual inheritance of these individuals. For example, appropriate hormonal treatment of an egg with a male genotype can lead to its development as a female with a normal female reproductive system and female secondary characteristics. A sex-reversed amphibian or fish shows mating behavior appropriate to its apparent sex and produces viable eggs or sperm. However, its offspring (if allowed to develop without hormonal treatment) will show abnormal ratios of sexes, for the sexual genotype of the parent is not altered by the hormonal treatments. For example, when a normal individual mates with a sex-reversed individual, two XX genotypes or two XY genotypes may be involved. In the first case, all of the offspring will be of the same sex. In the second case, there will be two offspring of the XY sex for each one of the XX sex and one abnormal YY individual, which probably will not develop beyond the early embryo stage.

Hormonal treatments of newborn mammals do not result in sex reversal because the critical periods of sexual determination occur while the embryo is within the mother's body. If large doses of hormones are injected

Figure 21.1 (right). Sex reversal in a genotypic female medaka (*Oryzias latipes*) as a result of androgen treatments. When mated with normal genetic females, sex-reversed genetic females produced all-female progenies.

Figure 21.2 (left). Mammalian sex hormones, showing the chemical structure of the four interlinking carbon rings.



into a pregnant mother, the pregnancy usually results in abortion. However, a condition of partial sex reversal occurs in cattle as a result of the simultaneous development of two embryos (twins) of opposite sex. If the blood supply of the two embryos is intermixed within the mother, the female often develops with undifferentiated sexual organs and with male secondary sexual characteristics. Such an abnormal female is called a *freemartin*, and she is sterile. A freemartin is apparently produced by hormonal influences from the male embryo causing masculinization of the female twin (Lillie, 1917).

Androgens and Estrogens

The hormones involved in the sex-determination phenomena described above involve a group of hormones called the steroid sex hormones, which may be divided into the androgens (typically male hormones) and the estrogens (typically female hormones). Like cholesterol and other sterol lipids, vitamin D, bile acids, and some adrenal hormones, the mammalian sex hormones have a basic chemical structure composed of four interlinking carbon rings (Figure 21.2).

Androgens and estrogens were isolated and identified chemically in the 1930s. About 30 steroid hormones have been discovered in vertebrates, and all are produced by the ovaries, the testes, or the outer portion (cortex) of the adrenal glands, which are located on the kidneys. The cells that secrete these hormones share with the tissues of the kidney a common origin in the embryo.

One important androgen is *testosterone*, which is secreted by some interstitial cells of vertebrate testes during the production period of ma-

Figure 21.3a (upper right). Interstitial cells. A typical arrangement of the interstitial cells in the vertebrate testis is seen at left. At right are shown interstitial cell homologues (boundary cells) in the walls of the testicular lobules of certain teleost fishes. Interstitial cells are responsible for the production of the androgen testosterone.

Figure 21.3b (middle right). The structural formula of testosterone.

Figure 21.4a (lower left). An ovarian follicle of the bat *Myotis lucifugus lucifugus* at two levels of

ture sperm. This hormone stimulates development of male secondary characteristics, such as body hair and other male features that appear during puberty in humans. If amphibian or fish eggs are treated with androgens, the eggs will develop into male individuals, regardless of the sexual genotype of the egg. Thus, it appears that initial development of male sexual organs and other male characteristics is determined by the presence of a high level of androgens in the tissues of the embryo at a critical stage of development. Presumably, in mammals the androgens are produced under the guidance of genes on the Y chromosome.

The follicle cells of the ovary secrete a number of estrogens. Like the androgens of a male, these hormones stimulate development of secondary sexual characteristics in other parts of the body. Maturation of female sexual organs in the embryo is stimulated by the presence of a high level of estrogens at the critical period of gonadal sexual determination. The estrogens and a number of other steroid hormones help to regulate the complex cycles of ovulation and pregnancy.

Experiments with mammals show that gonad transplants or gonadal hormone injections can cause changes in reproductive tract structures and in secondary sexual characteristics, but they do not cause transformation of the gonads themselves. Nevertheless, animals subjected to such treatments early in life often are sterile. As in the amphibians, the hormones produce effects in mammals only if present during a critical period of development. In some small rodents, such as mice and rats, this period occurs within the first few days after birth. A female injected with either androgen or estrogen during this period will develop into a sterile adult female. The sterility is caused by the animal's inability to release mature eggs and, in many cases, a failure to exhibit normal behavioral responses to hormones secreted from the ovary at the time an egg is matured and ready for fertilization (Barracough, 1966).

In mammals with longer gestation periods, such as guinea pigs and monkeys, a similar period of sensitivity to hormones occurs before birth

magnification. The micrograph to the right shows the follicle in relation to the whole ovary at lower magnification; the lefthand portion is the same follicle at higher magnification. The follicle cells (surrounding the ova) of the ovary secrete a number of estrogens, which stimulate development of secondary sex characteristics.

Figure 21.4b (lower right). The structural formula of estrogen.

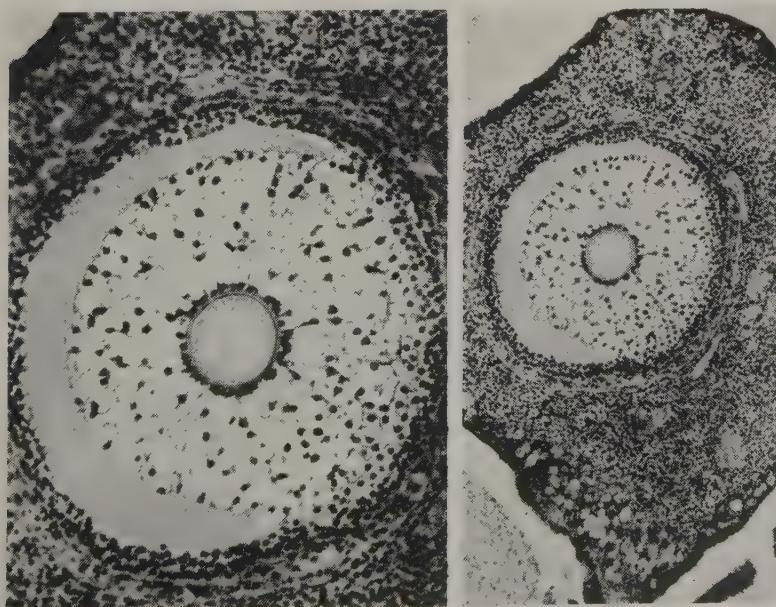
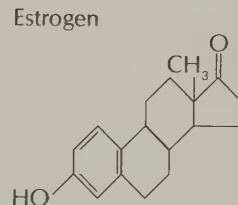
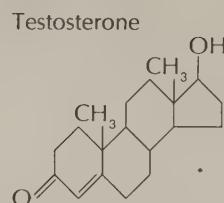
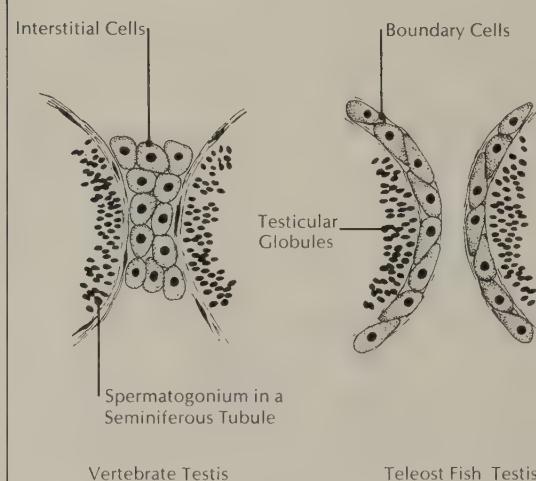
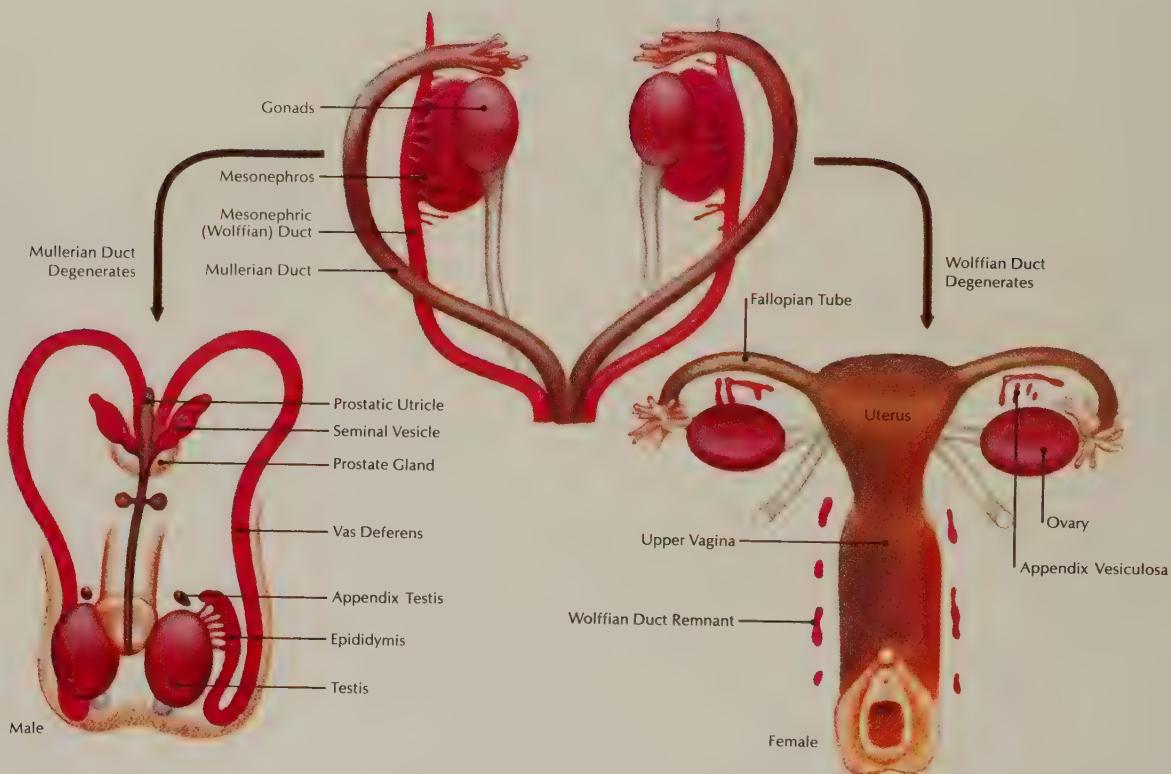


Figure 21.5. Diagram showing transformation of the genital tracts in mammalian embryos in transition from an undifferentiated stage (above) to the male and the female condition.



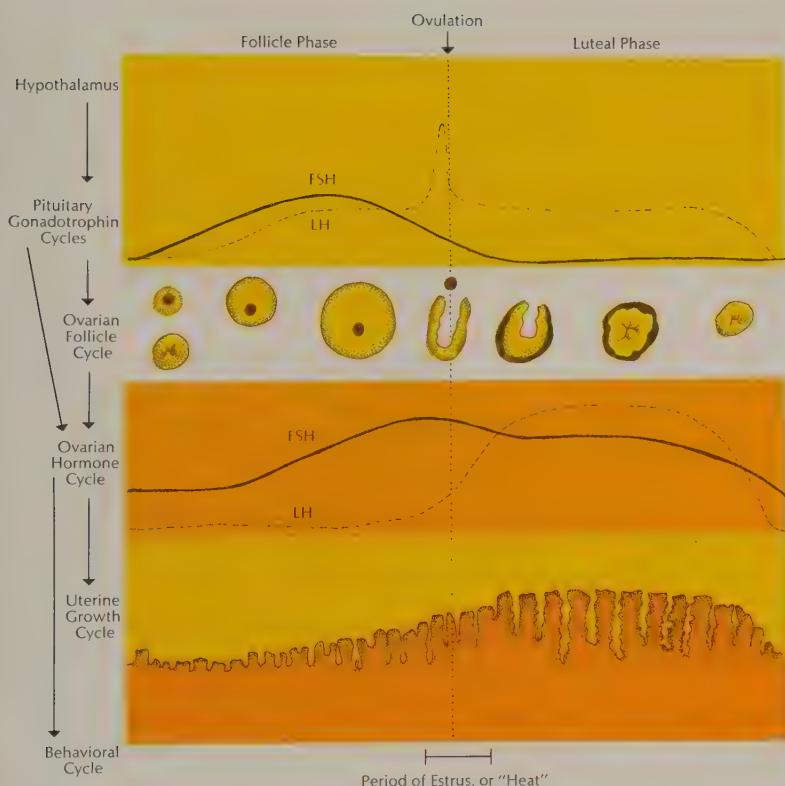
and lasts about five to ten days. A pregnant female injected with androgen during this critical period will produce sterile female offspring.

At an early stage in development of mammalian embryos, immature structures for both the oviducts of the female and the sperm ducts of the male are present. In the normal individual, only one of these embryonic structures develops into the mature reproductive tract; the other degenerates and disappears. If the gonads are removed from the early embryo, both structures remain in the undeveloped form. Thus, secretions from the gonads are apparently responsible both for the development of one reproductive tract and for the breakdown of the primordia of the other (Jost, 1955; Price, 1956). In general, a vertebrate embryo of either sexual genotype contains the primordia for formation of both male and female reproductive systems (Burns, 1961). The development of one or the other system is largely controlled by hormonal concentrations, which presumably are regulated by factors under the control of the genes on the sex chromosomes. It does appear, however, that the sensitivity of tissues to hormonal treatments depends upon the genetic constitution of the individual and that the sexual genotype plays some role in sex determination in addition to the control of hormone levels. In some cases, the primordia for the structures of the sex not genetically indicated are poorly developed and cannot be stimulated into normal growth by hormone treatments.

OVULATION

In most mammals, the release of matured eggs, or ovulation, occurs at regular intervals—for example, every four days in mice, rats, and hamsters. This

Figure 21.6. Estrous cycle of the rat. The hormonal cycles are somewhat idealized.

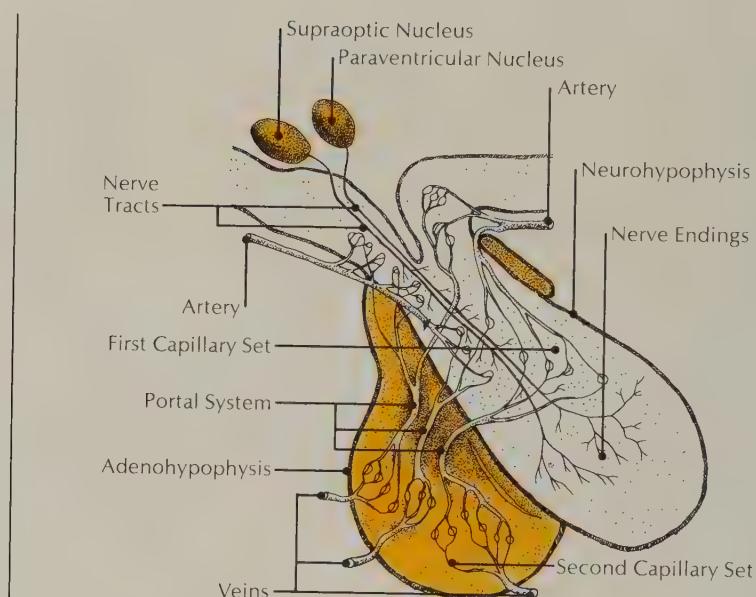


estrous cycle represents the time the ovary requires to mature and release eggs. Because the primary germ cells are determined and cease mitotic division early in development, the ovary has received its entire quota of ova long before the organism reaches sexual maturity. At sexual maturity, when the ovary begins periodically to release the number of eggs characteristic of the particular species, there is a finite population of ova that will be drawn upon throughout the reproductive life of the organism.

Completion of oogenesis and ovulation is dependent upon hormones manufactured by the pituitary gland, which is attached by a stalk to the base of the brain. Although it is a small gland, the pituitary secretes a large number of important hormones involved in the control of many body functions. The activity of the pituitary gland is controlled by chemical "factors" that are produced by neuroendocrine cells in the brain and are passed along to the pituitary through a series of special blood vessels, the pituitary portal system. This system extends from a series of fine capillaries at the top of the pituitary stalk. These capillaries run through a region of the brain projecting from the hypothalamus (which connects the pituitary to the main part of the brain), gather into larger vessels that convey the blood down the pituitary stalk, and then redivide to form capillaries that distribute the blood to the cells of the anterior pituitary. The chemical factors released by neuroendocrine cells in the hypothalamus travel through this blood system to the pituitary and stimulate the release of pituitary hormones into the general blood circulation.

The chemical factors produced in the hypothalamus have been called releasing factors. There is evidence for six different releasing factors, one

Figure 21.7. Schematic diagram illustrating the neural and vascular interrelationships within the pituitary gland. Neuroendocrine cells in the brain secrete chemical factors that pass through the pituitary portal system to the pituitary, where they stimulate the release of pituitary hormones into the general blood circulation.



for each of the six hormones manufactured by the anterior pituitary. Two of these hormones, *follicle stimulating hormone* (FSH) and *luteinizing hormone* (LH), are involved in ovulation. The corresponding releasing factors are *follicle stimulating hormone releasing factor* (FSH-RF) and *luteinizing hormone releasing factor* (LH-RF) (McCann and Dharival, 1966).

The maturation of the follicle surrounding the oocyte requires the presence of FSH, which also stimulates the activity of follicle and nurse cells in supplying nutrients to the growing oocyte. These cells are stimulated by FSH and LH to release estrogens into the bloodstream. When the oocyte is mature, its release from the follicle is triggered by a short surge of LH from the pituitary gland. This LH surge may last less than half an hour, but it results in the rupture of the follicle some 10 to 12 hours later.

In some species, a membrane guides the released egg into the reproductive tract. In other species, the ova are released into the body cavity and are carried by ciliary currents into the funnel-shape opening of the oviduct, or *fallopian tube*, which leads into the upper part of the uterus. In mammals the ova remain in the fallopian tube for three to four days, and penetration of the sperm into the ovum (fertilization) occurs within the fallopian tube.

Fertilization is possible for only a short period after the egg is first released into the fallopian tube. Sperm released into the female reproductive tract have a relatively short life because their meager food reserves are soon exhausted by the effort of swimming the length of the uterus and through the fallopian tube toward the ovum.

Blastulation begins immediately after fertilization and is completed by the time the embryo moves from the fallopian tube into the uterus. The tissues of the uterus walls have been prepared for implantation by hormone-stimulated changes that result in thickened walls and increased blood supply. These changes begin under the stimulation of the estrogens released by the follicle cells during oogenesis. When the egg is released from the follicle, the follicle cells form a body called the *corpus luteum*, which secretes the hormone *progesterone*. This hormone stimulates a final thickening of

the uterus wall and the development of a rich network of blood capillaries within the wall tissues. By the time the blastula arrives in the uterus, this preparation has been completed, and the blastula becomes embedded in the blood-rich lining, or *endometrium*, of the uterus. The embryo soon develops its own system of blood vessels, including a group of vessels, the *placenta*, that make contact with the capillaries of the endometrium. Through the placenta, nutrients are passed from the mother to the embryo and waste products are removed from the embryo. Normally, there is no direct union of the maternal and embryonic blood supplies; they are separated by the walls of the blood vessels, so that only small molecules are able to pass between mother and embryo.

Ovulation and Sexual Behavior

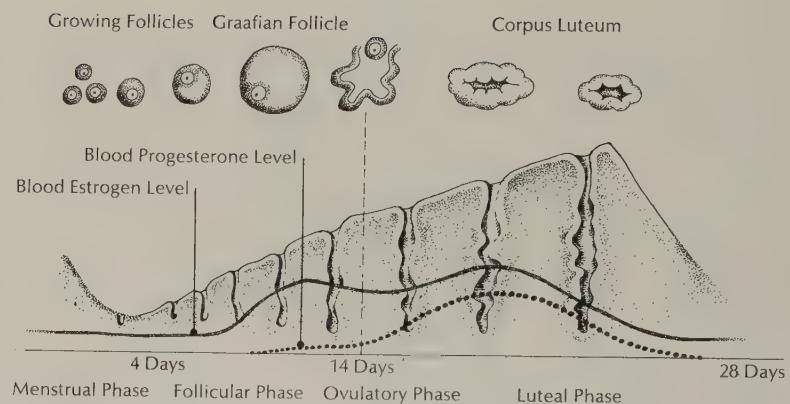
In all vertebrates, the estrogens act as a signal system to the brain cells, the pituitary, and the reproductive tract. As estrogen production increases in the maturing follicle, the estrogen concentration in the blood reaches a certain critical level and triggers the release of LH-RF in the hypothalamus. The LH-RF, in turn, stimulates production of LH in the pituitary, and LH stimulates ovulation. LH also stimulates the follicle cells to begin immediate production and release of progesterone. About two hours after the beginning of progesterone release, the female rodent stops fending off advances by the male and allows copulation to occur. The egg is not released from the follicle until several hours after the production of progesterone begins.

If the level of estrogens in the blood increases above the critical level, release of LH is inhibited and ovulation is prevented. If high levels of estrogens are maintained, release of both FSH and LH from the pituitary is inhibited, causing the ovarian follicles to remain immature and the reproductive tract to atrophy. The hypothalamus monitors the level of estrogens in the blood, sending appropriate signals to the pituitary to adjust indirectly the rate of estrogen production. There are two regions of estrogen-monitoring cells in the hypothalamus. One is involved in regulation of the pituitary gland, and the other is involved in regulation of sexual behavior (Everett, 1964; Lisk, 1967). Estrogen levels in the blood are increased above the critical level by steroid contraceptive pills, causing the monitoring cells in the hypothalamus to inhibit release of FSH and LH from the pituitary—a process that would normally lead to reduction of blood estrogen levels as maturation of follicles is prevented. The steady intake of estrogens in the pills maintains the high estrogen levels and thus prevents follicle maturation and ovulation as long as the pills are taken.

The corpus luteum is a round mass of cells larger than the follicle and is formed by division and growth of the follicle cells after ovulation. The cells of the corpus luteum respond to LH and FSH stimulation by releasing some estrogens and much progesterone. If fertilization does not occur and an embryo is not implanted in the uterus, the corpus luteum stops producing hormones after a time period characteristic of the species. The estrous cycle can thus be divided into a phase of follicle maturation (follicular phase) followed by a phase of corpus luteum activity (luteal phase).

When the corpus luteum ceases to secrete hormones, the tissues of the endometrium break down and blood is released from the rupturing capillaries. In humans and some other primates, the mass of tissue breakdown causes some of the materials shed from the uterus lining to be released from

Figure 21.8. Diagram showing progressive changes in the endometrial lining of the ovaries, the uterus, and the circulating ovarian hormones.



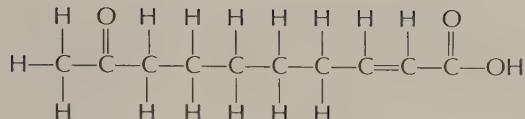
the external opening of the vagina. This process is called *menstruation*, and the estrous cycle in menstruating animals is often called the menstrual cycle.

After the corpus luteum ceases to produce hormones, the estrogen level of the blood normally decreases, causing the hypothalamus to trigger FSH release from the pituitary gland. FSH stimulates the maturation of new follicles, which release estrogens that act upon the uterus lining to halt menstruation and upon the hypothalamus to inhibit further FSH release. With most birth control pills, the intake of estrogens is halted briefly during each cycle, allowing the estrogen level to drop sufficiently to trigger menstruation, but intake of estrogens is resumed before the estrogen level has fallen sufficiently to cause release of FSH from the pituitary. Thus, the enlarged tissues of the uterus lining—whose growth is stimulated by the high estrogen levels—are removed by the normal process of menstruation, but follicle maturation and ovulation are prevented.

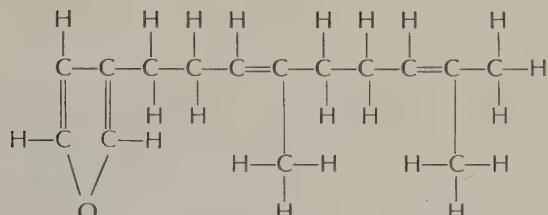
Intraspecies Regulatory Mechanisms

Not all species ovulate spontaneously in a regular cycle. Rabbits and members of the cat family produce a set of mature follicles that ovulate only after intense sexual excitement, usually resulting from copulation. In these species, the female becomes sexually receptive, or comes into heat, when follicles are mature. She remains in heat until mating occurs or until the mature ovum breaks down after a few days. If no mating has occurred to cause ovulation, the cat or rabbit comes back into heat after one or two days when another set of follicles has matured. The female thus continues to come into heat throughout the breeding season until mating occurs. In these species, the act of copulation provides the signal for a surge of LH from the pituitary, and ovulation follows some 10 to 12 hours later. These species are *reflex ovulators*, in contrast to the more common *spontaneous ovulators*. The dividing line between spontaneous ovulators and reflex ovulators is not a sharp one, and many outside stimuli can trigger the brain.

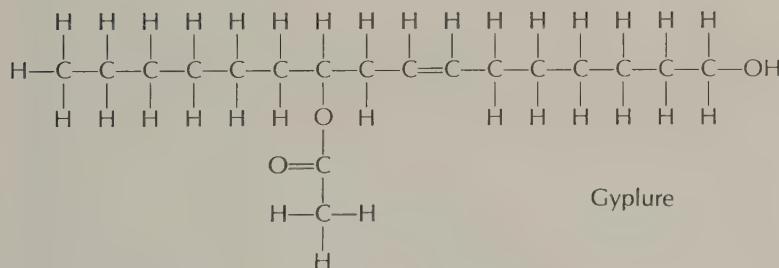
When female mice are kept in cages isolated from males, the estrous cycle lengthens from four days to six or seven days. If female mice are kept in large groups—for example, about 30 animals per cage—estrous cycling ceases. If males are introduced into such a cage, a significant number of females mate on the third night after introduction of the males. It is not necessary for the males and females to have contact to cause this readiness in the females but only for odor from the males to reach the females. Appar-



Honeybee Queen Pheromone



Dendrolasin



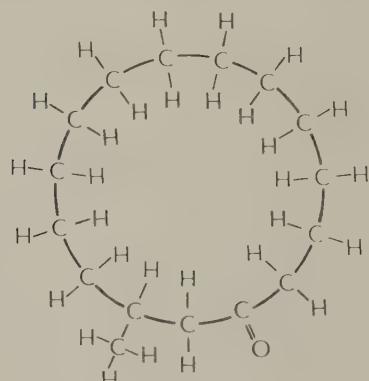
Gyplure

ently, some active substance in the odor from the males causes synchronous reinitiation of the estrous cycles in the females. These substances are present in the urine of normal males but not in urine from castrated males.

Chemical substances that regulate sexual activity among members of the same species have been termed *pheromones*. Another example of pheromone action has been observed in mice. If a recently mated female mouse is exposed to a male of a different strain, chances are extremely high that she will not become pregnant but will undergo a new ovulatory cycle and return to heat in three to four days. If the "strange" male remains with her and she mates on returning to heat, she will successfully bear the litter, unless she is again immediately exposed to a different male after mating. This effect can be produced merely by placing the female in a cage containing bedding soiled by the strange male, showing that the signal that results in the so-called pregnancy block is an odor cue (Bruce, 1966).

Such experiments make it clear that ovulation and sexual behavior are highly regulated phenomena whose timing is controlled not only by internal hormonal signals but also by chemical signals between members of the species. Many of the regulatory mechanisms function to increase the likelihood that fertilization will occur just before or after ovulation. In most species, the onset of breeding-season activity in males is triggered by the same environmental signals that act in females (Davidson, 1966). Maturation of

Figure 21.9. Structural formulae of some representative pheromones, or sex attractants. At left are formulae for the following: the pheromone secreted by the honeybee (above); dendrolasin—the pheromone produced by the *Lasius fuliginosus* ant (middle); and gyplure—the pheromone for the gypsy moth. At right is the formula for muskone, the pheromone produced by the musk deer. Most pheromones have a high molecular weight, which accounts for their high potency and narrow specificity.



Muskone

Figure 21.10. The pituitary gland and gonads of a newborn animal can be transplanted into a mature host, where they will rapidly complete development and become capable of normal sexual activity. Here, the testis of an 11-day-old mouse embryo is shown after being grafted into the scrotal testis of an adult host for 30 days. The seminiferous tubules of the graft are approximately the same size as those of the host testis. Interstitial tissue is well developed, and many of the tubules contain spermatids. Mature spermatozoa appear in such grafts after about 35 days.

sperm depends upon adequate stimulation of the testes by LH, which also stimulates production of androgens by the interstitial cells. Some FSH may be necessary in the male to complete the maturation of viable sperm.

Mating activity in the male requires an appropriate level of androgens, particularly testosterone, in the circulation. Excessive androgen levels can inhibit LH release from the pituitary, which will result in decreased testosterone production and atrophy of the reproductive tract. As in females, monitoring regions in the hypothalamus control both pituitary activity and sexual behavior, responding to changes in the level of steroid sex hormones in the blood.

PUBERTY

Sexual maturation, often called puberty, is reached at an age that is characteristic for each species. At puberty, the gonads become capable of producing eggs or sperm, and various secondary sexual characteristics appear or are more strongly developed. The immediate cause of these changes is an increased level of androgens or estrogens in the blood. The release of these hormones from the gonadal cells is triggered by release of increased amounts of FSH and LH from the pituitary.

The pituitary gland and gonads of a newborn animal can be transplanted into a mature host, where they will rapidly complete development and become capable of normal sexual activity. Because the maturation of the gonads can occur rapidly in the proper hormonal environment, it appears that this maturation is normally delayed by a lack of releasing factors that would trigger the production of LH and FSH in the pituitary. The hypothalamus-monitoring cells of the immature animal are extremely sensitive to low levels of estrogens or androgens in the blood and inhibit the release of FSH-RF and LH-RF long before the hormone levels in the blood can rise sufficiently to cause gonadal maturation (Critchlow and Bar-Sela, 1967). At puberty, the monitoring cells cease to be inhibited by the low hormone levels, releasing factors are sent to the pituitary, FSH and LH are released by the pituitary, maturation of the gonads and development of secondary sexual characteristics are triggered, and the increased level of estrogen or androgen production from the mature gonads eventually raises the level of these hormones in the blood to the adult critical level for the monitoring cells. The cause of this change in the monitoring cells is not yet known.

Ovaries implanted in male rats that were castrated on the day of birth and then allowed to grow to adult size show periodic ovulations. However, if male rats retain testes until the fifth day after birth, similarly implanted ovaries fail to ovulate. This experiment indicates that the neural mechanism necessary for ovulation can develop in the absence of any hormonal stimuli from the gonads after birth, that a functional ovulatory mechanism can be developed in any animal regardless of its sexual genotype, and that this mechanism normally fails to function in the male as a result of some factor produced by the testes after birth.

Further confirmation comes from experiments with substances called antiandrogens, which prevent androgen from acting upon its target tissues. If antiandrogen is injected into a pregnant female rat throughout pregnancy and treatment to the young is continued for a few weeks after birth, all members of the resulting litter have the external appearance of females (Neuman and Elger, 1965). If an ovary is implanted, ovulation and female

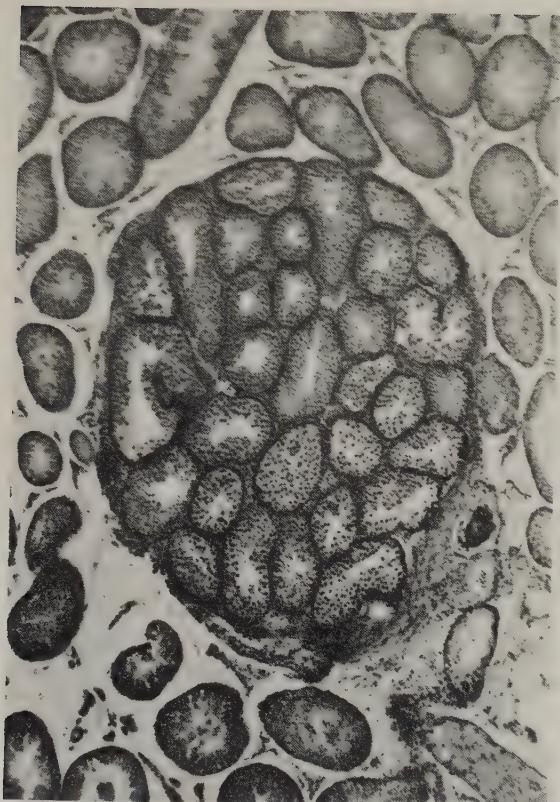


Figure 21.11. The structural formula of cAMP.

sexual responses are observed in the genetic males. It seems clear that the condition of maleness is determined by the presence of androgen at certain sensitive periods of early development.

Radioactive hormones can be injected into an animal, and the radioactivity of various tissues from the animal can be measured a few hours later. The radioactivity of the various target tissues—such as uterus, fallopian tube, pituitary gland, and hypothalamus—is many times the level found in the blood or in nontarget tissues. Apparently, the target tissues are able to capture and hold the hormone molecules, presumably as a result of special hormone receptor molecules in the target cells. Adult females that are sterile because of an injection of androgen during early development lack normal responses to injections of female sex hormones. When these animals are tested for hormone receptors by injection of radioactive hormone, little or no retention of the hormone is found in the target tissues. Early treatment with androgen apparently has destroyed the animal's ability to produce receptor molecules for the female sex hormones. This inability may be a major factor in the normal development of maleness.

THE BIOCHEMICAL ACTIVITY OF HORMONES

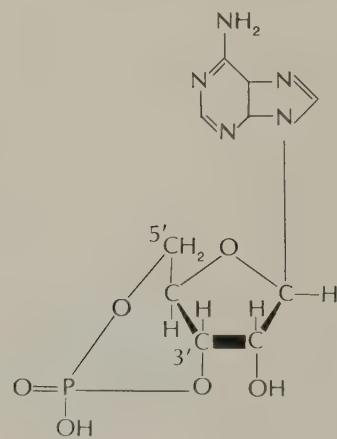
In man and other vertebrates, many different types of molecules have hormonal activity, and new hormonally active substances continually are being discovered. In addition to the traditional hormones—compounds produced by the endocrine glands—there are other regulatory substances (some not yet chemically identified) that influence the behavior of blood-forming organs, the immune system, and other cellular systems usually regarded as insensitive to hormonal control. Despite this overwhelming diversity, certain generalizations can be made about the chemical basis of hormonal action.

Scientific and medical researchers first became interested in hormones because of certain human diseases associated with malfunctions of the endocrine glands—diabetes, hyperthyroidism, and the often rather spectacular conditions associated with steroid hormone abnormality. It has become clear that hormones play an important part in the functions of virtually every living system, including microorganisms. This universality is fortunate for the biochemist because it offers a possibility for examining the biochemical operation of hormones in simpler organisms, where genetic and environmental factors may be more readily controlled.

Prothormones

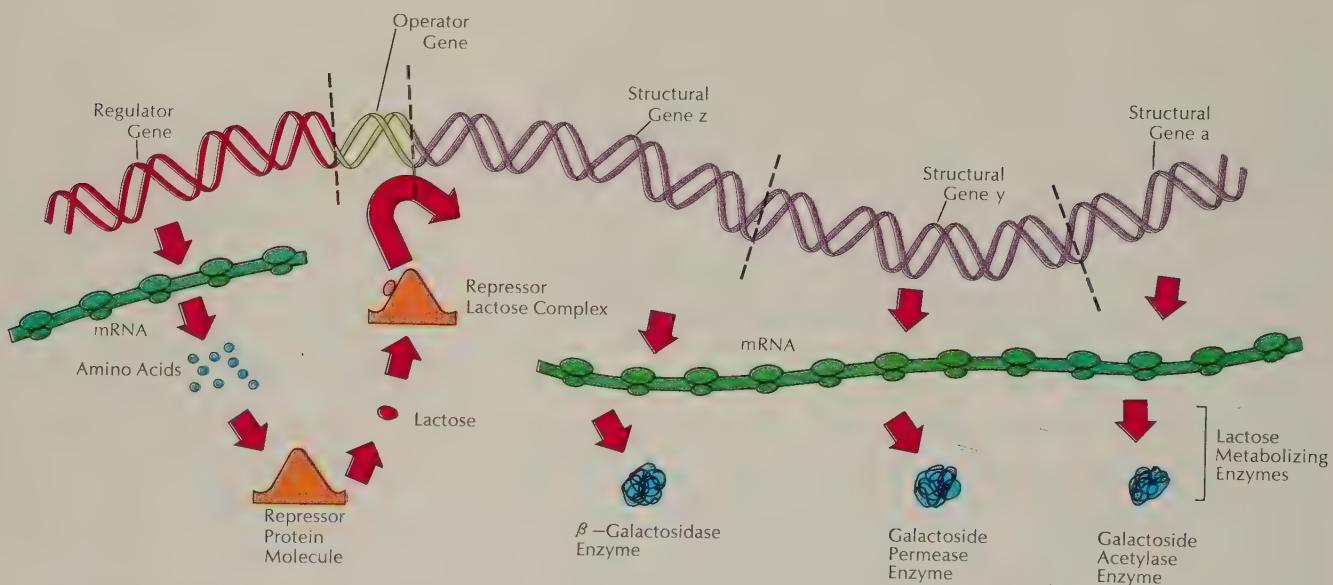
The probable evolutionary precursors of hormones have recently been discovered in single, free-living microorganisms. In these small organisms, most metabolic regulation is accomplished by the metabolites themselves. As the concentration of a particular precursor or end product increases, that substance acts either to inhibit the action or synthesis of enzymes involved in its own formation or to induce the synthesis of enzymes that metabolize it. Such compounds have both metabolic and regulatory functions. They may be sugars that are oxidized for energy, amino acids that are incorporated into proteins, and so on. In microorganisms, biochemists have found a few small molecules that have only regulatory functions and that do not play a role in metabolic pathways (Figure 21.11). One of these prothormones is adenosine 3'5' monophosphate, or cyclic AMP (cAMP).

The presence of cAMP in animal cells was detected some time ago, but



Cyclic Adenosine 3'5' Monophosphate (cAMP)

Figure 21.12. The *lac* operon mechanism of *E. coli* represented schematically, with lactose present in the system.



it has been found only recently in bacterial cells. Cyclic AMP is synthesized from ATP in a reaction catalyzed by the enzyme adenyl cyclase, which is found inside bacterial cells. The cyclic nucleotide is degraded in a reaction catalyzed by a specific phosphodiesterase enzyme. Thus, the concentration of cAMP in a bacterial cell depends upon (1) its rate of synthesis, (2) its rate of degradation, and (3) its rate of escape into the medium.

The *lac* operon of *E. coli* provides a good example of the regulatory function of cAMP in the bacterial cell. When lactose is present in the cell, it interacts with the repressor and removes the repressor from the operator site of the operon. With the repressor gone, RNA polymerase can begin transcribing mRNA from the structural genes that code for three enzymes involved in lactose metabolism. Under the influence of these enzymes, lactose is hydrolyzed to a mixture of glucose and galactose. The inducing action of lactose has been understood for some years. It also has been known for some time that *lac* enzymes will not be produced—even in the presence of lactose—if there is sufficient glucose in the medium to sustain bacterial growth. This effect of glucose is called catabolite repression. It ensures that the bacterium will not waste energy in degrading lactose so long as sufficient glucose is available for the taking.

The molecular basis of catabolite repression, elucidated only very recently, is relevant to an understanding of hormonal action in higher organisms. When bacteria run out of glucose, the concentration of cAMP within cells increases. In the presence of cAMP, RNA polymerase forms a complex with a specific protein called CR, or CAP. In this complex form, the RNA

Figure 21.13 (above). Schematic diagram illustrating the role of cAMP as a general mediator of carbohydrate metabolism in bacteria.

Figure 21.14 (below). The structural formula of epinephrine.

polymerase attaches more readily to the promoter site of the *lac* operon (Figure 21.13). Thus, cAMP acts as a positive regulator of the *lac* operon, just as the repressor is a negative regulator. Many bacterial operons are controlled by catabolite repression, and cAMP acts as a positive regulator in a similar fashion for each operon. Therefore, cAMP is a general mediator of carbohydrate metabolism in bacteria.

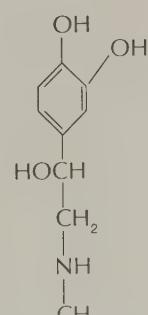
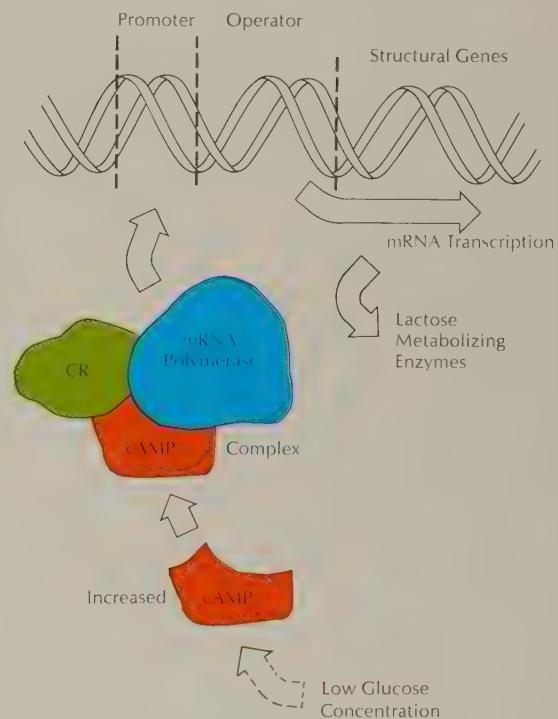
In the case of the *lac* operon, cAMP promotes transcription of mRNA from certain genes. It is also known to stimulate production of the enzyme tryptophanase, which catalyzes the hydrolysis of tryptophan to indole and serine—a step in a pathway that also leads to formation of glucose. Here also, an increase in cAMP concentration leads to an increase in glucose production. Preliminary evidence, however, suggests that cAMP can augment tryptophanase production even when RNA synthesis is prevented. Therefore, cAMP also must stimulate specific protein synthesis at steps in the process other than mRNA transcription. The mechanism of this post-transcriptional effect of cAMP is not yet understood, but it illustrates the fact that increased cAMP concentration mobilizes many different types of cellular responses to meet a deficiency of glucose.

MECHANISMS OF VERTEBRATE HORMONE ACTION

In the cells of vertebrates, adenyl cyclase is found as a component of the plasma membrane rather than free in the cell interior as in bacteria. In this location, cAMP production can be stimulated by various external influences. In fact, cAMP was first detected during studies of the mechanism by which the hormone epinephrine promotes glycogen breakdown in the liver. Earl Sutherland found that epinephrine acts by enhancing cAMP production at the plasma membrane of the liver cells. The cAMP functions within the cell as a “second messenger,” triggering the actual metabolic changes associated with the presence of epinephrine at the cell surface. In the liver, as in bacteria, cAMP promotes the production of glucose from a precursor.

In bacteria, cAMP acts within an individual cell, promoting glucose production when the glucose concentration decreases. In cells of vertebrates, however, cAMP functions as the mediator within the cell for other chemical signals arriving at the cell surface. As research has continued, it has become apparent that an astonishing number of vertebrate hormones function simply by stimulating cAMP production in particular target cells. The specificity of response to hormones is therefore determined by the presence of specific hormone receptors at the cell surface and by specific mechanisms within the cell that react to increased cAMP concentration.

For example, the actions of the hormones epinephrine and ACTH (pituitary adrenocorticotropic hormone) seem very different (Figure 21.14). Epinephrine is produced by the adrenal medulla and stimulates glycogen breakdown in liver cells. ACTH is produced by the pituitary gland and stimulates steroid production in cells of the adrenal cortex. Yet each hormone stimulates adenyl cyclase activity at the surface of its target cells, thus increasing the concentration of cAMP within the target cells. The actions of the two hormones differ because the liver cells have receptors that respond to epinephrine, whereas the adrenal cortex cells have receptors that respond to ACTH. They also differ because the mechanisms within liver cells respond to increased cAMP concentration by more rapid glucose breakdown, whereas the mechanisms within adrenal cortex cells respond to



Epinephrine

Figure 21.15. The structural formula of cGMP.

increased cAMP concentration by more rapid steroid production. The chemical mechanisms by which hormones act on the cell surface to stimulate cAMP synthesis are not known.

The mechanism by which cAMP acts within the cell is better understood, at least in some cases. In the liver cell, cAMP stimulates the phosphorolysis of glycogen to glucose 1-phosphate. Detailed studies of this process have revealed the general mechanism by which cAMP modifies all such reactions. Cyclic AMP activates a protein kinase. When activated by phosphorylation, the second kinase catalyzes the phosphorylation of the enzyme phosphorylase, which thus becomes activated as the catalyst for glycogen breakdown.

Subsequent research has shown that cAMP is active in virtually every type of mammalian cell, affecting many different types of biochemical reactions. In many cases, cAMP acts to modify enzyme activity, as in the liver cells. In other cases, cAMP stimulates synthesis of specific proteins. In some of the latter cases, inhibitors of RNA synthesis block the stimulating effect of cAMP, indicating that cAMP affects mRNA transcription. In other cases, the stimulating effect of cAMP is unaffected by inhibitors of RNA synthesis, indicating that the cAMP affects posttranscriptional processes.

It is still too early to say whether this unitary hypothesis of cAMP action will be substantiated. Nevertheless, it is an appealing hypothesis to explain the multiple biochemical effects of the cyclic nucleotide.

OTHER POSSIBLE MEDIATORS

The evidence at present suggests strongly that cAMP is not the only mechanism through which hormones act. Steroids, thyroxin, growth hormones, and insulin apparently operate independently of the cAMP concentrations within target cells. It is possible that there are different "second messengers" for these other hormones. In this case, these hormones would react with specific membrane receptors that promote the synthesis of the other intermediate messengers within the target cells. This hypothesis has been rendered more plausible by the discovery of another cyclic purine nucleo-

Guanosine 3'5' Cyclic Phosphate (cGMP)

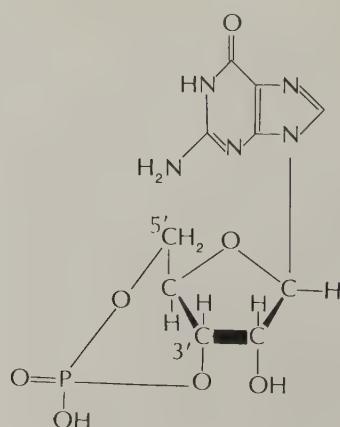


Figure 21.16. The structural formula of thyroxin.

tide, guanosine 3'5' cyclic phosphate, or cGMP (Figure 21.15). Much less is known about cGMP than about cAMP, but its enzymatic synthesis has been studied. The cGMP concentration within cells is affected by manipulations of hormone concentrations in living animals in a way that strongly suggests a role for cGMP as another mediator of hormone action.

Any other type of molecule that responds to membrane-bound hormones could act in the same way. Recent experimental results suggest that there may be specific membrane receptors for hormones that do not stimulate adenyl cyclase activity. For example, insulin—a relatively large polypeptide—remains hormonally active even when it is bound to an inert polysaccharide carrier so large that the insulin molecule is prevented from entering its target cell (in this case, a fat cell). As with other membrane-active hormones, researchers have investigated the possibility that cAMP is involved in the actions of insulin, but that possibility has been excluded in at least one case. In a line of rat hepatoma cells growing in tissue culture, insulin stimulates synthesis of an internal enzyme. Furthermore, neither cAMP nor its more penetrable dibutyryl derivative affects the rate of enzyme synthesis in these cells. Because insulin stimulates this enzyme even when prevented from penetrating the cells, either the membrane itself regulates the synthesis of the enzyme or, more probably, a molecule other than cAMP acts as an internal mediator for the action of insulin.

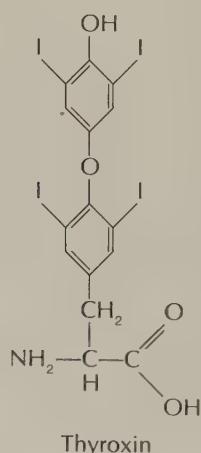
Much less is known about the actions of thyroxin and growth hormone. Thyroxin, a small molecule derived from the amino acid tyrosine, has profound effects on metabolism in a number of different vertebrate systems. For example, it is responsible in some way for the metamorphosis process that changes a tadpole into a frog. Thyroxin ultimately influences the synthesis and degradation of many types of macromolecules, but very little is yet known of the mechanisms under direct control of thyroxin.

STEROID HORMONES

Perhaps because of their importance in clinical medicine, the steroid hormones have aroused a great deal of scientific interest. Steroid hormones recently have been shown to have hormonal activity in some organisms simpler than the vertebrates. Apparently, the use of steroid hormones evolved before vertebrate organisms made their appearance, but steroid hormones appear to be a more recent evolutionary development than cAMP. Biosynthesis of steroid compounds has been observed only in eucaryotic cells, whereas cAMP plays an important regulatory role even in prokaryotes.

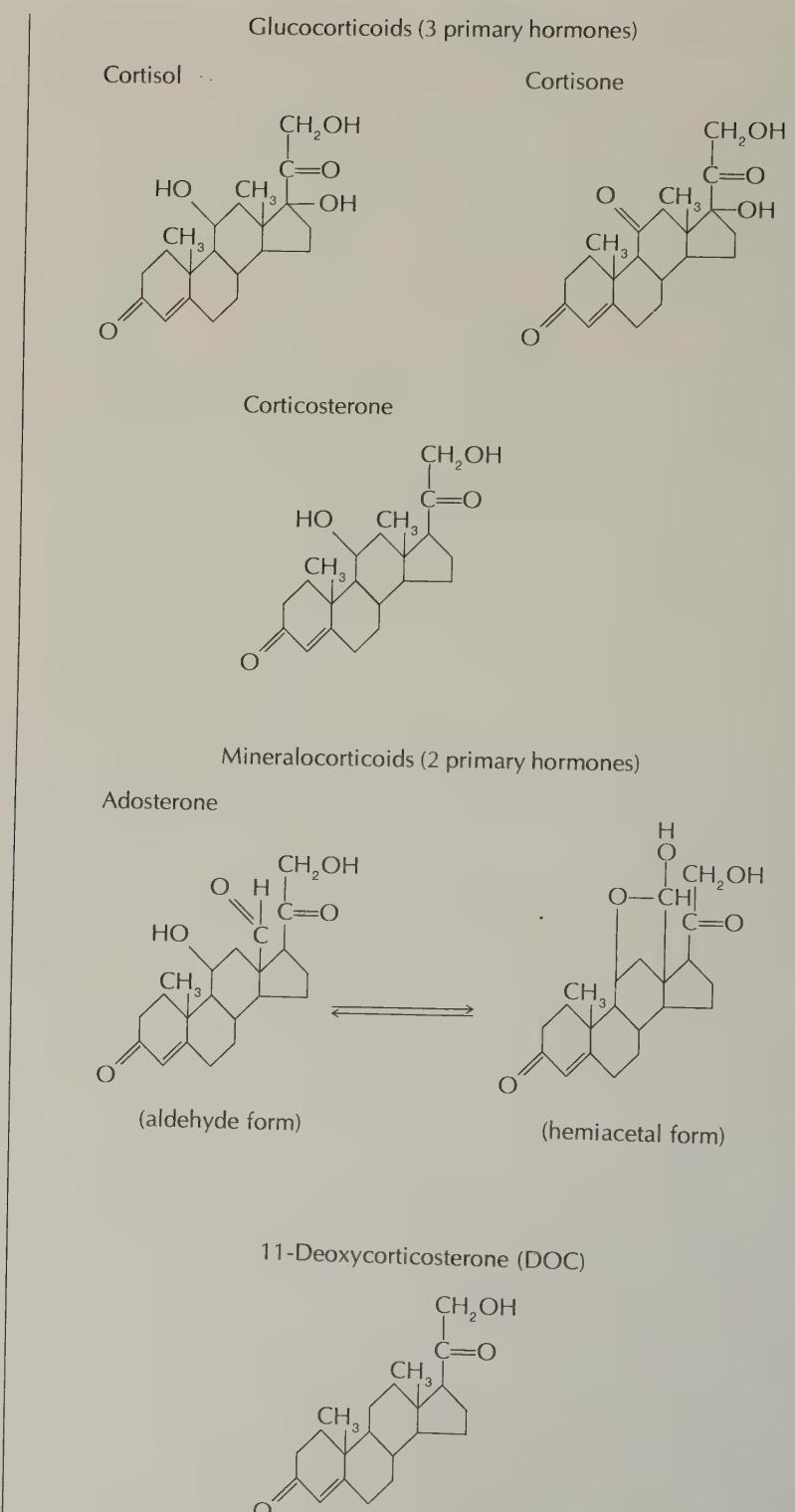
The principal steroid hormones of mammals are the sex hormones (androgens, estrogens, and progesterone) and two types of adrenal hormones (glucocorticoids and mineralocorticoids). A metabolite of the sterol vitamin D has biological activity similar to that of the steroid hormones.

The steroid hormones are generally discussed in terms of their effects on specific target tissues. For example, the reproductive tract is an obvious target tissue for the action of the sex steroids. It is becoming clear that many other tissues—for example, the liver, kidneys, and the nervous system—may also be influenced by sex hormones. Adrenal glucocorticoids usually are regarded as regulators of carbohydrate and protein metabolism in various target tissues. However, it has long been known that glucocorticoids also form the negative part of a feedback control loop by suppressing ACTH release from the pituitary. Glucocorticoids also induce lysis of lymphoid



Thyroxin

Figure 21.17. Structural formulae of adrenal hormones.



cells, inhibit growth of fibroblasts and of regenerating liver cells, promote development of the pancreas and certain parts of the nervous system during embryonic life, stimulate growth-hormone production by the pituitary, enhance release of free fatty acid from fat tissues, and regulate many other cellular responses as well. Aldosterone—the best studied of the mineralocorticoids—is usually regarded as a regulator of water and ion metabolism in the kidney. It influences sodium transport activity in the toad bladder and probably in other organs as well. In short, the concept that each steroid hormone affects only one or a very few target organs is difficult to defend.

A particular steroid does seem to act primarily as a direct regulator of the synthesis of a few specific macromolecules in cells that are sensitive to the hormone. The precise mechanisms of steroid regulation are unknown, largely because the mechanisms by which protein synthesis is controlled in eucaryotic cells are not yet understood. It is not yet known whether control is normally most prominent at the stage of specific gene transcription, mRNA transport and degradation, or mRNA translation. Until the normal internal control mechanisms of the mammalian cell are better understood, it will be difficult to evaluate the effects of steroids on those mechanisms.

One step in the action of steroids on cells does seem to be common to all systems thus far studied. The first step is the formation by noncovalent bonds of a complex between the hormone and a specific protein receptor molecule, probably in the cytoplasm of the cell (King, 1970). This complex apparently migrates into the nucleus of the cell. The receptors in every case have a number of similar properties, although they respond specifically to different steroid hormones. All receptors yet studied are proteins of about the same size; all form aggregates when studied at low ionic strength in cell-free systems; and all require the presence of free SH groups for the formation of a steroid-receptor complex. Much of the current research on these hormones is directed toward a search for a common underlying mechanism of action for all steroid hormones.

The outstanding question in present steroid research is the exact step at which the steroid-receptor complex affects the process of protein synthesis. Studies of posttranscriptional enzyme induction by steroid hormones in tissue cultures have led to the development of a model of the control mechanism that seems to account for more observations than does a modified

Figure 21.18. Schematic diagram of a theoretical model of hormone-gene interaction. Sex hormones (H) enter the cell and become bound to a receptor molecule (P). The bound hormone can then enter the nucleus and activate specific genes to produce proteins. These proteins in turn bring about the cellular changes triggered by the hormone.

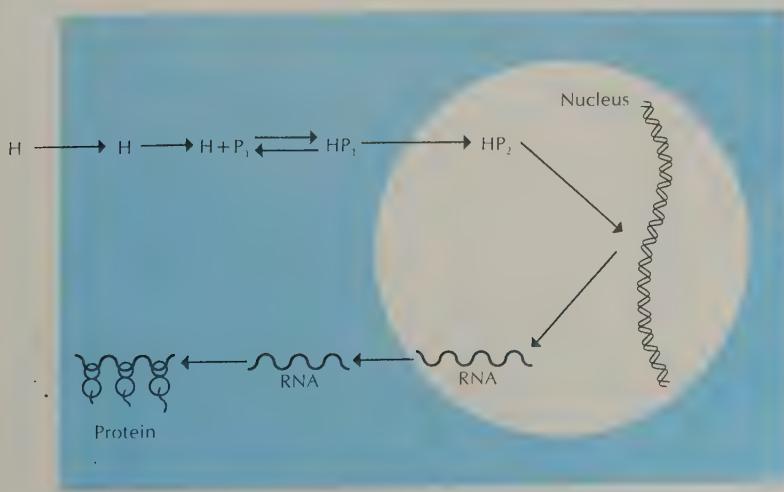
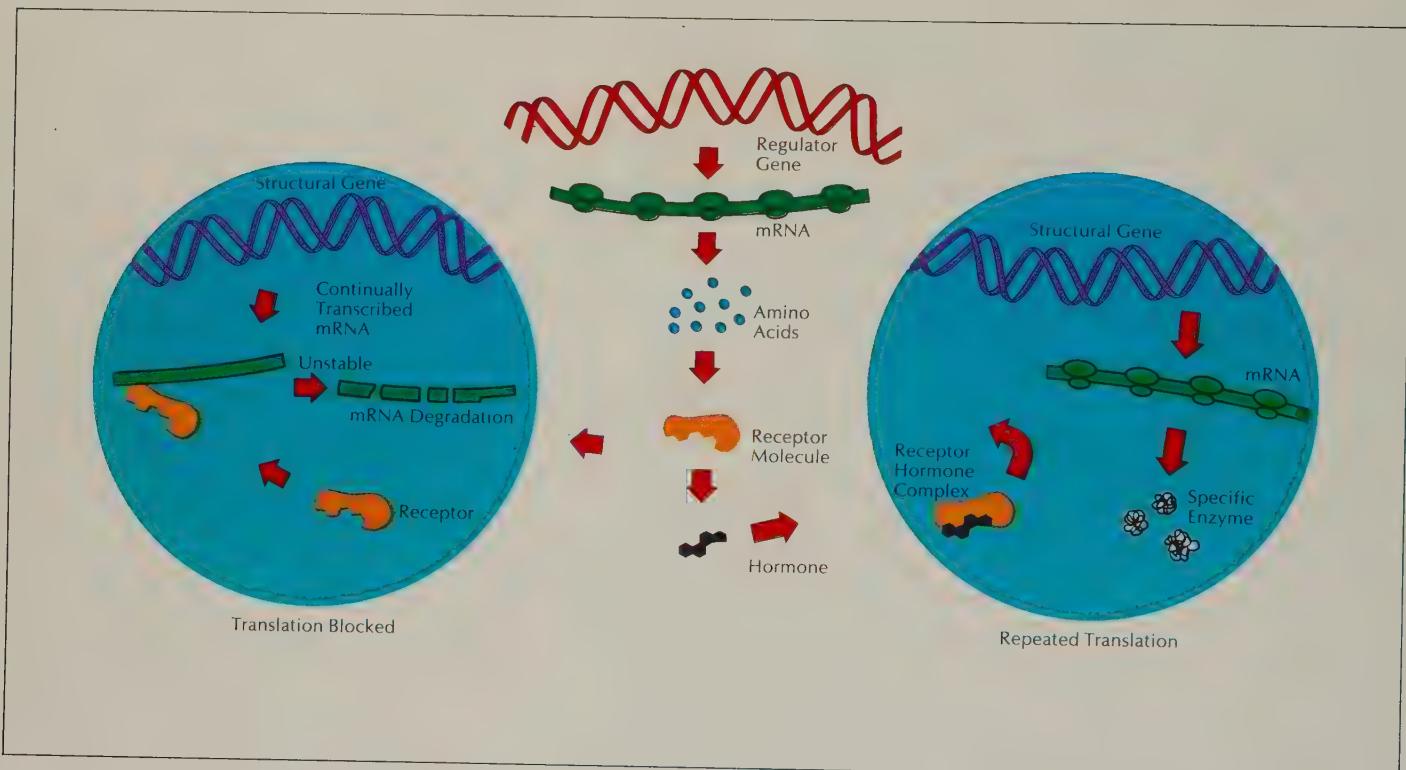


Figure 21.19. Posttranscriptional model. In this model, the hormone receptor alone acts as a repressor for specific mRNA. When the receptor binds to mRNA, it prevents translation of proteins from the mRNA and also makes the mRNA more unstable and subject to more rapid degeneration. Formation of the hormone-receptor complex removes the receptor from mRNA and permits repeated translation of induced protein molecules from the now stable mRNA.



Jacob-Monod model. The basic features of this mechanism are shown in Figure 21.19.

The posttranscriptional model has been proposed in an attempt to account for certain basic facts about the behavior of tissue cultures in response to steroid hormones. The effect of a hormone is to increase the activity of a specific enzyme through an increase in the rate at which that protein is synthesized. However, the hormone does not alter the total levels of protein and RNA synthesis of new RNA, although evidence on this point in various systems is not yet clear. If protein synthesis is inhibited by certain substances, there is no increase in enzyme activity upon addition of hormone but there is an accumulation of RNA (presumably mRNA specific to the induced enzyme), and synthesis of the protein is induced as soon as the inhibiting substance is removed, even if the hormone is no longer present. The hormone must be constantly present in order to maintain the fully induced rate of protein synthesis under normal conditions. However, if specific protein synthesis is first induced by addition of a hormone and then RNA synthesis is inhibited, formation of the induced protein continues at a high rate for some time, even if the hormone is removed.

The posttranscriptional model postulates that the hormone receptor alone acts as a repressor for specific mRNA. When the receptor binds to mRNA, it both prevents translation of proteins from the mRNA and makes the mRNA more unstable and subject to more rapid degradation. Formation of the hormone-receptor complex removes the receptor from mRNA and permits repeated translation of induced protein molecules from the now stable mRNA.

Further research will almost certainly reveal facts that will further modi-

fy this model or even precipitate development of entirely new models of hormonal control mechanisms. However, there is good reason to hope that relatively simple control mechanisms will be found responsible for most hormonal actions in eucaryotic cells and that these mechanisms will reveal important features of the normal control mechanisms of eucaryotic cells.

FURTHER READING

General information about animal hormones will be found in books by Barrington (1963), Gorbman and Bern (1959, 1962), Pincus and Thimann (1948–1964), Scharrer and Scharrer (1963), and Turner (1966). More detailed information about sex hormones is given by Van Tienhoven (1968) and W. C. Young (1961). For more information about the cellular slime molds, see the book by J. T. Bonner (1965).

Useful introductory articles on animal hormones include those by Constantinides and Carey (1949), Csapo (1958), Davidson (1965), Fieser (1955), Gray (1950), Jones (1968), Levey (1964), Levine (1966), Rasmussen (1961), Tanner (1968), Williams (1950), Wilson (1963), and Wurtman and Axelrod (1965).

22
Immune Responses



The first great book on immunology, by the Belgian Jules Bordet (1898), opens with the sentence "Life is the maintenance of an equilibrium that is perpetually threatened." The exquisitely balanced system of hormonal and neural regulations involved in the reproductive cycle (Chapter 21) is only one example of the normal mechanisms of self-correcting maintenance and control found in a multicellular animal. The immune responses of vertebrates function primarily to see that no foreign substances or cells disrupt these delicate systems.

In a sense, immunity—as it exists in man and other warm-blooded vertebrates—is a communications system. Foreign material most frequently enters the body as a result of the invasion and multiplication of microorganisms. Other foreign material may enter when a surgeon implants skin or an organ from another individual or when certain types of cancer arise—either spontaneously or in some region of chronic irritation. The first step in the chain of communication is recognition that the invading material is foreign—is not-self rather than self. The second step involves multiplication and activation of the cells appropriate to combat the particular alien material present. Finally, these cells or their specialized chemical products must find and destroy the foreign cell, microorganism, or protein. This three-stage process represents the simplest outline of a *primary immune response* to any sort of foreign material.

Immunity, however, is usually thought of in terms of the resistance to further infections that follows an attack of infectious disease. For centuries it has been known that a man with a face marked by smallpox never contracts the disease again. Allowing for an immense variety of details, immunity to smallpox is a prototype of all *secondary immune responses*. Once foreign material has been successfully dealt with, there is an enlarged population of cells able to deal with that particular foreign substance and able to be called into action more rapidly and more effectively.

THE INFLAMMATORY RESPONSE

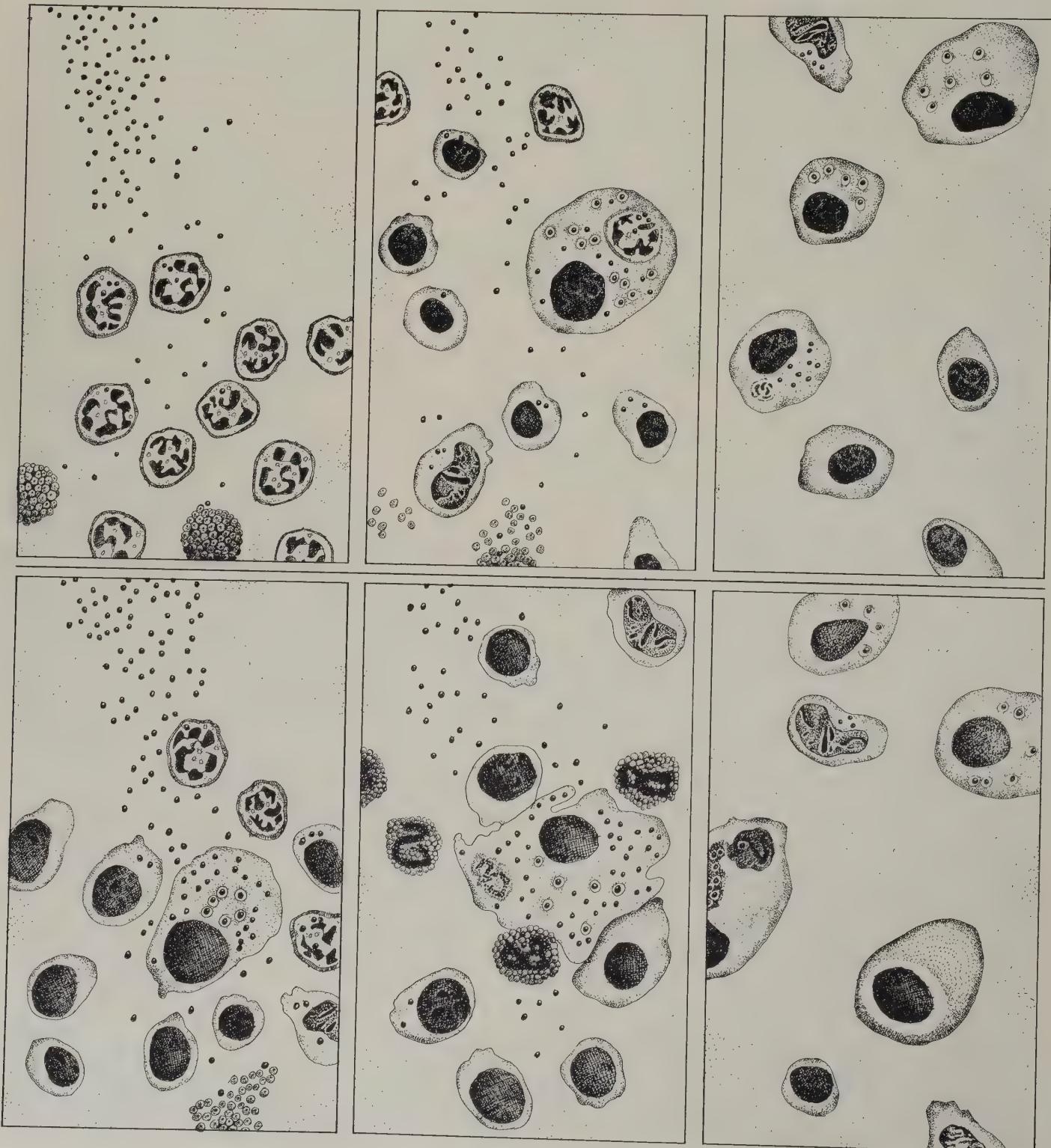
The presence of a foreign substance in the body triggers a dramatic sequence of events known as the *inflammatory response*. The major participants in this response are the white blood cells, or *leucocytes*, which are amoeboid cells capable of moving through intercellular fluids and tissues of the body as well as the bloodstream. Lymphocytes, granulocytes—neutrophils, eosinophils, and basophils—and monocytes are all types of leucocytes. Most of the leucocytes in the body under normal conditions are lymphocytes and *neutrophilic granulocytes (neutrophils)*. Leucocytes are manufactured in bone marrow, the lymphatic system, and the thymus gland. In general, leucocytes combat foreign materials by engulfing and digesting them, but the details of the inflammatory response are relatively complex.

The process can be analyzed by examining what happens when the tissues of a mouse, for example, are injected with a foreign protein. The injection first causes the breakdown of certain cells in the tissues. These *mast cells* have large granules in their cytoplasm, and the granules appear to be lysosomes filled with hydrolyzing enzymes. The release of enzymes from disintegrating mast cells causes the destruction of other cells in the vicinity, beginning the development of an inflammation (Figure 22.1). Soon a number of neutrophils begin to arrive at the site of inflammation. The neutrophils engulf some of the foreign particles by phagocytosis or endocytosis

Figure 22.1 (above). Primary immune response of inflammatory cells to antigen injected into a mouse (not previously exposed to this antigen). First (upper left), large-granule mast cells in the body tissues come in contact with the antigen and break down. This cellular disintegration releases large numbers of lysosomes, whose enzymes and other substances destroy other cells in the vicinity, initiating inflammation. The first moving defensive cells to arrive at the site are neutrophils, which swallow up some of the foreign particles but soon disintegrate themselves. Next (upper

middle), lymphocytes and monocytes reach the area and feed upon the foreign particles and cellular debris. This process causes some of the lymphocytes to enlarge and become macrophages (upper right). Eventually, these cells ingest all the foreign matter and the inflammation subsides. Most of the antigen is broken down into amino acids and sugars by enzymes, but some is maintained in the macrophages by combining with RNA. (After Scientific American, 1964)

Figure 22.2 (below). Secondary immune response of inflammatory cells to antigen injected into a mouse



(previously exposed to this antigen). Neutrophils arrive at the site but in fewer numbers, while macrophages arrive in larger numbers (lower left). Some of the macrophages contain antigen in combination with RNA, and these cells interact with eosinophils, which cause them to be broken open. More macrophages then move in and engulf pieces of broken cells (lower middle). Some antigen escapes destruction in combination with RNA in the macrophages (lower right). The rapid arrival of macrophages and the ease with which they ingest

and internally digest the particles. Within a few hours, however, the neutrophils themselves begin to disintegrate, adding their contents to the growing amount of fluid in the inflamed area.

Among the substances released by the disintegrating mast cells are histamine, serotonin, and heparin. The histamine and serotonin act as local hormones, causing dilation of arterial vessels and constriction of venous vessels near the inflamed site and thereby increasing the supply of blood to the invaded tissues. Heparin prevents clotting, or coagulation, of the blood in the area. The region where the foreign protein was injected becomes swollen, flushed, and suffused with the various products of cell breakdown and secretion that are collectively called pus.

After the neutrophils have begun to break down, lymphocytes and monocytes begin to arrive in the area of inflammation. In their normal condition, these cells have large nuclei surrounded by relatively small amounts of cytoplasm. As they feed upon the foreign particles and cellular debris in the vicinity, many of the monocytes become enlarged to the form called macrophages. Some macrophages multiply by cell division, greatly increasing the population of leucocytes in the inflamed area. Eventually, all the foreign material and cellular debris is ingested by macrophages, and the inflammation subsides.

If the mouse is later injected with the same foreign protein, the inflammatory response follows a somewhat different pattern. Again, neutrophils arrive at the invasion site first, but in smaller numbers than responded to the primary infection (Figure 22.2). On the other hand, macrophages arrive sooner and in much larger numbers, and eosinophils also arrive in greater numbers. Some of the macrophages become immobile, swell as they form large fluid vesicles in the cytoplasm, and attract to themselves large numbers of eosinophils, which penetrate and disintegrate the swollen macrophages. The eosinophils and fragments of macrophages are then ingested by other macrophages, which continue to arrive at the site. Other cells that appear in the inflamed area include a large number of plasma cells, which contain large amounts of rough endoplasmic reticulum and which secrete proteins rather than ingest materials.

Because of the rapid arrival of macrophages and their greater effectiveness in ingesting foreign particles, the secondary inflammation is less

foreign particles result in a shorter period of secondary inflammation and one that is less severe. (After Scientific American, 1964)

Figure 22.3 (right). Ehrlich's "lock and key" hypothesis explaining the mechanism of immunity. Blood cells with different "locks" will bind to specific antigen-antibody "keys" causing agglutination, or clumping, to occur. Thus, when the key fits the lock, immunity is established.

Figure 22.4 (left). White blood cells.

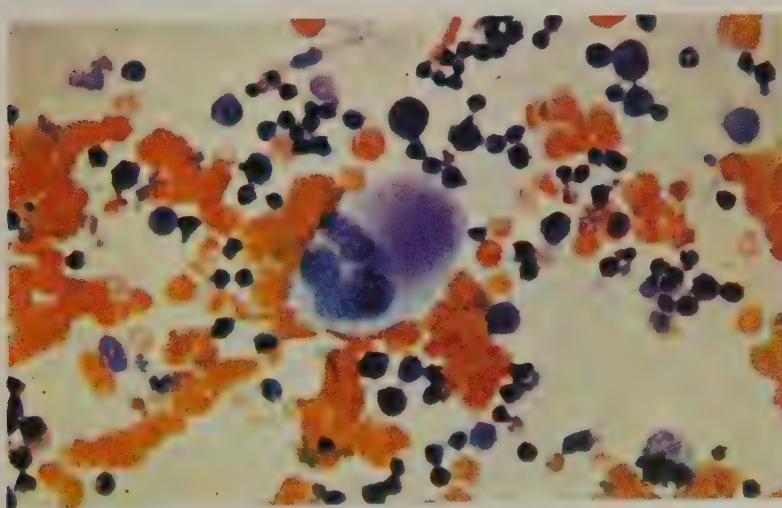
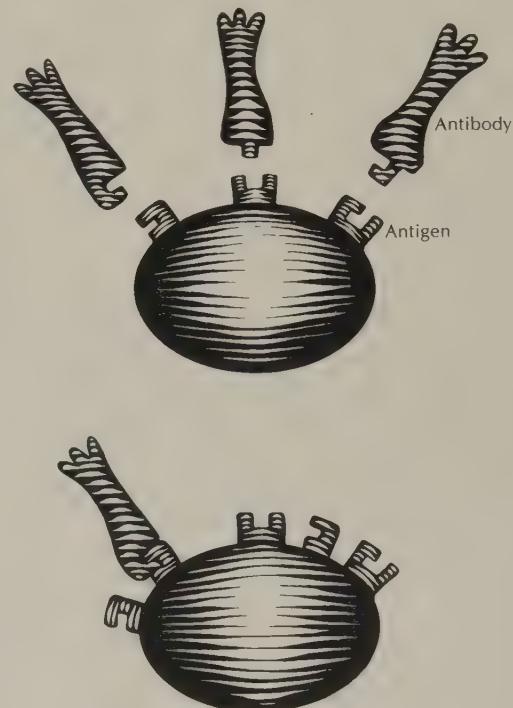


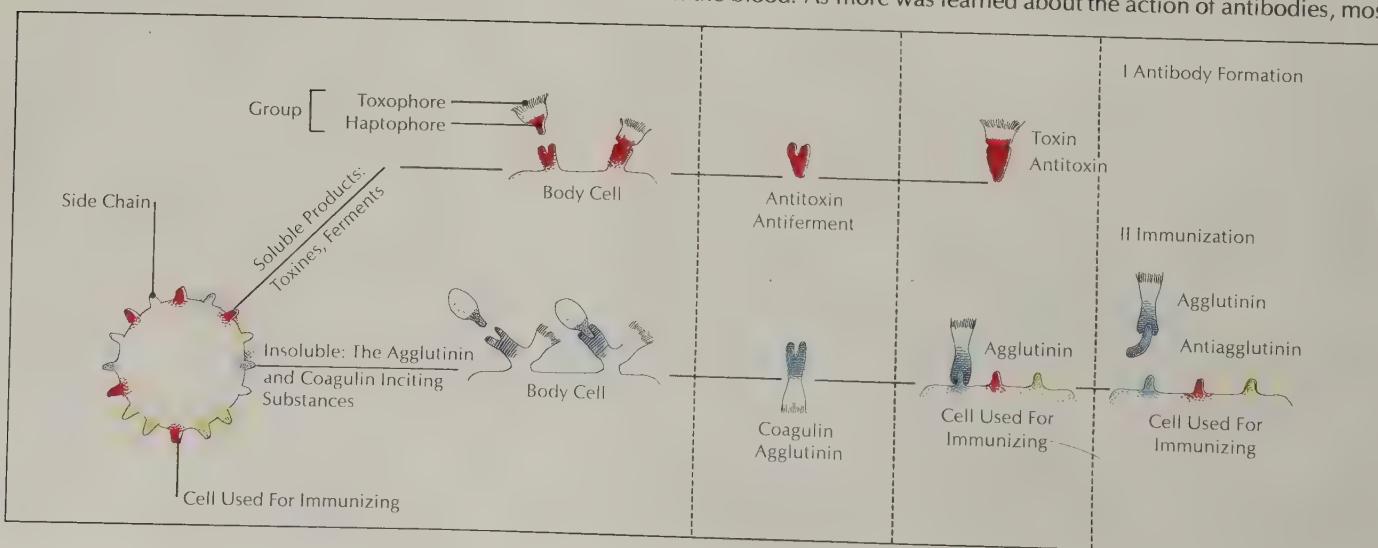
Figure 22.5. The formation of antibodies and the immunization process, according to Ehrlich's theory. Ehrlich suggested that certain cells possess food-capturing receptor side chains. If these receptors capture an antigen and survive, they may be replicated by the cell and released into the blood plasma. These released receptors would then serve as antibodies.

severe and is cleared up more rapidly than was the primary inflammation. As in the case of the primary inflammation, some of the pus and white blood cells may drain out of the wound. The balance is processed through the lymphatic system (Chapter 19). The ultimate fate of the foreign material and cellular debris is either to be ingested and used as food by leucocytes or eventually to be processed through the excretory system of the organism. The damage caused by the inflammation is repaired by the normal processes of wound healing (Chapter 18).

ANTIBODIES

The role of leucocytes in digesting bacteria and other foreign particles was first described by Elie Metchnikoff in the closing decades of the nineteenth century. Metchnikoff regarded the phagocytic action of leucocytes as the body's primary line of defense against foreign materials. At about the same time, however, Emil von Behring and Shibasaburo Kitasato showed that the noncellular, fluid portion, or *plasma*, of the blood contains substances that play a role in the immune response. These substances, which came to be called antibodies, appear in the blood after the primary invasion of a foreign substance, or *antigen*, and are apparently essential to the increased effectiveness of the secondary response. The antibodies were clearly related to the development of immunity against particular diseases or other kinds of invasion, as shown by the success of inoculation as a defense against disease. Therefore, immunologists during the early part of this century turned their attention largely toward the actions of antigens and antibodies.

In the case of bacterial invasions, antibodies immobilize the bacteria or cause them to clump together (agglutinate). Antibodies may immobilize other microorganisms by binding together their cilia or flagella. In the test tube, an antibody may combine with an antigen to form a solid precipitate. In the body, a similar reaction presumably causes neutralization of the antigen, preventing chemical damage to the cells. In each case, however, an antibody acts only against the specific type of antigen that caused its formation in the blood. As more was learned about the action of antibodies, most



biologists came to regard the ingestive action of the leucocytes as simply a mopping-up operation that followed actual immobilization of the invading materials by antibodies.

One early attempt to explain the formation of antibodies was made by the German bacteriologist Paul Ehrlich in the late 1800s. He suggested that molecules of protoplasm possess side chains that normally serve as receptors in capturing food molecules. If the side chain captures a molecule of bacterial toxin or other antigen—and if the living molecule survives the toxic effects of the antigen—the side chain might be replicated in large numbers by the molecule and released into blood plasma. These released side chains would then serve as antibodies, binding to antigen molecules and immobilizing them.

In 1917 Karl Landsteiner (who had earlier discovered the ABO blood groups) succeeded in causing animals to produce antibodies against artificial antigens, which were prepared by binding small organic molecules called haptens to large carrier proteins. Landsteiner's experiments showed that antibodies can react with antigens other than those against which they were formed only if the new antigens are very similar in molecular shape to the antigen that stimulated antibody production. Furthermore, he showed that a single animal can produce specific antibodies against a great variety of antigens—even artificial antigens that could never have been encountered in nature by this animal or any of its ancestors.

Because so many different antibodies can be produced as needed, the weight of opinion began to shift away from theories that involved formation of antibodies from already existing receptors on molecules or cells. Instead, it began to seem more and more likely that the antibody molecules are somehow created in response to the presence of a specific antigen. By the 1930s, it had become clear that the antibodies are among the proteins of the blood plasma, the so-called *immunoglobulins*. Several biologists independently suggested that antibody proteins might be synthesized in contact with the antigen, taking a complementary shape that would lead to formation of a firm bond between the antibody and any antigen of similar shape.

This "instructive theory" of antibody formation was given a more complete biochemical background by the work of Linus Pauling (1940). At the time, little was known of the process of translation or of the fact that protein shape is determined by amino acid sequence. Pauling suggested that a polypeptide chain was shaped against the antigen template and then held in the new shape by formation of hydrogen bonds. The instructive theory remained the dominant explanation of antibody formation for several years. According to this theory, large globular-protein molecules exist in *immunocytes* (white blood cells involved in antibody formation) in relatively unformed condition. The presence of an antigen causes a cavity (*combining site*) to be shaped in the globular protein, complementing the shape of an active part of the antigen. The release of the specifically shaped protein, now an antibody, then leads to the binding and inactivation of antigen molecules of the same or similar shapes. The shaped antibody molecules can remain within the plasma after the primary infection is eliminated; thus providing a ready reserve of antibodies to combat any further infection of the same antigen without delay.

During the 1940s and early 1950s, extensive experience with blood transfusions and transplantation surgery emphasized the ability of the

Interleaf 221

HUMAN BLOOD GROUPS

Many deaths following serious injury or hemorrhaging are due to loss of blood. Why not replace the lost blood with blood from another person or from an animal? This obvious treatment was tried by many physicians over the ages, but the results were often disastrous. In a few cases, the blood transfusion was successful and the patient showed a speedy recovery. In most cases, however, the patient reacted violently to the transfusion and soon died. By the end of the nineteenth century, most European nations had outlawed attempts at blood transfusion. There seemed to be no way to predict in which cases the treatment would prove beneficial.

In the laboratory, experimenters showed that agglutination, or clumping, of red blood cells almost always occurs when blood samples from two animal species are mixed. Similar agglutination occurs often—but not always—when blood samples from two humans are mixed. The Austrian physician Karl Landsteiner began investigating this phenomenon in 1900. He took red cells from the blood of one person and mixed them with blood serum from another person. In some cases the serum caused agglutination of the cells; in other cases it did not. At first, Landsteiner suspected that the blood of some persons lacks the agglutinating factor, either because of illness or because of a hereditary abnormality. To explore the phenomenon further, he obtained blood samples from all the workers in his laboratory and mixed cells and serum in all possible combinations.

Landsteiner soon discovered that serum from a single individual may agglutinate cells from some individuals but not from others. He was able to classify individuals into three groups, each having blood of a type that reacts in particular ways with blood from individuals of other groups. In further studies, Landsteiner found a fourth blood group.

The German bacteriologist Paul Ehrlich recognized this phenomenon as being very similar to antibody-antigen reactions involved in bacterial infections. Ehrlich suggested that agglutination is caused by a “lock-and-key” fitting together of antibodies in the serum with antigens on the cells. He thought that each of Landsteiner’s four types of blood contains a different set of antibodies and antigens, with agglutinating combinations possible only between certain pairs of antibodies and antigens.

Landsteiner modified Ehrlich’s explanation somewhat, showing that his observations could be explained with only two kinds of antibodies (α and β) and two kinds of antigens (A and B). A antigens combine with α antibodies, and B antigens combine with β antibodies. The blood of a person in group A contains β antibodies in the serum and A antigens on the cells, whereas that of a person in group B contains α antibodies and B antigens. A mixture of these two blood types will always produce agglutination. The blood of a person in group AB contains both A and B antigens but no antibodies. Thus, serum from AB blood can be mixed with either A or B cells without producing agglutination, but the AB cells will be agglutinated by serum from persons of either the A or B groups. The blood of persons in the fourth group (O) contains both kinds of antibodies, but there are no antigens on the corpuscles of O blood.

It was soon demonstrated that blood types are inherited according to simple Mendelian principles (Chapter 12). The production of cell antigens is coded by a particular gene. One allele (I^A) codes for production of A antigens, and the other (I^B) codes for the production of B antigens. Thus, a person with genotype $I^A I^A$ will have type-A blood, and a person with genotype $I^B I^B$ will have type-B blood. The blood of a person with genotype $I^A I^B$ contains both types of antigens and is called group AB. The existence of type-O blood is due to the presence of a third allele for this gene. This allele (i) does not code for either kind of antigen and thus acts as a Mendelian recessive. A person of genotype ii has neither type of antigen on his cells and belongs to group O.

Subsequent research has revealed that the antigens are glycoproteins (called agglutinogens) that form part of the surface coating of red blood cells. The combinations of antibodies (called isoagglutinins) and agglutinogens in the blood of the four basic groups are summarized in Table 22.1.

Landsteiner’s blood groups made possible the reliable prediction of the outcome of a transfusion. The blood types of donor and recipient can be determined by simple

tests with standard serum samples. It is then easy to predict whether the transfusion will cause agglutination. Soon after Landsteiner's research was published, blood transfusion became a standard and indispensable medical treatment.

An understanding of the genetic determination of blood types often has been useful in settling questions of relationships among people. If, for example, a rare mix-up in a hospital nursery leaves some doubt as to which baby belongs to which parents,

Table 22.1.
Genetics of Human Blood Groups

GENOTYPES	BLOOD GROUP	AGGLUTINOGENS	Isoagglutinins
$I^A I^A$ or $I^A i$	A	A	β
$I^B I^B$ or $I^B i$	B	B	α
$I^A I^B$	AB	A and B	none
ii	O	none	α and β

the analysis of blood types may help to resolve the problem. Parents both having type-A blood (genotypes $I^A I^A$ or $I^A i$) could not possibly have a child with type-AB or type-B blood. Such genetic analyses also play important roles in many court cases involving disputed paternity. In this case, as in most situations that involve deduction of genotype by observation of phenotype, it should be noted that combined effects of other genes may rarely produce phenotypes that mimic the characteristics produced by the single gene being discussed here. Undoubtedly, several genes are involved in production of proteins associated with blood cells and serum antibodies. The combined effects of changes in such other genes might produce the appearance of type-A blood in a person with a genotype that includes the I^B allele. Thus, there are rare cases where a person whose blood tests as type-B or type-AB may be the offspring of parents with blood that tests as type-A.

Blood-type genetics have been of use also in the study of the races of man. Different populations show different proportions of the three alleles I^A , I^B , and i . For example, the I^B allele appears with a very high frequency in Central Asia and in parts of India, but it becomes less and less frequent in populations farther and farther away from these centers. The corresponding phenotypes (type-B and type-AB blood) show a similar decrease in frequency in populations farther away from Central Asia and India. Among Australian aborigines, nearly 70 percent of the population is of blood type A, and the remainder is of type O; the I^B allele is entirely absent from this population. There are no sharp boundaries between races or population areas. Instead, the frequency of one allele rises and that of another drops as population samples are tested along any particular line.

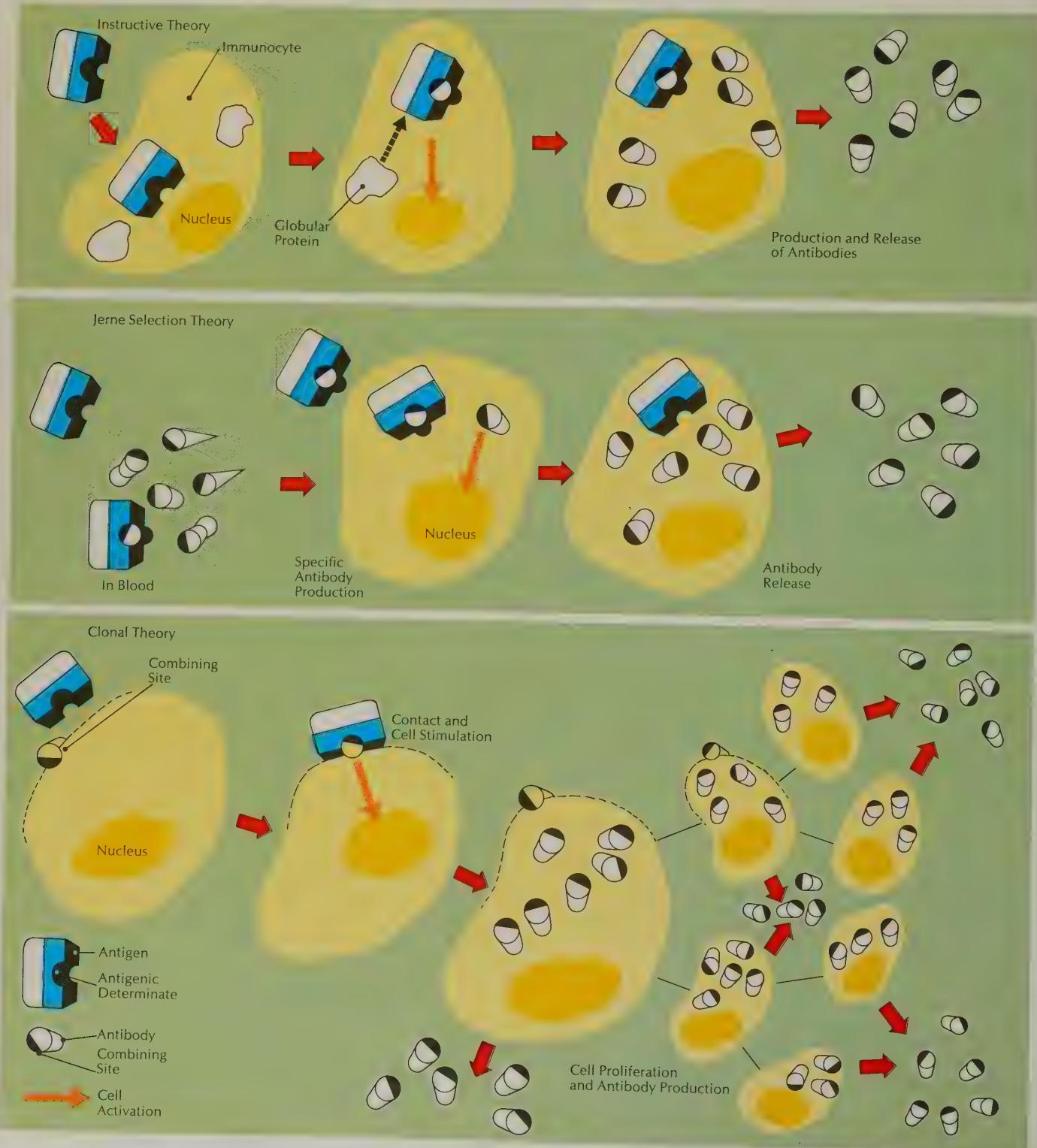
Landsteiner continued his research on blood types for many years. He found a number of other antigen-antibody pairings that are controlled by other genes. These have less extreme effects in normal transfusions but are of importance in many special cases. Landsteiner discovered the M and N blood groups, and in later work with apes and monkeys he found the Rh factor, or Rh antigen, which plays an important role in certain previously unexplained birth difficulties (Chapter 42).

Figure 22.6 (above). Instructive theory of antibody formation (1940). Unformed globular proteins were thought to exist in the immunocyte, which, in the presence of an antigen, conformed to the antigenic determinant (AD). This AD then activated the cell to produce an antibody of a specific shape.

Figure 22.7 (middle). Selective theory (1955). This theory postulated that different antibodies exist in normal blood. The presence of an antigen "selected"

the specific antibody whose combining site bound to the AD. Immunocyte activation then leads to specific antibody release.

Figure 22.8 (below). Clonal theory (1959). The most recent theory postulates that there are specific antibody combining sites on the immunocyte cell surface. Antigen contact (via the AD) causes cell stimulation and proliferation. This action produces a clone of similar cells that all produce the same antibody.



immune system to refrain from attacking materials that are part of the body, even when these materials are transplanted to a new location. A skin graft from another donor is soon attacked and destroyed by leucocytes, whereas a graft of skin from the recipient's own body is not attacked. The instructive theory offered no explanation for this ability of the immune system to distinguish between self and not-self, between particles formed in its own body and alien particles.

The final blow to Pauling's instructive theory came when studies showed that antibody proteins could be completely denatured with accompanying loss of antigen-binding ability. Renaturation then was carried out in the absence of the antigen, and the refolded antibody was found to have reacquired much of its original antigen-binding activity. Thus, antibody proteins are like other proteins, where the chain folding is determined by the primary structure.

Among the theories developed to replace Pauling's were some additional instructive theories and some selective theories. Instructive theories hold that the antibodies are in some way shaped to complement the structure of the particular antigens present. Selective theories hold that the capability for producing any particular antibody is already present in some immunocyte of the body and that invasion by an antigen causes selective reproduction of that cell and production of antibodies by it and its descendants.

One of the earliest selective theories was offered by N. K. Jerne (1955), who suggested that traces of each kind of antibody exist in normal blood. When an antigen enters the blood, it binds to the specific antibody whose combining site complements some part of its structure (the so-called *antigenic determinant*, or AD). The antigen-antibody complexes are then taken into macrophages, which produce more antibodies of the specific form included in the complex. According to this theory, any natural antibodies capable of reaction with parts of the organism would be combined and removed from the blood early in life, thus accounting for the failure of the immune system to attack tissues transplanted from one part of the body to another.

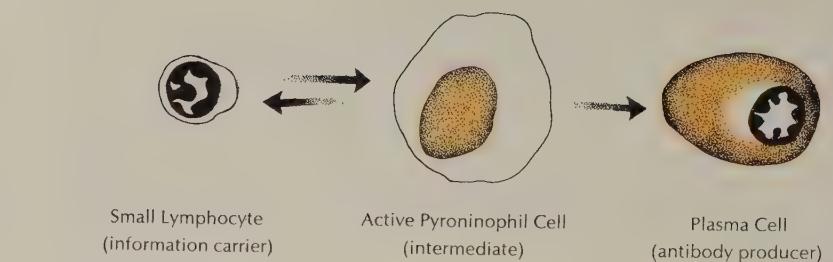
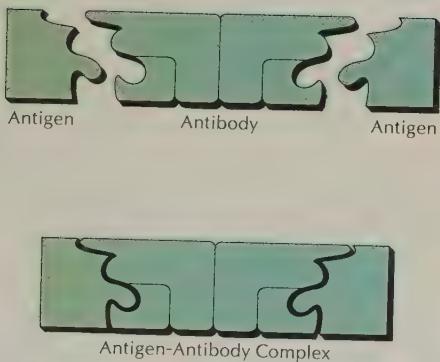
Jerne's theory, however, did not account for the newly discovered phenomenon of tolerance for foreign antigens that were implanted before a certain stage of development. If an antigen is injected into an embryo or—in some species—a newborn animal, the adult will not show an immune response against this antigen in later injections. A modified selection theory was proposed independently by D. W. Talmage (1959) and Macfarlane Burnet (1959). At present, the *clonal selection theory* proposed by Talmage and Burnet—as subsequently broadened and modified—is generally accepted as the most satisfactory explanation of the immune response (Figure 22.8). This theory and some of the outstanding problems yet unsolved will be described in the following sections.

THE MOLECULAR BASIS OF IMMUNITY

Immunology is one of the biological fields in which research is proceeding at top speed. As in any active biological science, new complexities emerge every few months. If general statements are to stand, they must be carefully chosen. In many cases, it is as well to be honest and say that there are a few observations that do not quite fit but that a particular generalization is so useful in understanding 99 percent of what is observed that it is worth using—at least for the present. There are many precedents for a confidence

Figure 22.9 (left). Lock and key antigen-antibody reaction. Evidence suggests that an antibody molecule has two identical halves, each structured from one large and one small component. A particular antibody reacts with a particular antigen because the configuration of its combining site interlocks with that of the antigen. When the antigen and antibody make contact at the proper angle to bring the complementary patterns together, a union between antigen and antibody is formed, thus immobilizing the antigen.

Figure 22.10 (right). The immunocyte concept of immunity. The surface of an immunocyte contains antibody-type combining sites that can combine with a specific antigen. Binding of an antigen to the cell surface may cause the cell to enlarge and proliferate to produce a clone of similar cells. These cells then become capable of producing large amounts of antibodies as well as multiplying as plasma cells.



that future work will straighten out the apparent discrepancies. With these minor reservations, it is useful to consider the relationships among antigen, immunocyte, and antibody in terms of *chemical complementarity*.

Each immunoglobulin molecule is a complex protein made up of at least four polypeptide chains. In the most common type of immunoglobulin with antibody activity (immunoglobulin G), there are two parts of the molecule that serve as combining sites. At these sites are two segments of polypeptide chains lying close together and providing a unique three-dimensional pattern—a pattern that, depending upon which amino acids are involved and their sequence, can take on thousands or even millions of different configurations. A particular antibody reacts with a particular antigen simply because the configuration of its combining site interlocks with the antigenic determinant of the antigen, rather like a key fitting into a lock (Figure 22.9). When the antigen and antibody collide at the proper angle to bring the complementary patterns together, a relatively firm union between antigen and antibody is formed. As in the similar relationship between an enzyme and its substrate, the union involves both the interlocking shapes of the molecules and weak bonds such as hydrogen bonds. The antibody will combine only with antigens that possess an AD of very close complementarity to the shape of the combining site.

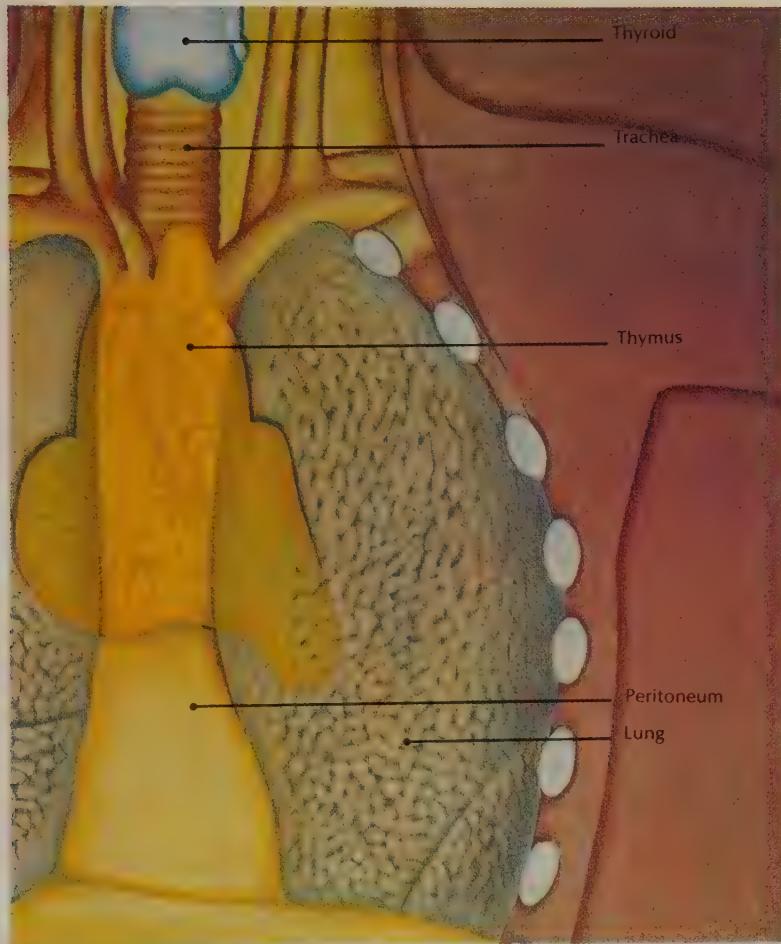
On the surface of an immunocyte are antibody-type combining sites that, like an antibody itself, can unite with the corresponding AD. On the cell surface, however, this "fixed antibody" acts as a receptor, not only binding the antigen to the cell temporarily but also stimulating the cell to activity. Most commonly, the stimulus causes the cell to enlarge and proliferate to produce a clone of similar cells (Figure 22.10). In some cases, the stimulated cells become capable of producing large amounts of antibody as well as multiplying—these antibody-producing cells are the plasma cells, or plasmacytes.

Recent studies have confirmed that each immunocyte produces only a single kind of antibody (Awdeh, et al., 1970). Perhaps the most important generalization in immunology is that when an immunocyte multiplies, its descendant cells all produce the same type of antibody, whether the antibody is present only as a few surface receptors or is synthesized in large amounts.

The Thymus Gland

One of the most important aspects of recent immunological research has been the recognition of the importance of the thymus gland. The thymus is unlike any other mammalian organ in that it is relatively large in infancy and fades away to a few fibrous shreds in old age. In babies, it is a mass of actively multiplying lymphocytes lying just behind the top of the breastbone (Figure 22.11). The first clues to its importance came in 1960, when Jacques Miller devised a method for surgically removing the thymus from newborn mice. Such mice show serious disturbances in their immune responses, and detailed analysis of their anomalies has been highly fruitful. But for an aid to understanding the function of the thymus, it has proved useful to use what Robert Good calls experiments of nature—genetic diseases that in one way or another involve the immune system. These observations have led to the same conclusion as a wide range of experiments in mice, chickens, rats, and so on. It is clear that there are two major families of immunocytes. One set is thymus-dependent (T-D) cells, whose ancestors

Figure 22.11. The human thymus gland. Immunocytes produced directly by the thymus or the ancestors of cells produced by the thymus appear to be concerned with cell-mediated immune responses.



have come from the thymus. The other immunocytes can be called thymus-independent (T-I) for the present, although eventually they will probably be found to be related to some other tissue that plays a role similar to that of the thymus. Some biologists have felt that the evidence justifies calling this analogue of the thymus the *gut-associated lymphoid tissue* (GALT), and this term is often seen in the literature of immunology.

The important feature is that there are two congenital diseases that neatly separate the two groups. In Di George's disease, the embryonic cells that should form the thymus and parathyroid glands fail to develop, and the child is born with no thymus at all. If the effect of the missing parathyroids is remedied by giving the appropriate parathyroid hormone, the remaining symptoms can be assumed to be due to the absence of T-D immunocytes. The second disease, congenital agammaglobulinemia, represents a complete failure of the T-I system with what appears to be a perfectly normal thymus. Both diseases are very rare and have been carefully studied. When they are compared, a clear division of immune function becomes visible.

A child with agammaglobulinemia, whose T-I system is lacking, shows the following differences from a normal child: (1) no antibodies are produced when antigens such as diphtheria toxoid and polio vaccine are

injected; (2) there are no plasma cells and only minute amounts of immunoglobulins in the plasma; (3) the child is highly susceptible to pneumonia and other bacterial diseases and—in the days before antibiotic drugs—always died in infancy. In the following types of immune response, however, the child will behave normally; (4) a graft of skin from another child is rejected; (5) the skin shows sensitive reactions to chemical substances, and the child can develop a positive reaction to a tuberculin skin test; (6) some virus infections, such as measles or vaccination against smallpox, run a normal course and are followed by immunity against another attack.

In a case of congenital absence of the thymus, Di George's disease, where T-D cells are absent, a different picture appears: (1) antibodies are produced in response to diphtheria toxin or polio vaccine injections, but in smaller quantities than in normal children; (2) plasma cells are present in normal quantities, and immunoglobulin is in nearly normal amount in the plasma; (3) the child is very "delicate" but is more prone to infection by fungi than by bacteria; (4) skin grafts from another individual are accepted and remain healthy indefinitely; (5) the skin shows no sensitive reactions to foreign substances; (6) measles develops no rash, is often fatal, and—if the child survives—no immunity follows. (The final part of the sixth statement is only a guess based on observations of a related disease.)

The comparison of these two genetic diseases indicates that there are two related but distinct immune systems. The first can be called the antibody-producing system—including the plasma cells—and its main function is dealing with bacterial infection. The second system is concerned with cell-mediated immune responses in which the immunocytes themselves carry out the entire process. Cells derived from thymal ancestors are wholly responsible for the cell-mediated responses and also play an important part in making some types of antibody response possible. In all probability, the thymus-dependent system is of earlier evolutionary origin, and it is in many ways more important than the antibody-producing system. However, because antibodies are more conveniently studied than living immunocytes, much immunological research has focused on the antibody-producing system, and a clear picture of the biological significance of immunity has been obtained from such work. Although the importance of the thymus-dependent system has been increasingly recognized in recent years, any summary of current knowledge and research in immunology must still emphasize the actions of antibodies.

STUDYING ANTIBODIES

Anyone with an interest in biology or medicine has a rough idea of what antibodies are. Before the days of antibiotics, a physician caring for a patient with pneumonia would watch for what was called the crisis, which was a sudden improvement occurring about a week after the onset of symptoms and signifying that adequate supplies of antibodies to deal with the germs—the pneumococci—had been produced. Once that supply of antibodies existed in the blood, the pneumococci would be controlled. There were various ways by which the antibodies could be recognized and their amount measured.

In light of modern knowledge of protein biosynthesis, biochemists have taken a particular interest in the nature of antibodies. Virtually all body proteins are synthesized to a particular, genetically determined pattern to serve

some particular function in an organism. Proteins are accurately produced to match patterns indicated in genes through the well-known processes of transcription and translation, involving mRNA, ribosomes, and tRNA. Antibodies, however, appear to exist in an almost infinite variety of forms—such that an antibody is produced to fit almost any sort of foreign organic material. It is not surprising that many biologists have concluded that the antibodies are custom-built to fit the antigens.

Two questions are of particular interest to biochemists: What are antibodies? How are they synthesized in such a vast diversity of patterns? It is not easy to obtain an antibody for study. If a rabbit is immunized with some pure protein, enough antibody is produced to react with the protein antigen. It might be reasonable to expect that injection of a pure antigen would lead to production of a pure antibody, but this expectation proves to be far from the case. In fact, what is obtained from the rabbit's blood is a very complex assemblage of immunoglobulin molecules whose one common feature is that they will all unite—in varying degrees of strength—with the antigenic determinants of the protein injected.

Myeloma Proteins

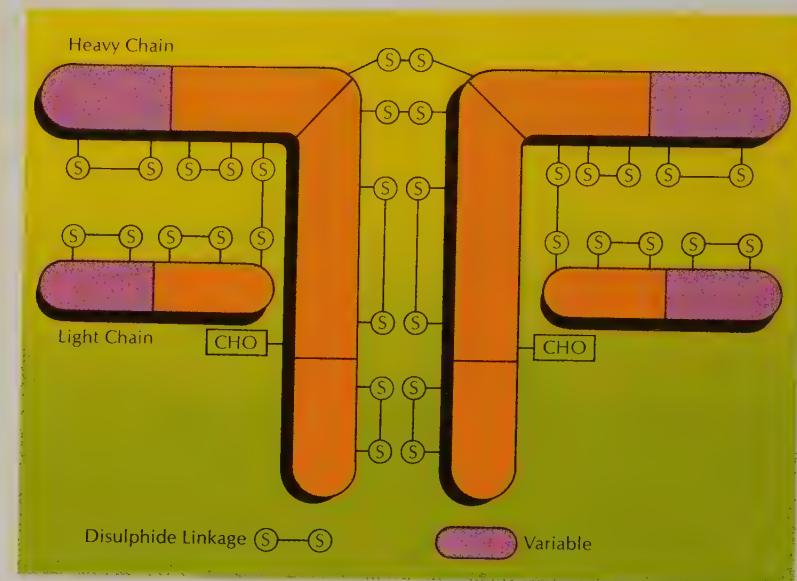
The difficulties of studying mixtures of immunoglobulin molecules were overcome by making use of another experiment of nature. There is a fairly common disease—a mild form of cancer called *myeloma*—in which a single plasma cell undergoes malignant change. It becomes cancerous in the sense that it enlarges and divides into two, the daughter cells continue to divide, and the process continues indefinitely until there are many billions of this sort of plasma cell in the body. The genetic information of the original plasma cell allows and compels it to produce one particular pattern of antibody, and, as the proliferation continues, each descendant acquires the same genetic information. When the disease is fully developed, the blood contains a vast excess of that particular type of antibody. Of special interest to the biochemist is the fact that this antibody is essentially pure. It is a homogeneous population of immunoglobulin molecules, each one made to the same pattern.

Although any random plasma cell may be provoked to cancerous proliferation, the event is so rare that it is most unusual for it to occur twice in a single individual. Each individual myeloma victim has his own unique pattern of antibody, and that pattern differs in some or many ways from the pattern of any other individual's myeloma protein. The antigenic determinant appropriate to unite with the combining site of a myeloma protein has been identified in only a few rare cases. It is relatively certain, however, that if tests were done with any two myeloma proteins, they would react with different determinants.

Application of techniques such as immunoelectrophoresis and comparative studies of normal immunoglobulins and antibodies have yielded a flood of information—so much that only a brief description of the most common and best-studied myeloma protein can be given here. This molecule is of the type called *immunoglobulin G*.

The molecule of immunoglobulin G is made up of four chains bound together with single disulphide linkages. It is a double-end molecule with a combining site at each end (Figure 22.12). Everything is symmetrical; the two light (L) chains are identical, and the two heavy (H) chains also form an

Figure 22.12. Structure of immunoglobulin G.



identical pair. The combining sites made by the association of the end segments of one L and one H chain are also equivalent, each reacting with the same antigenic determinants.

When myeloma proteins from a number of different people with this same type of the disease are compared in detail, a new feature becomes evident. Some parts of the molecule, as judged by the sequence of amino acids, are identical in all the proteins or show only one or two discrepancies in amino acids. However, the sections shown in Figure 22.12 as "Variable" differ widely from one protein to another, although they have a basically similar structure. At a position nine places along from the end of this variable segment, for example, the amino acid may be any one of at least six—serine, threonine, glycine, aspartic acid, alanine, or leucine. It is very significant that the combining site is produced by the interaction of the variable segments of the L and H chains. Here, surely, is the chemical basis for the diversity of antibodies.

The Origin of Antibody Diversity

The second and more difficult question remains: How can the plasma cells of the body produce many thousands of different combining-site patterns, each presumably involving a different amino acid segment in the variable segments? How is it that the body can build an appropriate antibody even against some synthetic antigens that neither the individual nor his ancestors could ever have encountered in the past?

According to modern theories of protein biosynthesis, the amino acid sequences of immunoglobulins must be genetically transmitted from each cell to its descendants, coded as nucleotide sequences in genes. How does the body develop a population of plasma cells, each carrying genes that specify a different variable region for its immunoglobulin? One of the biggest puzzles left for molecular biologists after the cracking of the genetic code is the explanation of the source of this genetic diversity. The answer has not been found, but the sort of answer that it must be is becoming clear.

According to the clonal selection theory developed by Burnet, the diversity must depend to a large extent on the presence of duplicated

genes carried in the germ cells and subject to individual mutations in the course of evolution. The varying evolution of these different (originally identical) genes could account for the presence of different types of immunoglobulins (having different sequences in the "constant" regions) in the same individual. According to Burnet's theory, diversification also arises through somatic mutations—genetic changes that occur in the body cells at various stages of embryonic and later life. These mutations in individual cells produce the variety of possible sequences in the variable regions of the immunoglobulins. These somatic mutations probably involve changes in a single nucleotide in genes specifying the sequence of the variable regions, although there are other possibilities. Finally, Burnet suggests that there must be a process called *phenotypic restriction* through which the cell "chooses" to produce only one of the possible immunoglobulins coded in its genes. Once this selection is made, the type of antibody to be produced by the cell and all of its descendants is fixed.

Various other theories under current consideration differ from Burnet's in certain details, but all postulate the steady production—especially in infancy and childhood—of a great variety of essentially random modifications of the standard antibody patterns inherited by the zygote. This variety of patterns is the raw material from which antigens, when they enter the body, "select" appropriate immunocytes to be stimulated through combination of the AD with the combining site of the immunocyte receptors. The stimulated cells proliferate and make antibodies that will react with the antigen that these cells have "recognized."

TOLERANCE: SELF OR NOT-SELF?

The immune system has to immobilize and eliminate foreign material, but it must have no harmful effect on the proper tissues and components of the body. Thus, there must be a natural immunological tolerance for all accessible components of the body. Tolerance to foreign substances can be induced artificially in various ways—by injecting foreign cells into newborn animals, by using x-rays, or by using drugs. The successful transplantation of organs, for instance, depends upon skillful use of drugs to "trick" the body into tolerating cells and tissues that it would normally reject.

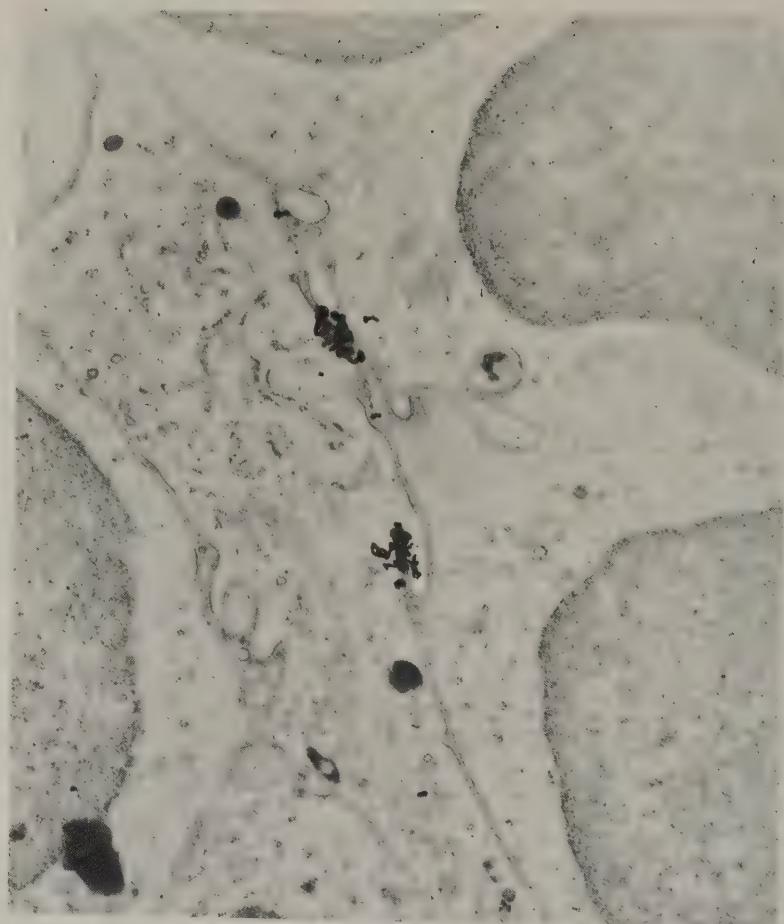
Tolerance is not fully understood, but a simple interpretation, suggested many years ago by Burnet, has not yet been disproved. For a time after its primary differentiation as an immunocyte, the cell is highly sensitive to contact with any antigenic determinant that matches its combining sites. Instead of causing proliferation, the stimulus at this time is so severe that it kills the cell. Such a sensitivity of "newborn" immunocytes would ensure that all cells capable of being stimulated by normal components of the blood and lymph are eliminated from the immunocyte population in this early stage. According to this view, tolerance results simply from the absence of immunocytes with combining sites that match normal components of the body.

Other immunologists have hypothesized the existence of *tolerant immunocytes*, which have the capacity to recognize normal components of the body fluids and to prevent the synthesis of antiself antibodies. However, the existence of such tolerant immunocytes has not yet been demonstrated.

IMMUNE RESPONSES

The complexities of immune responses make it impossible to give a simple summary that includes all known facts about the sequence of events

Figure 22.13. Electron micrograph showing antigens that have become trapped on the branches of dendritic phagocytic cells (DPC) within a lymph node. Also present are the nuclei of small lymphocytes.



involved. All that can be attempted here are an outline of two well-studied examples and a reasonable—but not unanimously accepted—interpretation of each.

The first example involves the injection into a rat of protein derived from bacterial flagella. This antigen provokes a strong reaction, and the antibody is easily detected and measured in the blood plasma. If this antigen is labeled with radioactive iodine and is injected into the footpad of a rat, the fate of the antigen can be followed in detail by autoradiography. The antigen passes quite rapidly to the lymph node that lies behind the knee-joint and drains the entire foot region. In the lymph node, the antigen is taken up by the phagocytic macrophages, which destroy most of it but may retain some of the antigen. Another part of the antigen is taken up by a different type of phagocytic cell that is present among the masses of lymphocytes in the node. These cells, called *dendritic phagocytic cells*, have long, branching extensions, which are particularly well developed if traces of the antigen are already present in the lymph. Antigen sticks to the surface of these branches and sometimes stays there for weeks (Figure 22.13).

In a living lymph node, lymphocytes are always coming and going and moving about “like a bag of worms.” In every lymph node there are ample opportunities for a particular antigen held on the branches of the dendritic

phagocytic cells to be contacted by a lymphocyte whose combining sites match its AD. Almost certainly, the stimulation of the immunocyte takes place in this fashion, but immunologists are uncertain of the subsequent processes by which large numbers of antibody-producing plasma cells appear, either in the draining lymph node or elsewhere in the body. A simple—probably too simple—description is that the stimulated lymphocyte enlarges, becomes more mobile, and moves by lymph or blood circulation to any place where the conditions are right for it to settle down and multiply, producing a clone of antibody-synthesizing plasma cells.

The second example—the rejection of a foreign skin graft—demonstrates cell-mediated immunity. Here again, various steps of the process are clear, but the connection between them is still uncertain. It is probably best to give the simplest interpretation that seems to be consistent with the known facts—remembering that future observations may prove this simple explanation inadequate.

When the foreign skin graft is placed in the raw bed from which the animal's own skin was removed (Figure 22.14), there develops a steady traffic of wandering cells from the blood vessels into the general area, some passing into or making frequent contact with the grafted tissue. Fragments of the foreign tissue lodge on these wandering cells, and some are carried to the draining lymph nodes.

In the lymph nodes, the cells carrying foreign antigen appear to meet thymus-dependent immunocytes with complementary combining sites near the central part of the node. There is the appearance of great activity in this area, where immunocytes multiply freely but produce more lymphocytes rather than plasma cells. Many of these active cells do not possess antibodies of the type that matches the antigen, but the stimulation of the "correct" immunocytes has a secondary effect on the others. Both sorts of cells pass into the circulation and, if they reach the site of the foreign graft, tend to lodge there.

The inflammatory response produced by the interaction of these immunocytes with the antigens present in the graft damages the tissues, particularly the newly formed capillaries that are beginning to extend into the graft. Damage to cells accumulates, the blood supply becomes inadequate, and eventually the graft dies and is sloughed off as a scab about two weeks after the implantation.

THE SCOPE OF IMMUNOLOGY

Immunology is a science that impinges on virtually every other aspect of biology. It is an area of extremely active research and exciting discoveries, as well as a field with extremely important medical implications.

Much interesting work is being done on the process by which immune systems have evolved and on their comparative forms in different vertebrates. There is growing evidence that the immune system can sometimes—perhaps much more often than yet known—nip an incipient cancer in the bud, and the nature of this immunological surveillance is of obvious relevance to cancer research. The nature of the immunological coexistence of mother and embryo during pregnancy and the occasional breakdown of this mutual tolerance are matters of great interest to many immunological and medical researchers.

The symptoms of measles are due not so much to the actions of the virus that infects the body as to sensitization—the disease is essentially a cell-

Figure 22.14. Sequence showing the procedures for experimental skin grafts in mice. (A–B) Dissecting the skin. (C) A fitted graft. (D) Open style grafts.

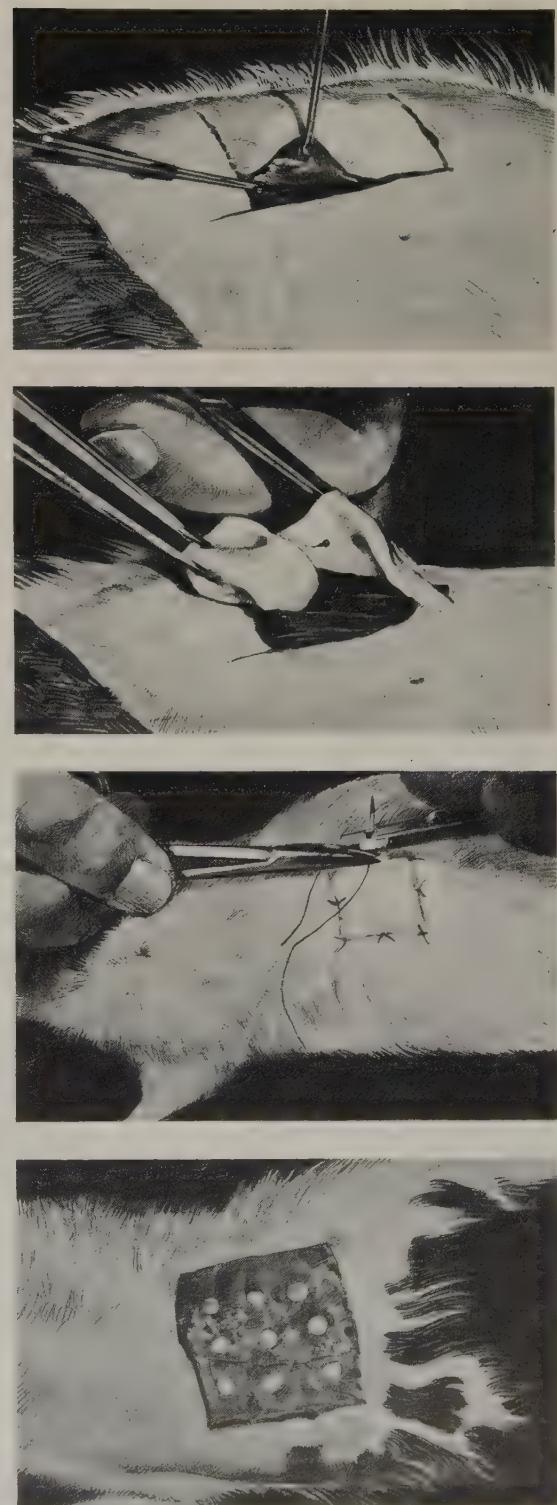


Figure 22.15. Bronze statue of Edward Jenner inoculating his son with cowpox germs.

mediated immune response. In fact, pathologists are beginning to suspect that many manifestations of infection are actually unfortunate effects of the immune responses that should deal with them. Of particular interest are the many diseases that appear to be due to an attack by the immune system upon normal body tissues. Among these *autoimmune diseases* are such serious conditions as haemolytic anaemia and lupus, and there is a great deal of evidence that many other conditions such as rheumatism and arthritis involve autoimmune responses. Allergic reactions involve abnormal immune responses; in most cases, tissue damage is caused by an immune response to a substance that would normally be harmless.

The story of immunology began with Edward Jenner's inoculation of a boy with cowpox germs, thus protecting him from smallpox infection. The protection of children against diphtheria, polio, and whooping cough by immunization represents one of the great medical advances of the century.



and has resulted in a dramatic decrease in childhood deaths around the world. Paradoxically, this great advance has been a major contribution to the explosive population growth that is a major problem today (Chapter 45). In the field of veterinary diseases, there has been even more effective use of immunization. Much current research is aimed toward development of more effective vaccines and of immunization techniques against diseases not yet controlled.

Immunology provides an enthralling mixture of interests that stretch across the spectrum of biology. Its limits have not been exhausted by any means. Among the problems recently investigated by immunologists—to mention a few of the bizarre but still useful extensions of the field—are the detection of horse meat in hamburgers, the explanation of why certain deodorants produce lumps in ladies' armpits, and the blood grouping of the remains of ancient Egyptians.

FURTHER READING

Burnet (1969) presents a readable summary of current knowledge and theories in immunology, with emphasis upon his own theoretical viewpoint. Kabat (1968) gives a more complete treatment of the molecular aspects of immunology, with thorough bibliographies. Killander (1967) includes reports on much of the research aimed toward an understanding of the synthesis of immunoglobulins. For a general review of current concepts of the nature of antibodies, see Edelman and Gall (1969). Relevant *Scientific American* articles include Allison (1967), Billingham and Silvers (1963), Boyden (1951), Burnet (1954, 1961, 1962), Crowle (1960), Duve (1963), Edelman (1970), Frei and Freireich (1964), Levey (1964), Nossal (1964), Porter (1967), and Williams (1960).

23

Building Blocks of Nervous Systems



The general anatomy of the nervous system and its function in the control of the animal body were worked out by physiologists over the centuries, culminating in Charles Sherrington's great book, *The Integrative Action of the Nervous System* (1906). But the nature of the nerve impulse, the mechanisms by which it is propagated in the *neuron*, or nerve cell, and the way in which impulses are passed from one neuron to the next have been deciphered only in the past few decades. The study of the nervous system is best begun with an examination of these neurons, the building blocks that make up the fantastically complex nervous systems of higher animals.

An animal's nervous system must gather information about the internal state of the organism and its external environment, evaluate this information, and coordinate activities appropriate to the situation and to the animal's current needs. The gathering of information is performed by *receptors* and *sensory neurons*. The coordinated activities are executed by *effectors*, which may be muscles or glands, and the effectors are controlled by *motor neurons*. The nervous system also contains an immense number of *interneurons*—neither sensory nor motor—whose role is to process the sensory input, evaluate it, and command the motor output. These cells perform an associative function; they form connective links between sensory and motor levels. They may be organized into interconnecting groups and levels with almost unimaginable complexity.

The traditional division of the nervous system into sensory, associative, and motor portions is useful because there are many properties shared by the elements within each division. There are, however, many properties common to particular elements of different divisions, and it is sometimes difficult to say where "sensory" ends and where "associative" begins. Certain cells, for example, originate within the brain but send processes to the peripheral sense organs—such as the auditory receptors—where they presumably modulate the reception of incoming information. Similar difficulties arise with the definition of motor elements. Any division of the complex nervous system into segments, regions, or subsystems must be an arbitrary one. In the living animal, the system functions as a magnificently complex and coordinated whole.

CELLS IN THE NERVOUS SYSTEM

Neurons are the best-studied cells of the nervous system. These highly specialized nerve cells are distinguished by the extremely long processes, or *axons*, that they send through the animal body. The general structure of the neuron is described in Chapter 11.

The vertebrate nervous system includes not only neurons but also closely associated *glial cells*—or *neuroglia*—and the blood vessels, membranes, and other structures closely associated with the nerves. Neuroglia exist in a wide variety of shapes and forms. Although their functions are largely unknown, they traditionally have been thought to function in the support and nourishment of neurons. Neuroglia pack the spaces between nerve cells and closely surround neuron bodies and processes. More than 90 percent of all cells in the vertebrate brain are neuroglia. They are generally smaller than neurons and thus make up only 50 percent of the brain's weight. Neuroglia contain far less RNA than do neurons, and they possess strikingly different kinds of RNA and enzymes.

Two well-studied types of glial cells are *oligodendroglia* and *astrocytes*.

Figure 23.1 (right). Photomicrograph of glial cells, or neuroglia, among the larger nerve cells.

Figure 23.2 (left). Depolarization of a glial cell produced by nerve impulses in the *Necturus* optic nerve (above). The glial membrane potential recorded with an intracellular electrode was 86 mV. In the upper record, depolarization after a single nerve volley peaks in about 150 milliseconds and declines with a half time of about 2 seconds. In the lower record, three stimuli at 1-second intervals produced a summation of glial

depolarizations. Glial depolarization effected by four different frequencies of nerve stimulation (below). Trains of electrical stimuli at 0.5, 1, 2, and 5 stimuli per second are applied for about 20 seconds. The amplitude of the glial depolarization, as measured with an intracellular electrode, ranged from about 6 to 7 mV with 0.5/s stimulation to about 17 mV with 5/s stimulation.

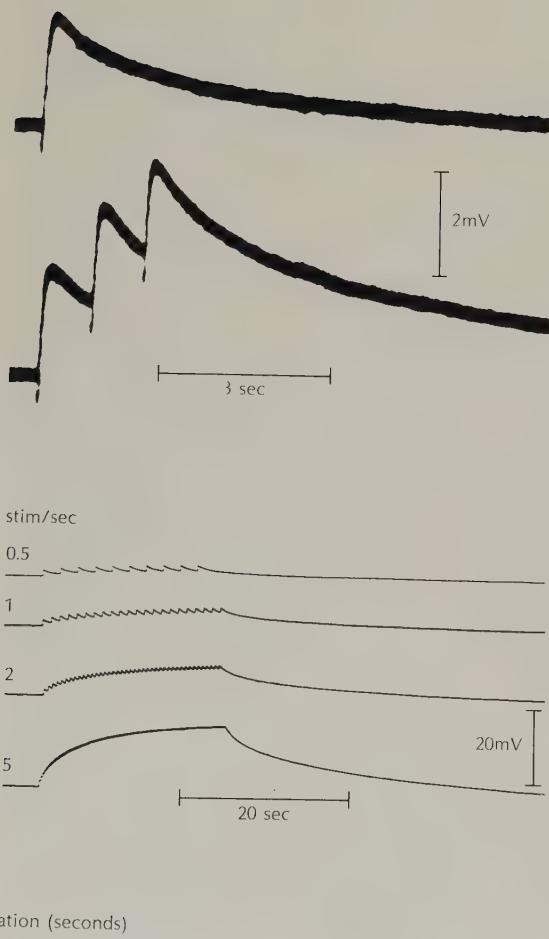


Oligodendroglia are instrumental in the formation of myelin in the central nervous system, as are the Schwann cells in the peripheral parts of the system. The myelin sheaths formed around axons by Schwann cells and oligodendroglia play an important role in speeding movement of impulses along the axons. Oligodendroglia may have other important functions as well, but none has yet been firmly established.

Some of the older suggestions about the functions of astrocytes still seem reasonable today. Undoubtedly, they provide mechanical support for neural tissue. They keep the brain free of sick neurons by phagocytosis—engulfing and digesting them. Astrocytes probably serve in a nutritive capacity because they are intimately associated with capillaries and with neurons. Electron microscope studies indicate that there is always a layer of glial cytoplasm between any neuron and the nearest blood vessel. The astrocytes may mediate transfer of proteins or metabolites between neurons and the bloodstream.

An interesting modern approach to the study of neuroglia has been taken by S. W. Kuffler (1967) and his colleagues at the Harvard Medical School. They used leech nervous systems and optic nerves from *Necturus* (the mud puppy, an amphibian) to demonstrate electrical and ionic relationships of neurons and neuroglia. They found that there is an electrical potential, or polarization, between the inside and outside of the glial cell, with the inside about 60 to 90 millivolts (1 millivolt = 1 mV = 0.001 volt) negative with respect to the outside. This resting potential depends only on the concentration of potassium ion (K^+) in the extracellular space. The greater the K^+ concentration outside, the less negative the resting potential. (A decrease in the resting potential reduces the polarization and thus is called depolarization.)

Kuffler and his colleagues found that the activity of nerve cells induces the glial cell to produce an electrical response, namely the depolarization resulting from the increase in K^+ in the extracellular space. This response is fundamentally different from the nerve impulse of a neuron. There is a



high electrical continuity between adjacent glial cells, but little electrical connection between the glial cell and the extracellular space or the adjacent neurons. Thus, neuroglia are not well suited for electrical signaling to nerve cells. An older suggestion that glial cells play a vital role in transmission of nerve impulses was disproved in an experiment in which the neurons were washed free of most glial cytoplasm yet maintained the ability to conduct impulses for hours. Thus, neuroglia are not absolutely necessary for conduction of nerve impulses.

The most interesting discovery made by Kuffler's group was that K^+ concentration increases in the region outside a glial cell as nerve impulses pass nearby. The increase of K^+ concentration was detected because it causes a prolonged depolarization of the glial cell membrane. This change in the ionic concentration of the extracellular fluid could have profound effects on the synapses, where one neuron affects the triggering of impulses in another, and possibly on nerve impulses moving through fine nerve fibers. The depolarization may also trigger chemical changes in the glial cells—changes that might ultimately lead to other effects on neurons. The role of glial cells in the nervous system is only beginning to be understood.

ACTIVITY OF NEURONS: THE NERVE IMPULSE

There are many different types of neurons, differing in shape and size of cell body (soma), in the presence or absence of specialized dendrite processes, in length of the axon, and in the presence or absence of a fatty myelin sheath covering the axon (Figure 23.3).

An account of the function of neurons must explain how stimuli arising

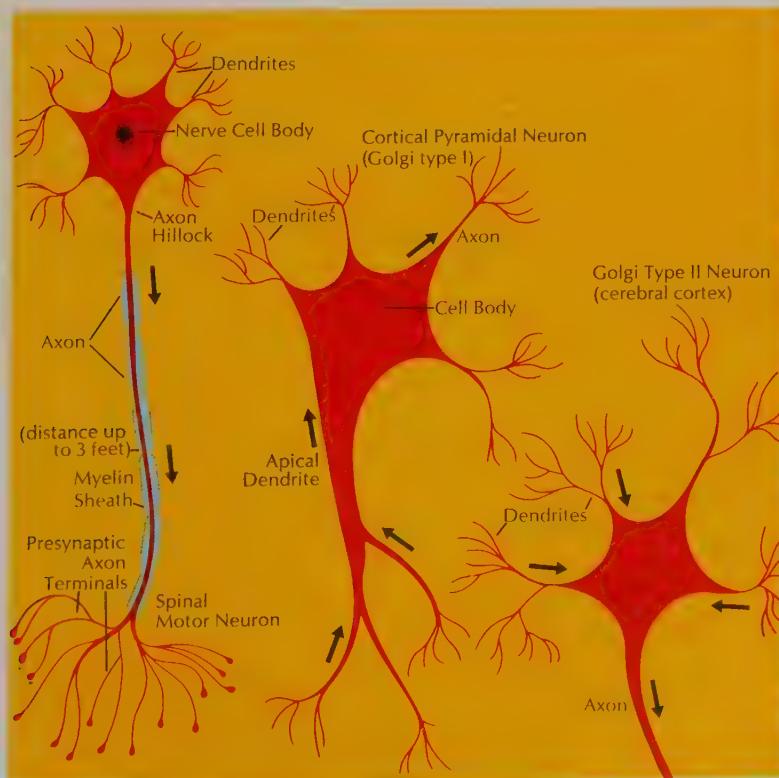


Figure 23.3a (left). Diagram illustrating the variety of types of neurons. Neurons may differ in shape and size of the cell body (soma), in dendrite processes, in length of the axon, and in presence or absence of a myelin sheath.

Figure 23.3b (right). A slice of cerebellar cortex stained to outline neurons.

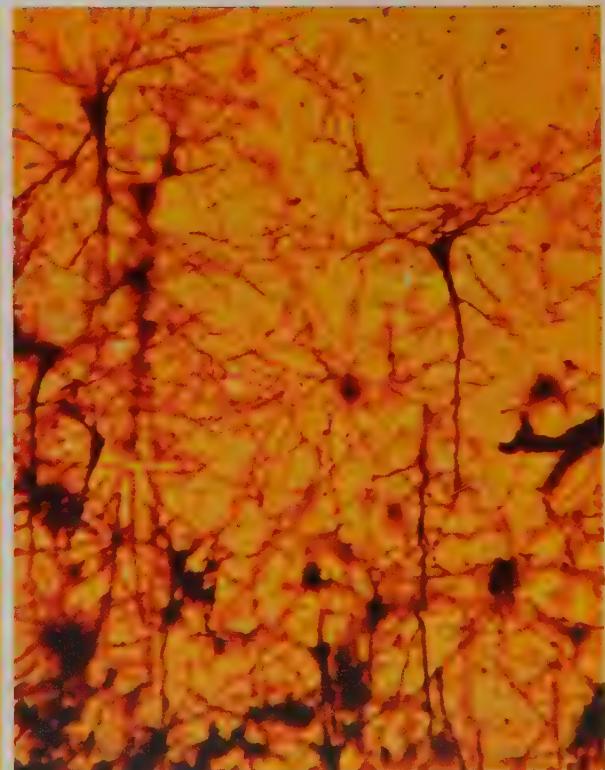
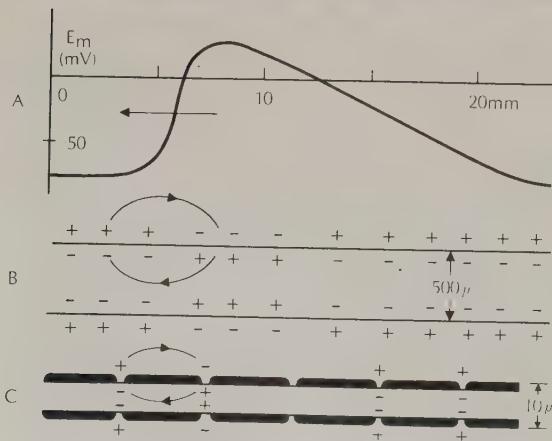
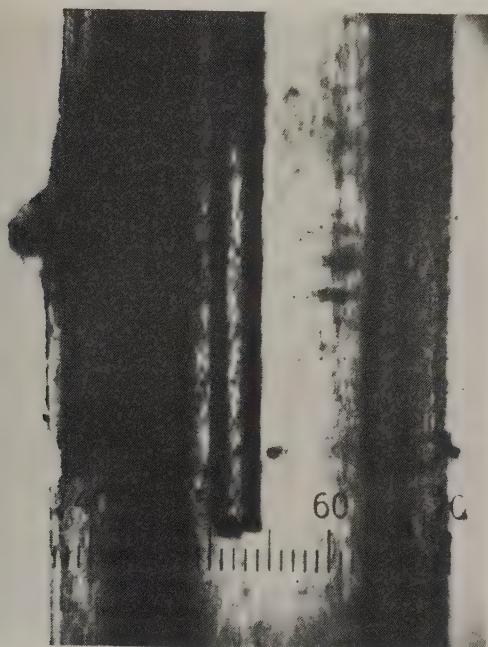


Figure 23.4a (upper left). Micropipette inserted into a very large axon found in the squid. Each scale division equals 33 microns.

Figure 23.4b (lower left). Propagation of action potential by local circuit stimulation. The spatial variation of an action potential at a fixed time is shown in A. Ordinate is transmembrane potential (E_m); abscissa is distance along fibers shown in B and C. The action potential is propagated at a speed of 20 meters per second to the left (arrow). An unmyelinated nerve fiber is shown in B. Transmembrane voltages given in A are presented here by plus and minus signs. Arrows indicate that current flow in a loop is due to the differences in transmembrane potential caused by different ionic permeabilities of the membrane; K^+ is highly permeable at either end, and Na^+ is even more highly permeable in the central region. Propagation is achieved by the depolarization action of local current flow. A myelinated nerve fiber is shown in C. Distance between nodes is shown as twice actual distance. Charge is shown only at nodes because of the very high electrical resistance of the sheath. Local circuit flow is thus from node to node as shown by the arrows.

Figure 23.4c (lower right). Nerve impulse-action potential.



outside the cell are converted into nerve impulses, how the nerve impulse travels along the axon to its destination, and how the impulse stimulates other neurons or effectors at the destination. The electrical and chemical mechanisms that accomplish these functions are similar in most types of neurons. The nature of the nerve impulse is similar in all neurons.

When physiologists first realized that the nerve impulse is electrical in nature, they assumed that nerves act like cables carrying electrical currents through the body. The axon is a cablelike structure, with a core of ion-containing fluid that acts as an electrical conductor, surrounded by a membrane that acts as an insulator. However, the electrical properties of a nerve fiber are extremely poor when compared with standard electrical cable structures. The electrical resistance of the fluid in the axon is about 100 million times greater than that of copper, and the axon membrane leaks electric current about 1 million times more than the sheath of a good cable. Thus, the cablelike performance of an axon is about 100 million million times poorer than that of a typical sheathed-copper cable. If an electric pulse too weak to trigger a nerve impulse is started in an axon, it dies out within a few millimeters' travel along the axon. However, a stronger pulse triggers a unique electrical event, a nerve impulse, that travels along the axon at high speed for an indefinite distance without distortion or loss of strength, something that cannot happen in a normal sheathed-copper cable. The nature of the nerve impulse depends upon the ion-transporting properties of the neuron membrane.

A neuron, like a glial cell, has a resting potential. That is, its membrane normally is electrically polarized, with the inside 50 to 90 mV negative relative to the outside. When a *nerve impulse*, or *action potential*, is triggered, the potential across the axon membrane falls toward zero (depolarizes) and then reverses with the inside positive. The positivity reaches a peak, and then the membrane potential decreases, passes through zero again, and eventually restores the resting potential of -50 to -90 mV (Figure 23.4). At any one spot in the neuron membrane, this complete cycle of polarity changes usually takes about 1 to 5 milliseconds (1 millisecond = 1 ms = 0.001 second). The position of the disturbance of membrane potential travels along the axon at a speed known as the *conduction velocity*. It is this propagation of the nerve impulse along the axon that allows information

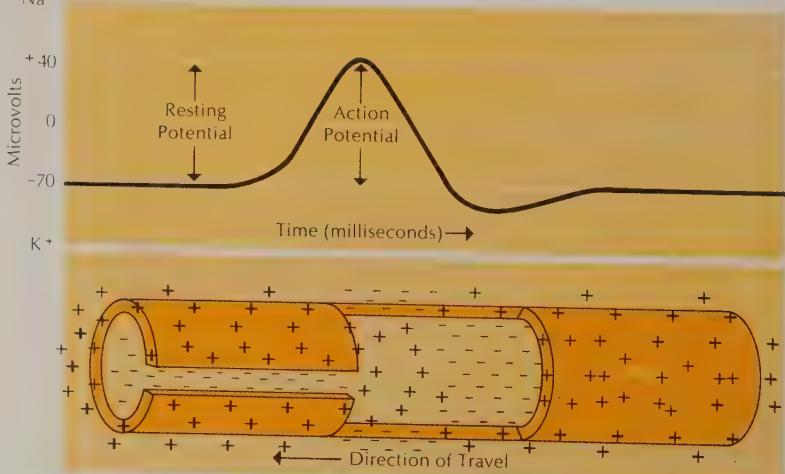


Figure 23.4d. Voltage clamp experiments. Schematic diagram of the apparatus and technique (A); schematic diagram of the feedback amplifier operation (B), and diagram showing component membrane currents (C). Voltage is applied by electrodes on either side of the membrane while an automatic feedback amplifier is used to keep the membrane potential constant at any value selected by the experimenter. Thus, currents resulting from ionic flow across the membrane can be measured when membrane potential is displaced to and held at some fixed value.

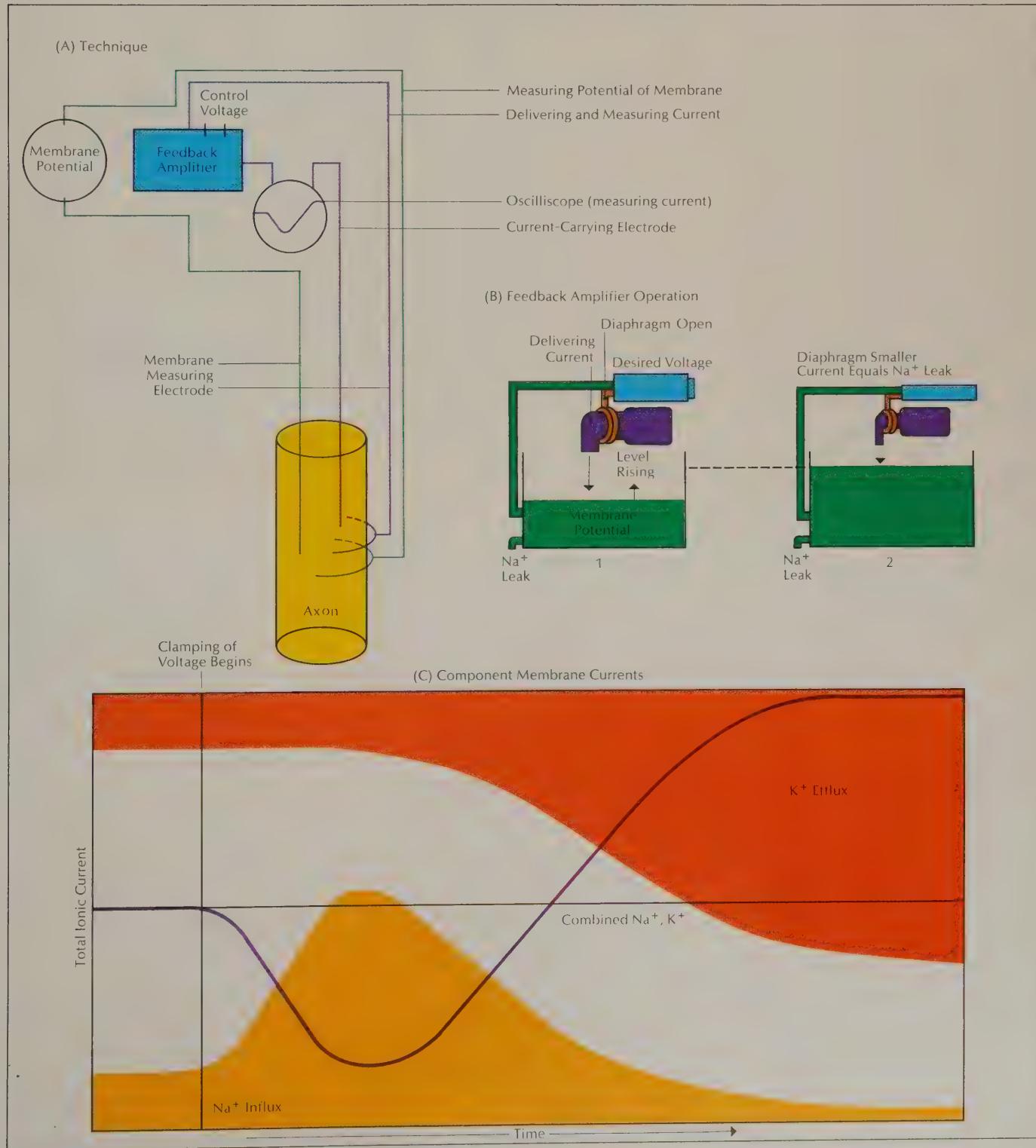
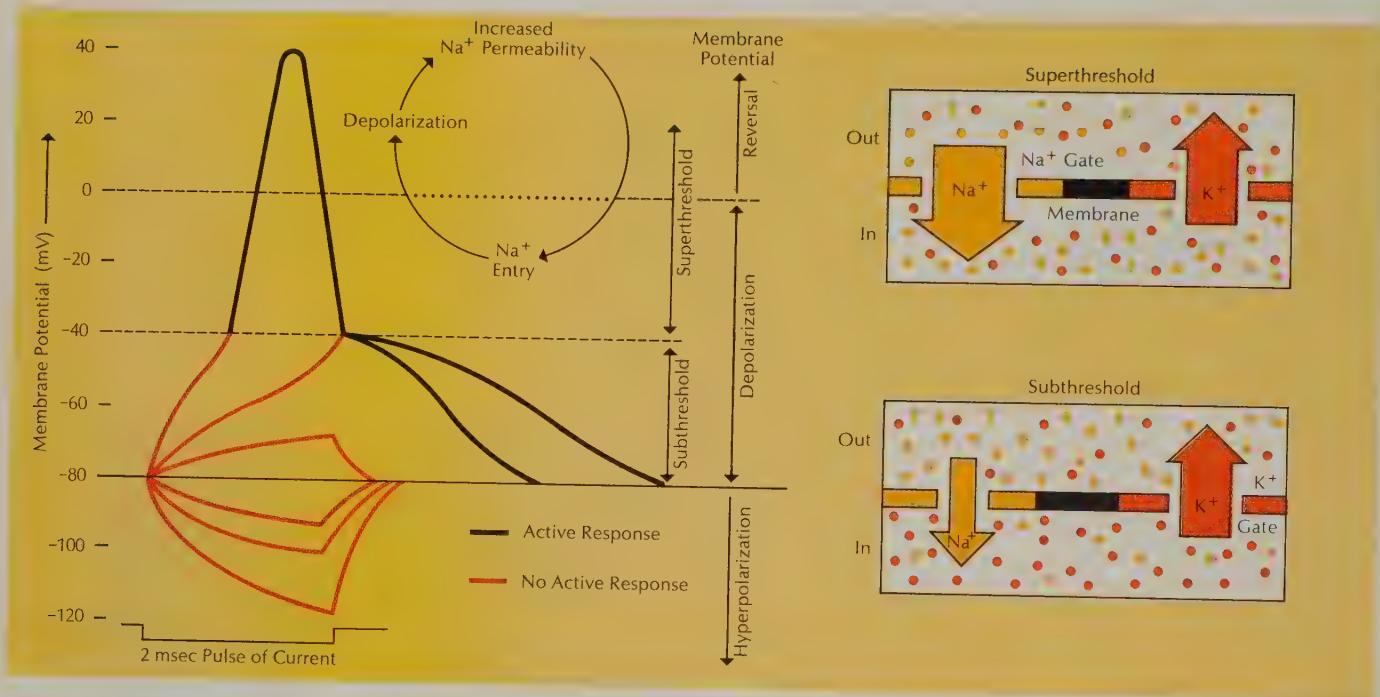


Figure 23.4e. Impulse initiation by local depolarization. Current pulses of fixed duration (indicated below the curves), but of variable size and polarity, cause membrane potential variations represented by the family of curves shown above. Inset diagrams indicate sodium (Na^+) and potassium (K^+) flow through the membrane.

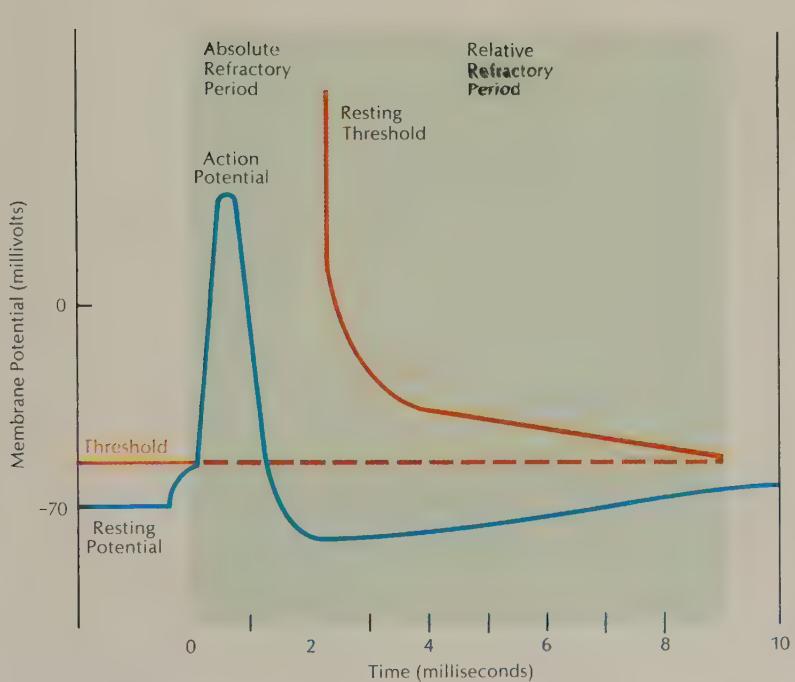


mation to move from one place to another in the nervous system. The nerve impulse is "all-or-none" — it either happens or it does not, and its size, duration, and velocity do not depend on the nature of the stimulus that triggered it. The nerve impulse is *nondecremental* — that is, it does not decrease its size as it travels along the axon from its origin to its destination.

The mechanism by which the impulse is generated and conducted along the axon is now relatively well understood, although there is still much to be learned about its biophysical mechanism. Information about the nerve impulse has come from studies of the giant axon of squid (Hodgkin and Huxley, 1939). Because this axon can be up to 1 mm in diameter, electrical and chemical studies are much easier to make with the squid neuron than with mammalian neurons, which have axons less than 20μ in diameter. Painstaking work with small neurons subsequently has confirmed that the processes of impulse transmission are much the same.

The resting potential is caused by differences in concentrations of certain ions inside and outside the nerve cell. In squid and in most vertebrate neurons, the sodium (Na^+) concentration outside the cell is 10 times greater than the concentration inside, whereas the potassium (K^+) concentration inside is at least 20 times greater than that outside. The Nernst equation, developed by physical chemists, tells what potential must exist across the membrane to keep any particular ionic type in equilibrium when it diffuses freely across the membrane — that is, to have the diffusion inward equal the diffusion outward. This *equilibrium potential* for each ion type is determined by the ratio of its concentrations outside and inside the cell. Another principle of physical chemistry states that the actual membrane potential will depend upon the equilibrium potentials of all the different ion types that can cross the membrane. The relative importance of each ion type in the determination of the membrane potential depends upon the ease with

Figure 23.5. Excitability potential. Ordinate is transmembrane potential; abscissa is time. The absolute refractory period of 0.5 ms follows the peak of an action potential. During this time, the neuron membrane cannot be made permeable to sodium (Na^+) ions. The relative refractory period represents a recovery period where the threshold depolarization necessary to turn on sodium permeability gradually returns to normal.



which that ion can cross the membrane—its *ion permeability*. Thus, if K^+ permeability is much higher in the resting axon than Na^+ permeability, the resting potential will be closer to the potassium equilibrium potential (typically about -90 mV) than to the sodium equilibrium potential (typically about $+60\text{ mV}$). The resting potential of the giant squid axon typically is about -70 mV (Hodgkin, 1958).

This explanation of the resting potential is important for an understanding of the action potential. If something caused the axon membrane to become much more permeable to Na^+ than to K^+ , then the membrane potential would change toward the $+60\text{ mV}$ sodium equilibrium potential. A. L. Hodgkin, A. F. Huxley, and Bernard Katz (1952) performed what is now called a voltage-clamp experiment. In this experimental technique, voltage is applied by electrodes on either side of the membrane. An automatic feedback amplifier is used to keep the potential across the membrane constant at any value desired by the experimenter. Thus, the currents caused by ions moving across the membrane can be measured when the membrane potential is displaced to and held at some fixed value.

In their experiment, Hodgkin, Huxley, and Katz rapidly depolarized the membrane by a certain amount and then held the membrane potential constant at the new level. If the depolarization exceeds a minimum, or *threshold* amount, the Na^+ permeability of the membrane suddenly increases. Sodium ions carrying positive charge rush into the axon. If no equivalent inward current is applied, the membrane potential would rapidly approach the sodium equilibrium potential of about $+60\text{ mV}$. However, with the membrane potential held constant, after a few milliseconds the Na^+ permeability decreases to almost its original level, whereas the K^+ permeability increases greatly. If the membrane voltage were not “clamped,” this change would cause the membrane to return to a potential more negative than its

resting level—nearly to the K^+ equilibrium potential of about -90 mV.

These findings about changes in ionic conductance enabled Hodgkin and Huxley (1952) to give an accurate description of what happens to a patch of membrane as an action potential passes. Currents from an adjacent patch of membrane depolarize it, causing Na^+ permeability to increase. Sodium ions rapidly move into the cell, further decreasing the voltage difference and further increasing the permeability to sodium ions. This process continues for a fraction of a millisecond; sodium ions moving into the cell make the interior more positive than the exterior, approaching the sodium equilibrium potential. At the end of this brief period of activation, the membrane returns to a normal permeability for sodium ions but has become more permeable to potassium ions, which now move out of the cell toward the lower K^+ concentration of the exterior fluid. As K^+ ions leave the cell, within a few milliseconds the potential difference across the membrane changes, first to a value close to the potassium equilibrium potential, which is more negative than the normal resting potential. The K^+ permeability returns to its normal value, and the membrane gradually returns to the resting potential.

In the voltage-clamp experiment, the Na^+ permeability of the membrane increases gradually as the membrane is depolarized. There is no sudden change in Na^+ permeability as the threshold potential is reached. The sudden development of the action potential is related to the balance between movement of Na^+ and K^+ ions. For depolarizations smaller than the threshold value, the current of K^+ ions flowing outward is greater than the current of Na^+ ions flowing inward, and the net current depolarizes the membrane to its resting potential. When the membrane is depolarized beyond threshold value, the inward current of Na^+ ions exceeds the outward current of K^+ ions, thus further depolarizing the membrane and further increasing the Na^+ permeability. Therefore, once the membrane is depolarized beyond the threshold value, further depolarization occurs at an accelerating rate and the action potential is rapidly developed. The triggering of the impulse is thus a self-regenerative process.

It is easiest to understand propagation of the impulse along the axon in the case of myelinated axons. Because the myelin sheath has very high electrical resistance except at the nodes of Ranvier, most currents must pass through the membrane at these nodes (Tasaki, 1939). As an action potential develops at one node, the sodium influx causes electric current to flow down the axon to the next node of Ranvier. Because of the membrane's electrical capacitance and resistance, the current causes the next node to depolarize beyond the threshold point, increasing Na^+ permeability and triggering an action potential. The action potential jumps from node to node and thus is propagated along the axon.

The propagation process is similar in an unmyelinated nerve. Currents caused by increases in Na^+ permeability at one patch of membrane cause depolarization of adjacent membrane patches, which become active and cause the process to be repeated and the impulse to be propagated. The speed of conduction, or propagation, is influenced by the diameter of the axon (the speed is greater for larger fibers) and by the extent of myelination. The speed ranges from less than 0.1 meter per second to 160 meters per second in different kinds of neurons found in various animal species.

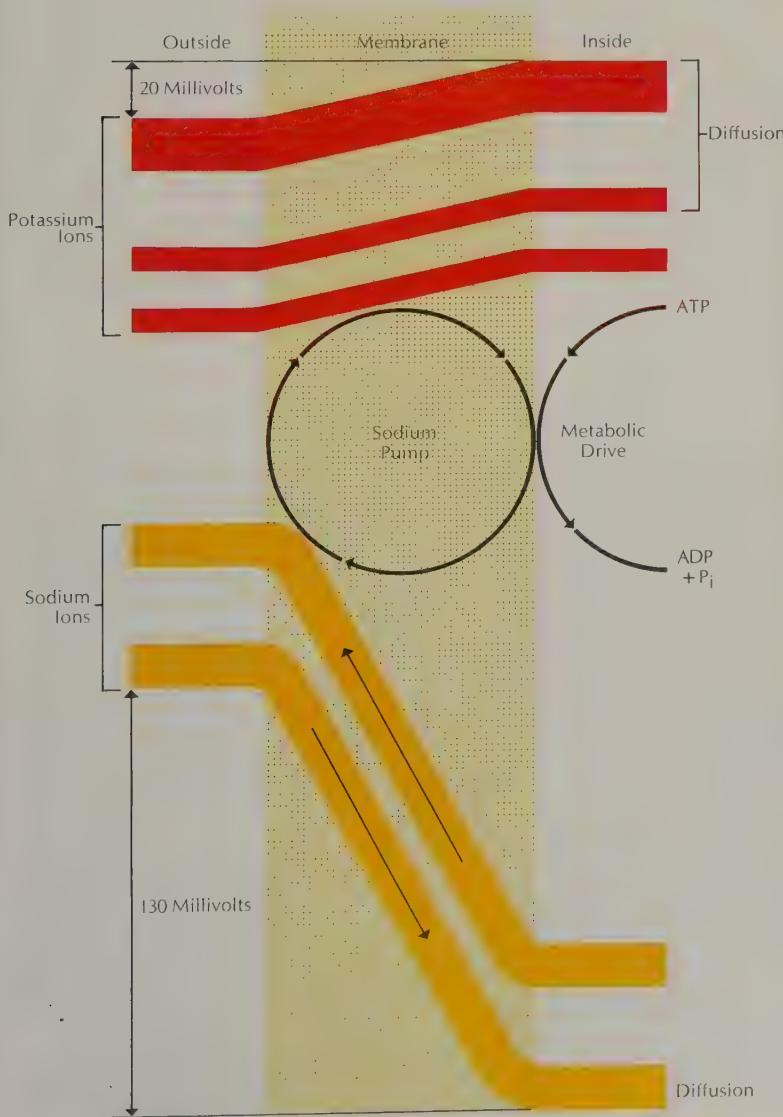
For a period of about 0.5 ms after the peak of an action potential, the neuron membrane cannot be made permeable to sodium ions, no matter

Figure 23.6 (left) Schematic diagram of the ionic pump mechanism. Sodium ions must be pumped out of the neuron and potassium ions pumped in. Because the resting potential is fairly close to the potassium equilibrium potential, but far from the sodium equilibrium potential, the major effect of the ionic pump is the movement of sodium out of the cell. As indicated in the diagram, ATP energy is used to operate the sodium pump active transport mechanism.

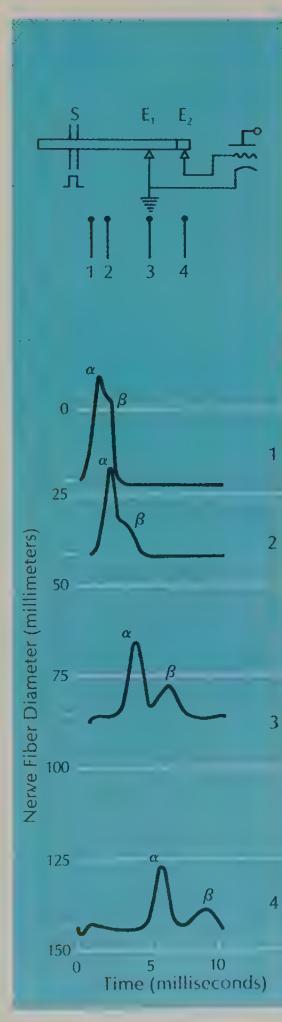
Figure 23.7 (right). Compound action potentials. The total action potential of the sciatic nerve of a bullfrog

what the depolarization. During this *absolute refractory period*, a new action potential cannot be generated, no matter how intense a stimulus is applied. In the next few milliseconds, there is a gradual recovery in the ability of membrane depolarization to effect an increase in Na^+ permeability. During this recovery period, or *relative refractory period*, the threshold depolarization necessary to turn on the Na^+ permeability (thereby causing another action potential) gradually returns to normal.

The changes during passage of an action potential do not result in large differences in the ionic concentrations inside and outside the neuron. Only about 0.1 to 0.0001 percent of the potassium ions inside axons are lost during the passage of an impulse. However, because neurons are known to be able to carry millions of impulses during their lifetime, it is clear that some mechanism must exist to pump sodium ions out of the neuron and to pump



shows several curves, which represent the impulses carried by different size classes of axons. Propagation velocity of the α and β waves is constant in this diagram. S is the stimulating electrode; E_1 and E_2 are recording electrodes, the latter at the killed end of the nerve. Ordinate is the distance from recording electrode E_1 ; abscissa is time. The starting points of the oscillograph trace show the distances at which the records were taken.



potassium ions in. This mechanism must also counteract the slower diffusions of ions that occur in the resting neuron, as Na^+ leaks in and K^+ leaks out along concentration gradients. Because the ions must be moved against concentration gradients by this mechanism, energy is required to operate the "ion pump."

Because the resting potential is fairly close to the potassium equilibrium potential but far from the sodium equilibrium potential, the major effect of the ion pump must be the movement of sodium ions out of the cell. Studies also suggest that the sodium pump moves potassium ions into the cell. The sodium pump operates slowly but steadily, so that the intermittent entry of sodium ions during passage of impulses is balanced by the continuous expulsion of sodium ions by the pump. Although several transport models have been proposed, the mechanism by which ions are moved across the membrane is not known.

Chemical energy (ATP) is used to operate the sodium pump (Hodgkin and Keynes, 1955; Caldwell, et al., 1960). Because a neuron can continue to generate and transmit impulses for a long time in the absence of oxygen and even when the process of glycolysis is inhibited, it appears that ATP is stored in large quantities near the membrane (Dunham and Glynn, 1961).

The movements of ions by action potentials and by pumps have been confirmed by radioactive isotope studies. Thus, the theory of an ionic basis for the nerve impulse has been generally accepted. The outstanding problem for current research is to discover the mechanism by which the ionic permeabilities of the membrane are changed during the passage of an action potential.

Action Potentials in Nerve Bundles

Axons differ in diameter and degree of myelination, even within a particular sensory nerve bundle in an individual mammal. It is a general property of neurons that conduction velocity increases as axon diameter increases. Often, axon diameters and conduction velocities within a single nerve bundle fall into several distinct classes. If an electrical stimulus is applied extracellularly to a nerve bundle, a larger current is needed to depolarize a small-diameter axon to threshold than is required to trigger an impulse in a large-diameter axon. These facts can explain many observations made about the conduction of impulses in a nerve bundle containing fibers of many different diameters.

First, the stronger the stimulus, the greater the amplitude of the response. Although each axon's action potential is all-or-none, the whole nerve acts in this graded manner because as the shock strength is increased, the number of smaller axons or fibers that reaches threshold increases. Second, several humps often are seen on the total action potential. Each hump represents impulses carried by one size class of axons. If the time of arrival of each hump is measured at two distances from the stimulating electrode and the distance between the two sets of recording electrodes is known, then the conduction velocity associated with each size class can be calculated.

These facts explain certain responses in sensory nerves. Different sense organs often have different sizes of axons, and not all sense organs of the same type have the same threshold of excitation or the same diameter of axon. If a person touches a hot stove, he feels the pressure of touching the object long before he feels pain or heat. This experience is largely because touch receptors have thick myelinated fibers, whereas fibers involved in

sensations of pain and heat are smaller in diameter and many are not myelinated. This latter group accounts for the delayed pain felt about one second after the pressure.

ACTIVITY OF NEURONS: SYNAPTIC TRANSMISSION

The action potential explains the conduction of information from one place to another along a single axon. However, there must be a way to pass information to another neuron or to an effector, such as a muscle. Similarly, the neuron must be able to receive information in order to initiate an action potential. Except for pacemaker cells, which generate impulses in the absence of known external stimuli, and some sensory cells, a neuron receives and transmits information at synapses. The most common type of synapse in the vertebrate nervous system is the *chemical transmitting synapse*; one neuron (the *presynaptic cell*) excites another (the *postsynaptic cell*) through the release of chemical transmitters from the terminal points of axon filaments. These chemicals diffuse across a small, fluid-filled space (the *synaptic cleft*) between the neurons, act on receptor sites, and thus induce electrical changes in the dendrite or cell body of the neuron with which they come in contact. The unit composed of the presynaptic terminal, the synaptic cleft, and the postsynaptic receptor membrane is known collectively as the synapse. Only since the development of the electron microscope has the structure of the synaptic junction been examined in detail (Figure 23.8).

Each axon filament ends in a presynaptic knob, or *bouton*. Hundreds or thousands of these boutons may lie on the surface of the cell body and dendrites of a single neuron. Electron micrographs reveal that each bouton is separated from the membrane of the postsynaptic cell by a synaptic cleft about 200 Å in width. At least three different types of synapses can be distinguished in electron micrographs (Figure 23.9). In *axodendritic* synapses, a small spine often extends from the surface of the dendrite toward the

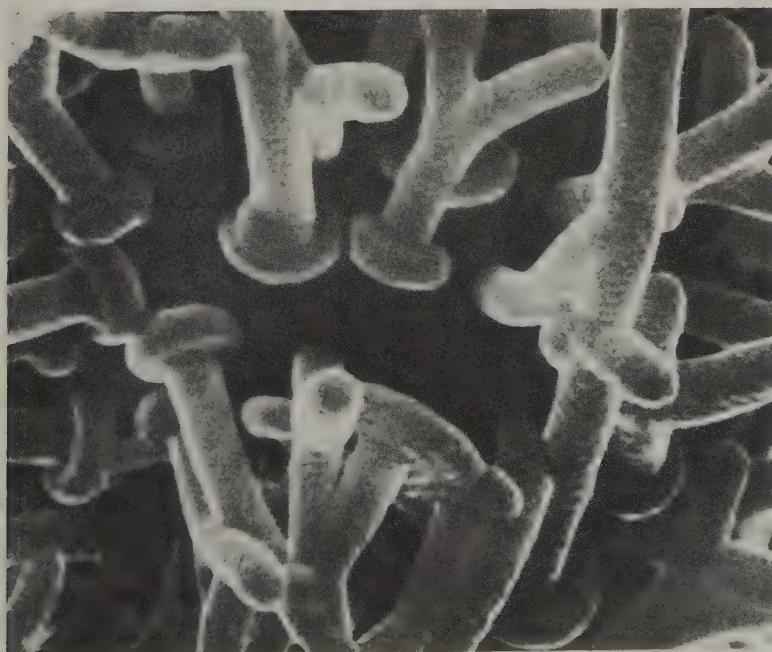


Figure 23.8 (left) Electron micrograph of the ultrastructure of a synaptic junction. Note the synaptic knobs or boutons.

Figure 23.9 (right). Various types of synaptic junctions.

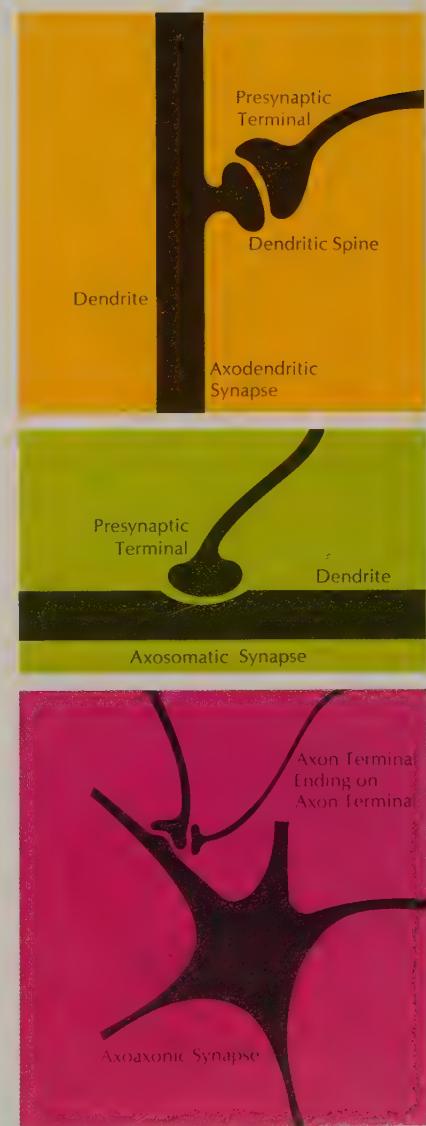
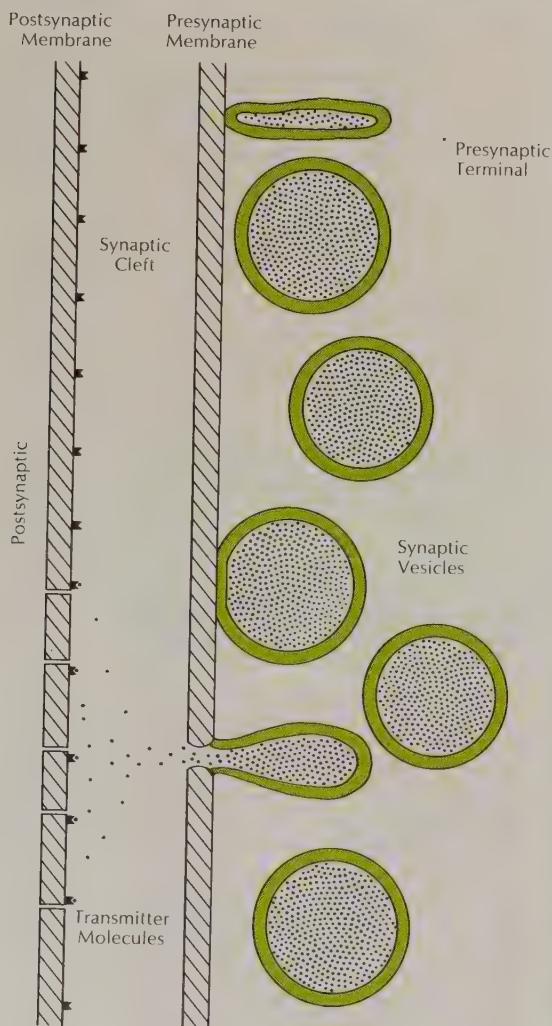
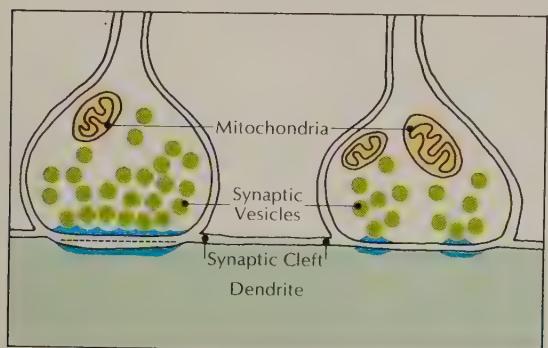


Figure 23.10. Synaptic knobs (above) release short bursts of chemical transmitter substance into the synaptic cleft, where this substance acts on the surface of the nerve cell membrane below. Molecules of the chemical transmitter are stored in vesicles prior to release. The schematic diagram (below) represents a portion of a synaptic cleft with synaptic vesicles in close proximity in the presynaptic terminal. Note the vesicles discharging transmitter molecules into the synaptic cleft. Some transmitter molecules are shown combined with receptor sites on the postsynaptic



membrane, thus facilitating membrane pore dilation. At the lower right is an electron micrograph of a synaptic knob containing synaptic vesicles (round bodies).

bouton; this type of synapse is found only on dendrites. In axosomatic synapses, which are found both on the cell body and on the large stumps of dendrites near the cell body, the bouton lies close to the postsynaptic cell membrane and there is no spine. A xoaxonic synapses involve a bouton that synapses on a bouton from another axon, but these synapses are relatively rare and have been observed mainly in sensory pathways.

Release of Transmitter Substance

When an action potential propagates to the axonal arborization of the pre-synaptic cell, the membranes there depolarize. Depolarization of the axon terminal causes transmitter substance to be released into the synaptic cleft. Although the details of this process are not completely understood, there is enough known to formulate a viable theory. P. Fatt and Bernard Katz (1950, 1951, 1952) studied the synapse between a motor nerve and muscle cell by recording intracellularly from single muscle fibers in the region of the synaptic junction. They discovered that when no action potentials are coming down the nerve, small depolarizations of about 0.5 mV occur spontaneously at random intervals. They called these depolarizations *miniature endplate potentials* (MEPPs). An increase in the concentration of calcium ions in the solution bathing a synapse causes an increase in the mean frequency of MEPPs. Fatt and Katz suggested that each MEPP represents the spontaneous release of a "quantum," or packet, of the chemical transmitter.

When a nerve action potential comes along, they theorized, the quanta are released at a higher rate than normal for a short time. A large number of MEPPs so evoked causes a large depolarization, the excitatory postsynaptic potential. Another experiment proved the accuracy of this hypothesis. Calcium concentration was reduced in the extracellular fluid, causing a drastic decrease in the frequency of MEPPs but no change in the size of each MEPP. When a nerve was stimulated, a small jagged potential occurred, which was clearly the sum of a few MEPPs. As the external Ca^{++} concentration was increased, the number of MEPPs composing the synaptic potential increased until a smooth normal potential was seen. This quantal mechanism of synaptic transmission has been verified for many types of synapses in vertebrate and invertebrate nervous systems. Apparently, calcium attaches to and passes through the presynaptic membrane when the axon terminals are depolarized. When many Ca^{++} ions reach the inner surface of the membrane, a nearby synaptic vesicle "fuses" with the

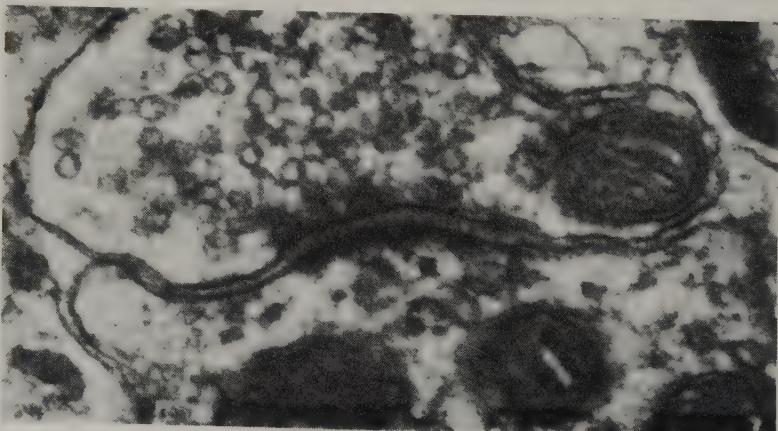


Figure 23.11. Presynaptic inhibition. Cell A has an excitatory synapse on a dendrite of cell C and causes an EPSP in C (as monitored by an intracellular microelectrode) whenever it fires an impulse. If A and B are fired together, a smaller EPSP is seen in C; thus B has an inhibitory effect. Because B fired by itself causes no IPSP in C, it does not have an inhibitory synapse on C but instead must inhibit the effect of B's excitatory synapse.

membrane, dumping its contents (one quantum of transmitter chemical) into the synaptic cleft. Hundreds of quanta may be emptied into the cleft in a single synaptic event at a single synaptic junction.

The release of some chemical transmitter substances into the synaptic junction results in excitation of the postsynaptic cell—a phenomenon called *postsynaptic excitation*—and leads to the production of an action potential in that neuron. However, movement of chemical transmitter substances across some synapses inhibits rather than excites the discharge of the postsynaptic cell. Such an occurrence is known as *postsynaptic inhibition*. In vertebrates, axodendritic synapses usually are excitatory and axosomatic synapses usually are inhibitory, although in a variety of complex synaptic structures in the central nervous system, these generalizations cannot easily be applied (Eccles, 1964).

This picture is complicated further because axoaxonic synapses, in which the axonal terminals of one cell make synapses on the axonal terminals of another cell, affect *presynaptic inhibition*. A special inhibitory transmitter substance from A may decrease the amount of transmitter substance released by an impulse in B, an effect called presynaptic inhibition. Probably there are cases where both excitatory and inhibitory synapses are modulated by these presynaptic interactions.

Throughout the central nervous system, inhibitory synapses counteract the generation of impulses by excitatory synapses. At every synapse of the central nervous system that has been thoroughly investigated, there is a conflict of excitatory and inhibitory action on a single neuron. Apparently, few excitatory synapses have the unchallenged power to excite a nerve impulse in the postsynaptic cell. If there were no inhibitory synapses, a single impulse might cause an explosive spread of excitation throughout the neuronal networks of the nervous system—in other words, convulsions such as those that occur in epilepsy.

In general, an individual neuron is converged upon by many axon terminals, each of which can contribute a quantity of transmitter substance to activate or inhibit the postsynaptic cell. The frequency of discharge for a particular cell at a particular time depends upon (1) the relative quantities of excitatory and inhibitory transmitter substances acting on the membrane of the postsynaptic cell and (2) the stimulus threshold of the cell at the particular time. More than 10,000 synapses may converge upon a single large neuron. The activity of the neuron is influenced by inhibitory and excitatory effects from a very large number of other neurons. Whether or not an impulse is generated in the postsynaptic cell depends upon the summation of the effects of activity in the hundreds of presynaptic cells, and their activity in turn is influenced by many thousands or millions of other neurons. For each synaptic event, only about one millisecond is required. The potentialities of the vertebrate nervous system for the control of complex behavior patterns can readily be appreciated.

Mechanism of Synaptic Transmission

The nature of synaptic transmission was a subject of debate for many years. The available evidence seemed to support the theory that an action potential in the presynaptic cell creates electrical fields that induce a potential in the postsynaptic cell. There are indeed many examples of *electrical synapses* in invertebrates and in simpler vertebrates. However, today an overwhelming amount of evidence indicates that in mammals the transmission

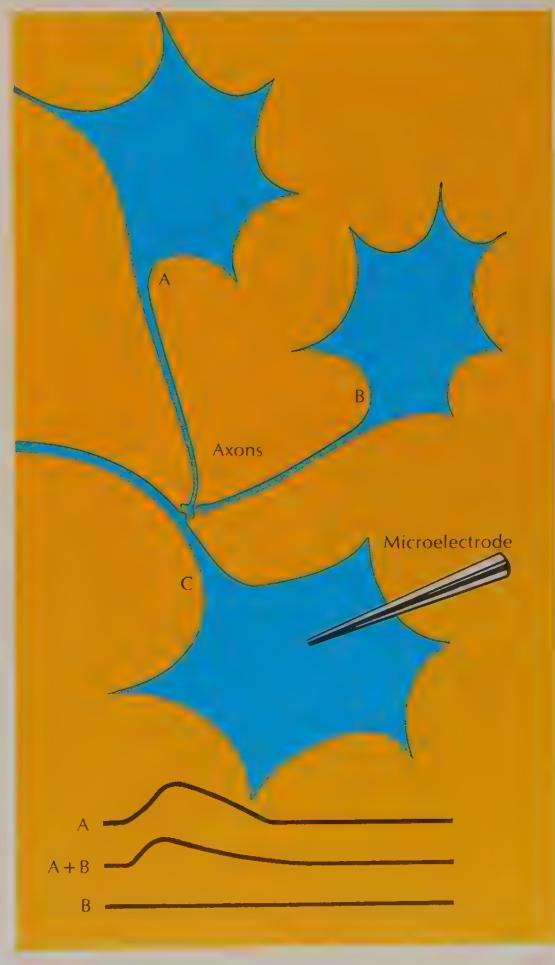


Figure 23.12. Photomicrograph of a motor neuron with its radiating dendrites and axon. Superimposed on the photograph is a drawing of a microelectrode enlarged 5 times but shown as it would be located for intracellular recording.



across the synaptic junction is accomplished by chemical rather than electrical means. It is interesting to note that John C. Eccles, who was a proponent of the electrical theory in the 1940s, shared the 1963 Nobel Prize largely for his research that provided firm evidence of the chemical mechanism of synaptic transmission.

Great advances in the study of synaptic transmission became possible in 1951 with the development of techniques for recording potentials within a single neuron. A fine glass pipette with a tip diameter of about 0.5μ is filled with a conducting salt solution. If such an electrode is carefully inserted into certain kinds of nerve cells that have been rigidly fixed in place, the cell membrane seals around the glass microelectrode, thus preventing the flow of a short-circuiting current from the inside to the outside of the cell. If this sealing takes place, the nerve cell appears to behave normally for hours. Figure 23.12 shows the dimensions of a microelectrode superimposed on a microphotograph of one of the large neurons that innervates muscle (a motor neuron). Early investigators were fortunate in choosing motor neurons for this research, because intracellular recording has proved to be much easier and more informative in these cells than in any other kinds of neurons. However, in recent years, many other kinds of neurons have been studied in this way with useful results.

Figure 23.13 shows intracellular records of a simple case of excitatory synaptic action. A single set of synaptic excitations causes the potential across the postsynaptic cell membrane to depolarize rapidly and then to return slowly to its resting value. As the number of activated synapses increases, the amplitude of the postsynaptic depolarization becomes larger. In fact, the total magnitude represents a simple summation of the depolarizations produced by each individual synapse. When the depolarization reaches a critical magnitude (in this case, a change of +18 mV, from the resting potential of -70 mV to a potential of -52 mV), an impulse is discharged in the postsynaptic cell. The only effect of further strengthening the synaptic stimulus is to cause a slightly earlier generation of the impulse, which occurs in every case when potential reaches -52 mV.

The depolarizing potential produced in the postsynaptic membrane by the excitatory synapse is called an *excitatory postsynaptic potential*, or EPSP. If the EPSP reaches the threshold potential of the postsynaptic cell, a nerve impulse is discharged. Extensive investigation of a wide variety of nerve cells in the central system indicates that this is the general mechanism of synaptic transmission.

Activation of an inhibitory synapse commonly has just the opposite effect; it causes a further polarization, or hyperpolarization, of the postsynaptic membrane. The effects of individual inhibitory synapses summate in exactly the same way as do excitatory synapses. Because the *inhibitory postsynaptic potential*, or IPSP, opposes the action of the EPSP, the effects of an excitatory and an inhibitory synapse counteract one another.

Excitatory synapses act by increasing the permeability of the postsynaptic membrane to sodium and potassium ions—an effect sometimes called opening the sodium and potassium ionic gates—which results in a net inward current that depolarizes the electrical potential across the membrane. This depolarization can be explained in the same way as the resting potential. In the resting cell, membrane potential is close to the potassium equilibrium potential because the membrane is much more permeable to K^+ than to Na^+ . An EPSP increases both K^+ and Na^+ permeabilities, causing

Figure 23.13 (below). Current flow and resulting membrane potential changes in excitatory (red) and inhibitory (blue) synapses. Transmitter substance at an excitatory synapse increases the membrane's permeability to both Na^+ and K^+ ions, resulting in more Na^+ entering the cell than K^+ leaving the cell. The net effect is thus an increase in membrane potential (red curve), which reaches a peak and slowly returns to normal. The inhibitory transmitter in many vertebrate synapses increases the membrane's permeability to K^+ (and sometimes Cl^-) and leads to an efflux of K^+ , which

generates a decrease in membrane potential (blue curve). If both excitatory and inhibitory synapses are activated in the same neuron, the resulting potential change (purple) will be located between the excitatory and the inhibitory responses. This result is called "spatial summation."

the membrane potential to rise to a value about halfway between the equilibrium potentials of these two ions. The membrane potential then returns to the resting potential as both Na^+ and K^+ permeabilities return to normal. If the synaptic membrane could be artificially charged to a potential value more positive than the halfway point between Na^+ and K^+ equilibrium potentials, the action of the transmitter substance should cause an inverted synaptic potential, which does happen.

The transmitter substance of an inhibitory synapse causes the postsynaptic membrane to become permeable to potassium and chloride ions. Opening the potassium and chloride ionic gates results in a net outward flow of current, hyperpolarization of the postsynaptic membrane, and development of an IPSP. The generation of the IPSP can be explained in a manner parallel to the explanation of EPSP. Potassium and chloride potentials are more negative than the resting potential, and when these ionic permeabilities increase, the membrane potential decreases toward the lower equilibrium potential. As the transmitter substance is dissipated, the K^+ and Cl^- permeabilities return to this level, and the resting potential is restored. Figure 23.14 shows how IPSPs can be turned upside down by artificially decreasing the membrane potential to a value more negative than the combined K^+ and Cl^- equilibrium potentials.

In the cells of the spinal column, the effect of an IPSP lasts only about 8 ms, slightly less than the 10 ms duration of an EPSP. In the brain, however, the effects of an IPSP last for 100 to 200 ms or more; in the neurons of the brain, a single activation of an inhibitory synapse may counteract many successive activations of excitatory synapses.

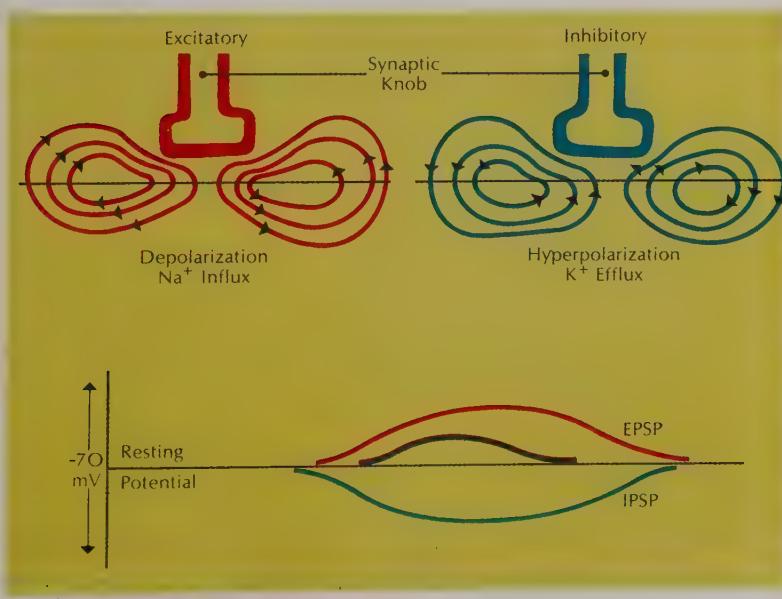


Figure 23.14 (right). IPSP reversal potential. In this diagram, two intracellular microelectrodes are placed in the same neuron. With one microelectrode, positive or negative current is transmitted into the cell, thus setting the membrane potential above (5–8) or below (1–3) the normal resting potential (4). With the other microelectrode, the additional potential change is recorded in the neuron as an excitatory synapse (red) is first stimulated, then an inhibitory synapse (blue). The height of the excitatory postsynaptic potential (EPSP) increases as the cell's membrane potential (3, 2, 1) is lowered. If the membrane potential (5) is raised, the height of the EPSP decreases until it disappears (6) and finally reverses its direction (7, 8). Level 6 is called the equilibrium potential for the EPSP (E_{EPSP}) because the EPSP can cause no further shift in membrane potential. Similarly, the size of the IPSP increases as the membrane potential is raised above the resting potential (5–8). The IPSP decreases in size as the membrane potential is lowered, disappears entirely (3), and, on being lowered still further, reverses its direction. Level 3 is therefore called the IPSP equilibrium potential (E_{IPSP}). This fact is explained by the finding that inhibitory and excitatory transmitter substances increase or decrease the synaptic membrane's permeability to different sets of ions.

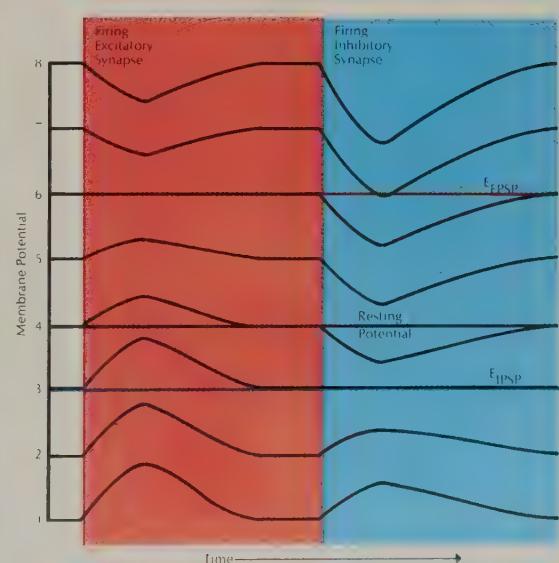
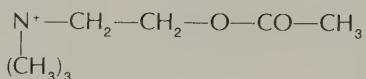
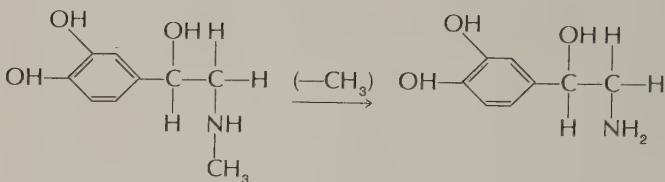


Figure 23.15. Some chemical transmitters.
Acetylcholine and norepinephrine are among the transmitters used at excitatory synapses. Glycine and gamma aminobutyric acid have been recently identified as inhibitory transmitters in the mammalian central nervous system.

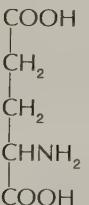
Acetylcholine



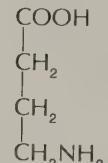
Epinephrine



Glutamic Acid



Gamma Aminobutyric Acid



The evidence for chemical transmission of signals across many synaptic junctions must be regarded as conclusive. With few exceptions (among invertebrates), a given presynaptic neuron has only excitatory or only inhibitory synapses with its several postsynaptic cells. Many neurophysiologists believe that a fundamental property of nerve cells is that a given neuron releases only a single transmitter substance from all of its axonal endings. However, it is difficult to establish exactly which transmitter is acting at a given synapse. To prove that a given cell releases a particular transmitter, it must be shown that (1) the cell has the material or machinery necessary to manufacture the transmitter; (2) the transmitter is present at the axon terminal in sufficiently great quantities; (3) stimulation of the neuron causes release of the substance into the synaptic cleft; (4) the postsynaptic cells are sensitive to the substance; (5) a molecular mechanism exists to break down or inactivate the substance after it has done its job, so that it will not accumulate at the synapse and continue to act, thus preventing further transmission of information; and (6) various chemicals that affect synaptic events in the body (such as curare, which prevents EPSPs in skeletal muscle neuromuscular junctions of vertebrates) also have parallel effects on the action of the substance when it is directly applied to the postsynaptic cell.

The advent of the technique of *iontophoresis* has given great impetus to the identification of transmitters. A solution of charged molecules (suspected transmitters) is used to fill a micropipette, which is then placed near a synapse. The micropipette releases a small amount of the substance when an electric current is passed through the pipette. Microchemical techniques, involving chemical assays of very small amounts of synaptic tissues, have also been important, as have histological techniques that show the localization of certain substances. As a result, acetylcholine (ACh) and norepinephrine were found to be among the transmitters used at excitatory

synapses. More recently, glycine and gamma aminobutyric acid (GABA) were identified as inhibitory transmitters in the mammalian central nervous system.

The identification of transmitter substances at the neuromuscular junction and in a few other kinds of synapses was relatively easy because large numbers of similar nerve cells exist together in these locations. In the spinal cord and brain, however, many different kinds of neurons exist in close proximity, and it is impossible to isolate pure samples large enough for ready chemical analysis. Even in the peripheral nerves of mammalian nervous systems, where ACh and norepinephrine were first identified as transmitters, it is not an easy job. ACh was accepted as the transmitter in one set of synapses, for example, but recently epinephrine was shown to have at least an indirect effect there (R. M. Eccles and Libet, 1961).

In the mammalian central nervous system, ACh was shown to be the transmitter at central synapses made by branches of a motor neuron, which also uses ACh at its peripheral branches where they innervate muscles (Eccles, 1957, 1964). Other candidates for central-nervous-system excitatory transmitters (of which there may be several) are ATP, epinephrine, histamine, glutamic acid, and some other amino acids. Identification of other transmitters may be expected as this research continues.

Longstanding candidates for inhibitory transmitters in the mammalian central nervous system have been 5-hydroxy-tryptamine, gamma aminobutyric acid (GABA), epinephrine, and glycine (an amino acid). Recently, glycine was shown to be an inhibitory transmitter in the spinal cord, and GABA was confirmed to be an inhibitory transmitter in the higher levels of the brain.

Drugs that affect normal synaptic function have been extensively used as tools to learn about transmitters and the processes of chemical transmission. Acetyl cholinesterase is an enzyme that inactivates ACh after it has generated an EPSP. The chemical prostigmine blocks action of this enzyme, thus causing excitatory synapses to produce prolonged synaptic depolarization. If prostigmine is applied to a synapse and has this effect, it provides evidence that ACh is the transmitter. Both botulin toxin (the bacterial toxin involved in some kinds of food poisoning) and curare prevent ACh synapses from functioning. Curare apparently acts by competitive inhibition—the drug reacts with the postsynaptic membrane. It does not depolarize the membrane, but it prevents the ACh from attaching to the same sites. The botulin toxin, on the other hand, apparently acts by preventing the release of ACh from presynaptic terminals. These substances produce muscular paralysis and interfere with involuntary functions of the body by preventing the transmission of impulses across synapses where ACh is the transmitter substance.

Small doses of the poison strychnine act as powerful stimulants of nervous activity by causing uncontrolled contractions of muscles and other symptoms of seizure activity. This effect is the result of the drug's action in blocking synapses involved in inhibitory pathways in the spinal cord. When the drug is present, the interneurons involved in feedback and feed-forward inhibition are activated, but their inhibitory synapses fail to exert inhibitory influences on the neurons of the main pathways. Strychnine acts through competitive inhibition of the normal transmitter substance (glycine) in these synapses. Another convulsant drug, bicuculline, antagonizes GABA and thus blocks inhibitory synapses at higher levels of the nervous

Figure 23.16. Diagrams of the two types of inhibitory pathways, recurrent inhibition (A) and afferent collateral inhibition (B). Inhibitory cells are shown in blue.

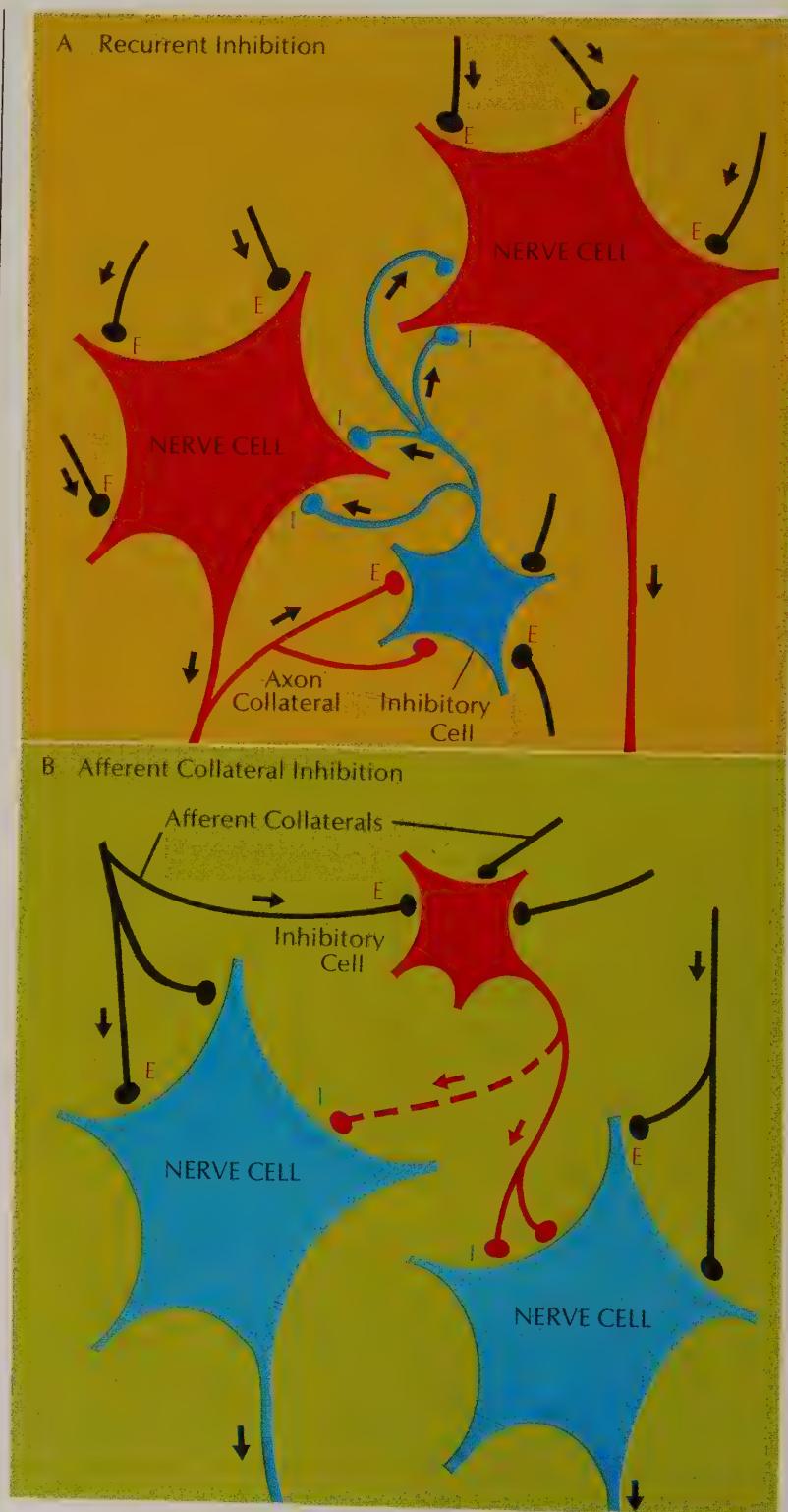


Figure 23.17. Schematic diagram showing pathway to the sensorimotor cortex for cutaneous fibers from the forelimb. Note the inhibitory cells in both the cuneate nucleus and the ventrobasal nucleus of the thalamus. The inhibitory pathway in the cuneate nucleus is of the feedforward type, whereas the inhibitory pathway in the thalamus is of the feedback type.

system. Tetanus toxin—which also inactivates inhibitory synapses—is thought to act by preventing release of transmitter substance from the pre-synaptic terminals of inhibitory synapses.

Many drugs that are commonly used as stimulants or depressants act at the synapses in the central nervous system. The exact nature of their effect cannot be known because not all of the separate connections in the brain nor all of the transmitter substances are known.

Most common nonprescription sleeping pills contain scopolamine, which acts to depress transmission at ACh junctions. Possible side effects of ingesting scopolamine, such as blurred vision and increased heart rate, are the direct results of the drug's action on synapses in parts of the nervous system. The sleep-inducing effects presumably result from some action on the central nervous system at a site or sites as yet unidentified. Barbiturates apparently act by depressing activity at central synapses. Some anesthetics, including ether, evidently act by reducing transmission through interneuron pathways. Still other drugs, such as cyanide, exert their effects upon general processes of cell metabolism, thus affecting the nervous system along with many other systems of the body. It is likely that hallucinogenic drugs also act at the level of the synapse, although little is known about them at this time.

TWO INHIBITORY CONTROL SYSTEMS

As a simple generalization, the two types of postsynaptic inhibitory pathways shown in Figure 23.16 can be regarded as the elementary constituents of all known neural pathways. In *recurrent inhibition*, the axon of a neuron gives off a recurrent collateral, or process, that ends in excitatory synapses on a neuron that has a widespread inhibitory action. Some of the processes from this cell end in inhibitory synapses on the first cell. In this way, whenever the nerve cell discharges an impulse, it automatically activates a recurrent inhibitory pathway, which tends to prevent further discharges from that cell and other similar cells.

In contrast to this recurrent, or feedback, inhibition is *afferent collateral inhibition*, where an afferent nerve fiber (one carrying impulses toward the central nervous system) synaptically exciting a nerve cell gives off a collateral branch that also excites an inhibitory cell. The inhibitory cell, in turn, exerts inhibitory synaptic action on other functionally related neurons—in effect, a feedforward inhibitory action.

After extensive investigations of mammalian central nervous systems, no exception has been found to the rule that nerve cells are always completely inhibitory or completely excitatory in action at all of their synapses; no ambivalent cells with some excitatory and some inhibitory synaptic terminals have ever been found. It is therefore justifiable to label cells as inhibitory or excitatory, according to the nature of their synapses. All primary afferent pathways—the pathways from receptor organs to the spinal cord and brain—are made up of excitatory neurons. These pathways exert inhibitory effects only by synaptic relays through inhibitory neurons (Figure 23.16).

Figure 23.17 illustrates in diagrammatic form the flow of information from skin receptors to the brain. Impulses from the arm travel up the spinal cord in the cuneate nerve tract to relay cells in the cuneate nucleus, where afferent collateral, or feedforward, inhibition may occur. The pathway then crosses to the opposite side of the brain and relays in the thalamus, where recurrent, or feedback, inhibition may occur. The main cells involved in

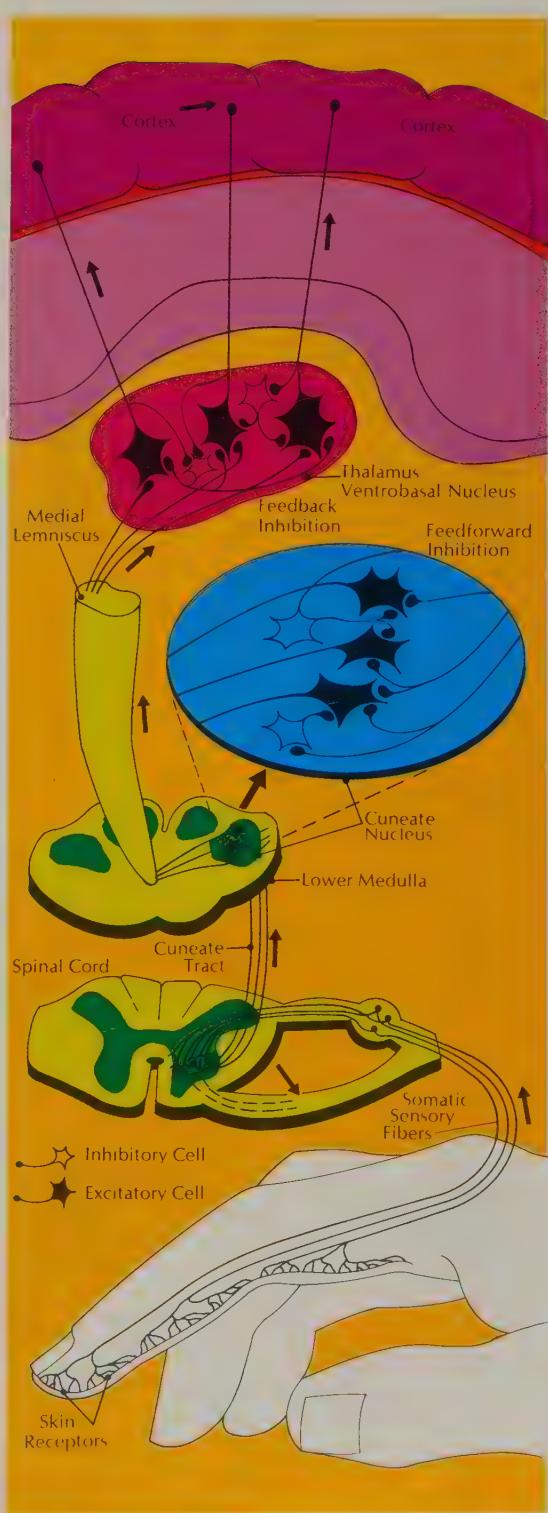


Figure 23.18 (above). Schematic diagram showing postulated connections of specific afferent fibers to the neocortical pyramidal cells. The inhibitory interneurons with their inhibitory synapses are shown in green. All other stellate cells and the pyramidal cells are assumed to be excitatory. Arrows indicate the directions of impulse propagation. Note that the inhibitory path is through inhibitory cells activated either directly by the afferent fibers or by mediation of excitatory interneurons. Also note the various degrees of complexity of the excitatory pathways to the pyramidal cells.

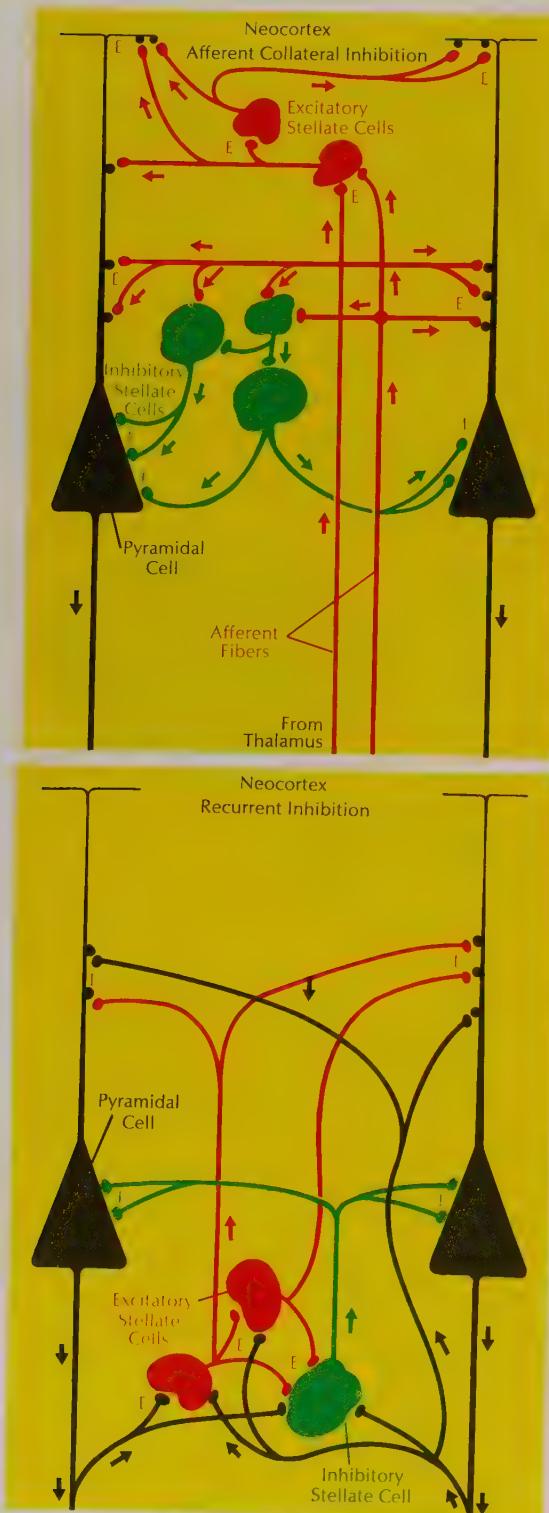


Figure 23.19 (below). Schematic diagram showing postulated synaptic connections of axon collaterals of pyramidal cells. The single inhibitory interneuron together with its inhibitory synapses on the somata of the pyramidal cells are shown in green. All other stellate cells and the pyramidal cells are assumed to be excitatory and are shown in red. Arrows indicate directions of impulse propagation. Experimental evidence suggests that both excitatory and inhibitory pathways can include interpolated excitatory interneurons.

the thalamus have processes leading directly to the cerebral cortex, where neural activity produces effects of conscious experience.

It may seem surprising that the information moving along neural pathways to the brain is subjected to inhibition at each of the synaptic relays. This arrangement serves to separate meaningful signals from "background noise," which originates from random discharges of neurons, and to neutralize the effects of abnormal connections in the nervous system. The nerve fibers grow and interconnect in the developing organism, and there is always the possibility that some disorganization will occur in such an immensely complicated growth process. The inhibition exerted at each synaptic relay tends to eliminate any isolated or random signals, causing the volleys of meaningful impulses from the receptors to stand out sharply.

Figure 23.18 shows schematically the pathways that carry impulses from the thalamus to the cerebral cortex of the brain. Both excitatory and inhibitory neurons lie on the pathways to the pyramidal cells of the cortex. However, this diagram is extremely simplified, and the actual patterns of neuronal activation must be infinitely more complicated. Similar pathways for afferent input to the cortex exist not only for the impulses relayed from various peripheral receptors through thalamic nuclei but also for looping associative fibers. These fibers carry impulses from the pyramidal cells of any area on the cortex to a wide surrounding area of cortex and also to the opposite hemisphere of the brain. Thus, pathways of an almost infinite degree of complexity are built up.

A further complication is illustrated in Figure 23.19, which shows the negative feedback through axon collaterals of the pyramidal cells. In fact, inhibitory feedback usually occurs not directly through an inhibitory interneuron but through one or more excitatory interneurons leading to and from the inhibitory interneuron. These complications make it almost impossible—at least with existing techniques—to trace the complete pathway of any particular afferent input through the brain. Although much has been learned about the general operation of the central nervous system, the preparation of a "wiring diagram" of any major part of the brain is still far in the future.

WHY SO MANY SYNAPSES?

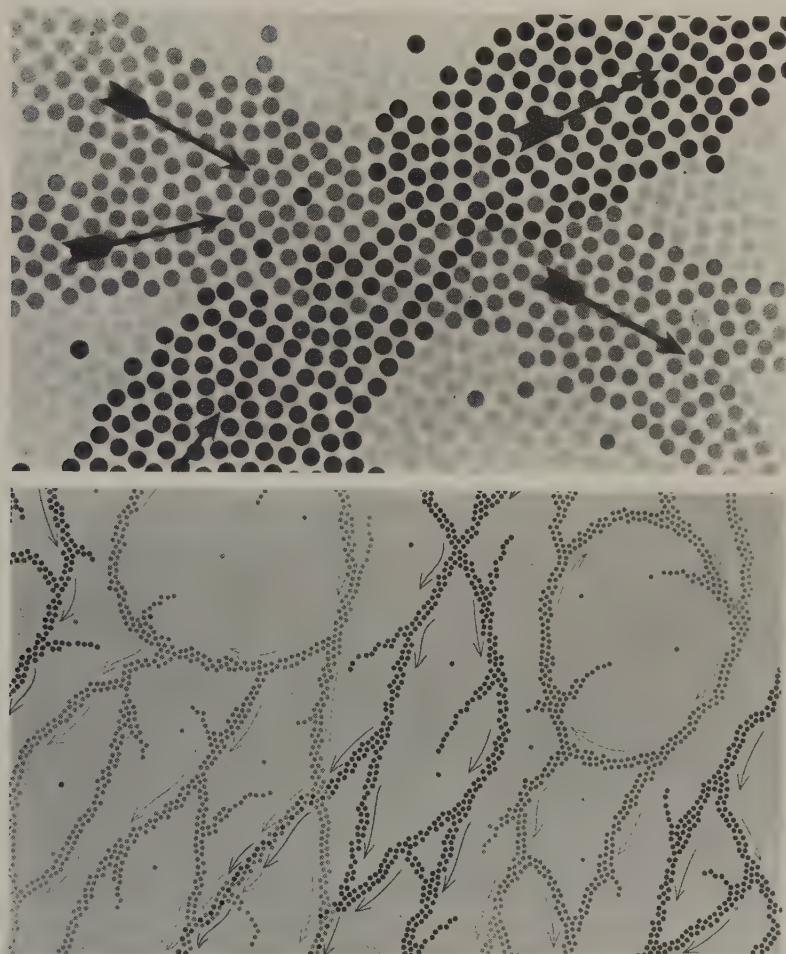
The basic operation of the nervous system depends upon the fact that many almost synchronous excitatory bombardments are necessary to generate an impulse in any neuron and thus to contribute to the further spread of neuronal activity. For an effective spread of activity, each neuron probably must receive synaptic activation from hundreds of neurons and must transmit to hundreds of others. Transmission through the nervous system is therefore more like a wave front than a single impulse (Figure 23.20). There is a kind of multilane traffic in hundreds of neuronal channels. The wave front of activity sweeps over at least 100,000 neurons per second, weaving a pattern in space and time in a way that C. S. Sherrington (1906) likened to the operations of an "enchanted loom." There is a great deal of evidence, for example, that a particular neuron may participate in the patterns of activity developing from many different sensory inputs.

The nervous system may be visualized as a complicated telephone exchange, constructed from some 10 billion unitary components or neurons. Unlike a telephone exchange, however, the nervous system does not merely carry a given message from a single input to a single output. Instead,

Figure 23.20 (above). Schematic diagram of an impulse wave front. The nerve cells of the cortex are laid out as dots on one plane. The multiline traffic in one, evolving a specific neuronal pattern, is shown in black and in another as dark gray. Light gray cells are not activated by either pattern. Note the very dark dots at the crossing of these two lanes, indicating participation of nerve cells in both patterns.

Figure 23.21 (below). Schematic diagram of impulse patterns among active neurons. The arrows show the directions of propagation for two separately evolving

patterns (black and dark gray). Note that where the black and gray patterns unite (2 sites), they propagate as one advancing wave.



the brain and the rest of the system act to correlate and integrate the incoming signals from an enormous number of sensory channels. For example, there are about 1 million separate nerve fibers leading to the human brain from each eye. The brain not only correlates the input from all of these channels to guide activity in relationship to the visual information but correlates the visual input with other sensory input, as when the eyes and the sense of touch are simultaneously used to guide and control movement.

Within the past decade, enormous advances have been made in the study of the cerebral cortex at very high magnifications in electron microscopy and also in the use of intracellular electrical recording devices to explore the nature of synapses and nerve impulses. However, all of this research has provided only a beginning for the understanding of simple perceptual awareness. The more complex problems of perceptual recognition and judgment have hardly been approached.

There is a great deal of neurophysiological evidence that a conscious experience is always accompanied by some specific activity in the cerebral cortex. For every experience, a specific pattern of impulses in time and space is woven by the meshwork of nerve cells in the brain (Figure 23.21). Any stimulus to a sense organ causes the repetitive discharge of impulses

along sensory nerve fibers to the brain. After various synaptic relays in the brain, specific spatiotemporal patterns of impulses are created in the neuronal network of the cerebral cortex. The transmission from sense organ to cerebral cortex utilizes a coded pattern of nerve impulses, rather like a Morse code with dots only in various temporal sequences. Certainly, this coded transmission is quite unlike the original stimulus to the sense organ, and the pattern of neuronal activity evoked in the cerebral cortex is different yet again. As a result of these cerebral activity patterns, people experience sensations that seem to represent events within the body, at its surface, or in the external world.

The investigation of the neuronal mechanism of the cerebral cortex is still at a primitive stage, and therefore it gives only a dim picture of the intricate pattern woven in space and time by the sequential activation of neurons in multilane traffic over the 10 billion components in the cortical slab of cells. It has been estimated that many millions of cells take part in the simplest cortical response. The human cerebral cortex presumably surpasses that of any other animal in its potentiality to develop subtle and complex neuronal patterns of the greatest variety. From this neural complexity must stem the richness of human behavior as compared with that of even the most intelligent of other animals.

The brain events associated with experiences may be caused by local stimulation of the cerebral cortex, by stimulation of some intermediate part of the sensory nervous pathway (as in the "phantom limb" experiences of amputees), or in the usual fashion by activation of sense organs by external stimuli. However, electric stimuli applied directly to the sensory zones of the cerebral cortex usually evoke only chaotic sensations—tingling or numbness in the skin zones, lights and colors in the visual zone, noises in the auditory zone (Penfield and Rasmussen, 1950). Such chaotic responses are to be expected, because electrical stimulation of the cortex must directly excite thousands of neurons, regardless of their functional relationships, thus initiating a spreading field of neuronal activation quite unlike the fine and specific patterns set up by normal input from the sensory organs. A familiar chaotic sensation—involving elements of touch, heat, cold, and pain—arises for a similar reason when a sensory nerve bundle is directly excited, as when the ulnar nerve in the elbow (the "funny bone") is stimulated by a sudden blow.

When gentle repetitive electric excitation is applied to the cerebral cortex, there is a relatively long period between application of the stimulus and the sensory experience. This time lag may be up to 0.5 second with a very weak stimuli, but a more typical lag is about 0.2 second. Each electric stimulus of the repetitive series must excite the discharge of impulses from nerve cells within a few milliseconds. The delay of conscious perception for 0.2 second or longer presumably represents the time needed to elaborate the spatiotemporal pattern that corresponds to the experience. This long time lag is surprising in view of the fact that reactions to stimuli can occur much more swiftly. However, it appears that such rapid reflex reactions—for example, the withdrawal of the hand from a hot object—are carried out before the accompanying sensations such as pain are experienced.

Transmission of an impulse from one neuron to the next takes no longer than 1 ms. Thus, the time lag of 0.2 second preceding a sensation could permit the serial relay of an impulse through as many as 200 synaptic linkages between neurons. Many thousands of neurons are probably activated by

the electric stimulus to the cortex, and each neuron in turn activates many nerve cells at each synaptic relay. Thus, it appears that millions of neurons are involved in the pattern that corresponds to a sensation.

This rich tapestry of neuronal activity is required for the perception of even the simplest sensation. Responses involving comparisons, value judgments, correlations with remembered experiences, aesthetic evaluations, and so on must take much longer and involve fantastic complexities in the patterns woven on the "enchanted loom."

FURTHER READING

For more detailed discussions of nerve impulses, see books by Brazier (1960), Eccles (1957), Hodgkin (1964), and Katz (1966). Synaptic transmission is discussed in books by Eccles (1957, 1964), Katz (1966), and McLennan (1969). Thompson (1967) provides a general survey of neurophysiological knowledge related particularly to the study of animal behavior.

Among the *Scientific American* articles particularly related to topics of this chapter are those by Baker (1966), Eccles (1958, 1965), Hubel (1963), Hyden (1961), Kandel (1970), Katz (1952, 1961), Kennedy (1963), Keynes (1958), Luria (1970), and Walter (1954).

24

Nervous Systems

The vertebrate brain is commonly subdivided into 3 regions: the forebrain, the midbrain, and the hindbrain.

Auditory nerve
Sound waves cause
small waves of pressure
amplitude and lead to one
impulse stimulation at the
hair cells and to the initiation
of a greater number of impulses
which pass over the auditory
nerve to the brain.

The nervous system is composed of two basic subdivisions: the central nervous system, comprising those structures enclosed in the brain and protection of the spinal cord, and the peripheral nervous system.

It contains the neurons that carry information from the periphery of the body to the CNS and vice versa, carrying the neuronal activity necessary

for activation of selected glands in muscle and organs.

Nerve fibers, like muscle fibers often contain hundreds of fine filaments.

Untwisted muscle filaments which are said to cable the fibres the nerve filaments are usually individual strands surrounded by a thin cytoplasmic membrane.

The simplest spinal reflex arc involves just two neurons: an afferent sensory neuron, conveying information about stimulus, the synapse, and an efferent motor neuron, which leads to a muscle group.

J. Dawson '71

Anyone attempting to describe briefly the structure and function of the nervous system faces much the same dilemma as an electronics engineer attempting to describe the workings of a television set. It is relatively easy—though tedious—to list all the components by name and to describe in precise terms the individual properties of each. It is also possible to describe in more general terms the roles played by various groupings of components in the television set—the tuner, the amplifier, the oscillator, and so on. However, it is far more difficult to show exactly how a given set of components with particular properties interacts to produce the exact effects needed for proper operation.

The scientist studying the nervous system faces a stiffer task. He would find it far more difficult to trace the circuitry and to discover all the components in the circuits than would an engineer studying a TV set. Neurobiologists have developed diverse techniques to explore nervous system function. An experimenter may deliberately damage or electrically stimulate parts of the nervous system. The resulting behavioral changes indicate some of the functions of the affected parts. He may make recordings from single nerve cells, nerve bundles, and large regions of the brain. These recordings provide other clues about the activity of the nervous system. He may also study the anatomy of the major interconnections among parts of the system, but he cannot today make a "wiring diagram" for any vertebrate.

Knowledge of the human nervous system has come from studies of persons with systems damaged by injury or illness. Correlation of behavioral defects with physical damage to parts of the nervous system provides some clues about the functioning of the normal system.

Such studies have revealed much about the functions of major groups of neurons and of major pathways. The properties of individual neurons and small groups of neurons have been investigated in nonhuman mammals, lower vertebrates, and invertebrates. In these animals, and especially in invertebrates, it is possible to see how properties of individual neurons contribute to the general functioning of the nervous system. The general organizational principles discovered in such simple nervous systems have proven useful in understanding the more complex vertebrate nervous systems.

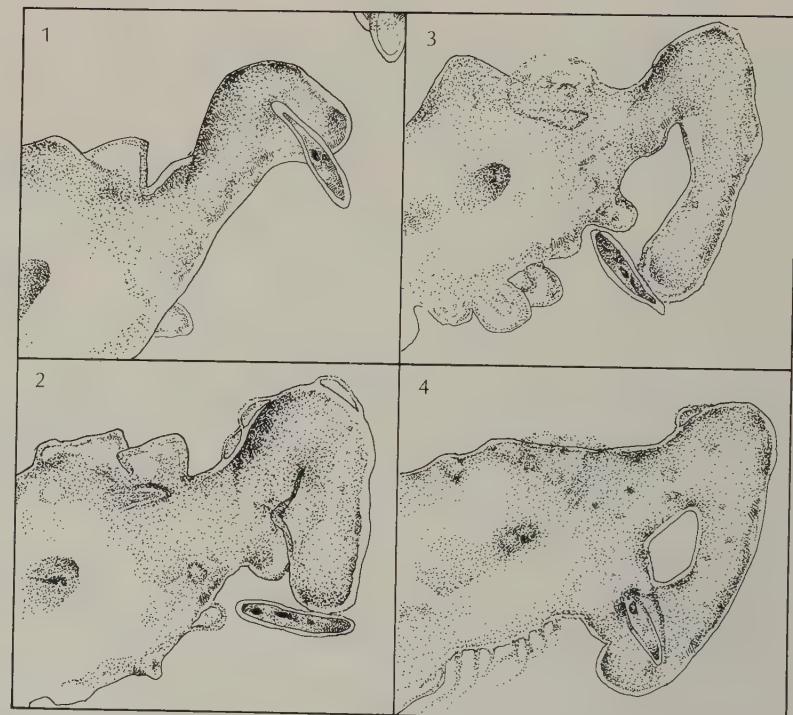
IRRITABILITY WITHOUT NERVES

Even the simplest unicellular organism exhibits some form of *irritability*—the capacity to respond with action to certain kinds of stimuli. Irritability is often listed as one of the basic properties of living systems.

An amoeba is a cell with relatively few organelles in what appears to be a generally formless cytoplasm. Although it lacks apparent specialized structures for sensory reception or response, the amoeba does show a regular behavior pattern. It responds to small food particles or certain chemicals with feeding behavior (phagocytosis) and responds to almost any other stimulus by withdrawing. The nature of its feeding activities varies with the chemical nature of the food stimulus, the degree of activity exhibited by the potential food, and the amount the amoeba has eaten recently. The mechanisms underlying these behaviors are not completely understood, but a stimulus apparently causes local changes in the protein structure of the cytoplasm, and these changes then lead to the general alteration of cytoplasmic properties that produces movement.

Unicellular organisms with organelles such as cilia, flagella, contractile fibrils, and sensory organelles exhibit far more intricate behavior patterns.

Figure 24.1. The giant amoeba *Chaos chaos* capturing and ingesting a paramecium. The nature of the amoeba's activities depends on the integration of such factors as the chemical nature of the food, the degree of activity exhibited by the potential food, and the amount of food the amoeba has eaten recently.



A paramecium that encounters an object or a noxious chemical as it swims will back up by reversing its ciliary beat and then move forward in a different direction. If microelectrodes are attached to these organisms, it is possible to depolarize the anterior end so that the paramecium causes its cilia to beat in reverse. If the posterior end is depolarized, the beat is accelerated. Evidently, some tactal and chemical stimuli cause such depolarizations, thus controlling the cell's behavior.

SIMPLE MULTICELLULAR ANIMALS

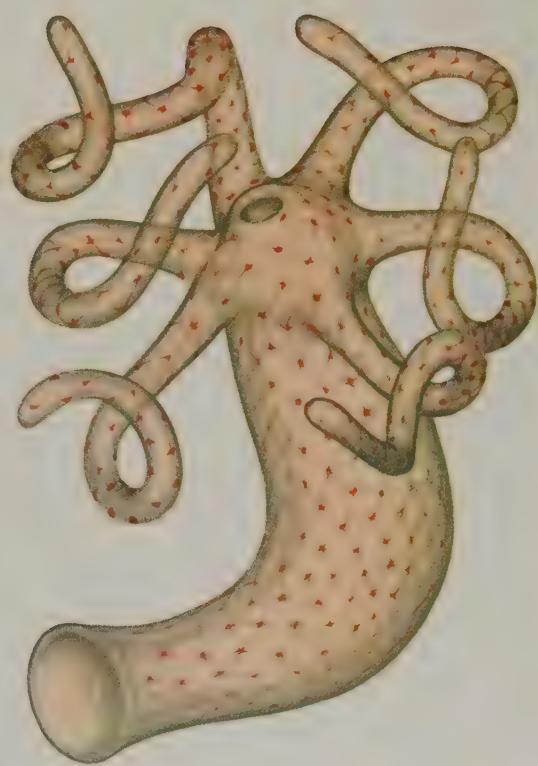
The simplest multicellular animals behave as if they are collections of relatively independent cells. Chemical signals spread quite slowly from one cell to another, and, except where contact stimuli occur between touching cells, little communication or coordination takes place. In most multicellular animals, however, specialized cells of the nervous system speed communication among the cells of the body. Neurons may be regarded as cells specialized for response to stimuli (irritability) and for rapid communication of the response through the organism along particular paths (conduction).

Coelenterates are the simplest animals that have well-developed nervous systems. The individual neurons of the coelenterate system are similar to those of higher animals, but the system itself is relatively simple in organization. The coelenterates have radial symmetry; they have neither a head nor anything that could be called a brain. The nervous system is best described as a collection of two-dimensional, interacting nerve nets. A *nerve net* is a group of neurons—in most cases scattered over a surface—whose processes (dendrites and axons) cross and intermingle in a netlike fashion. Synapses occur at many points of contact between processes. Electron micrographs of synapses reveal that there are synaptic vesicles on both sides



Figure 24.2 (left). A “simple” unicellular organism such as *Euglena* is really a complex integrated group of organelles coordinated by chemical signals. A positive phototactic response ensures adequate light to maintain photosynthesis; however, intense bright light will elicit a negative phototoxic response.

Figure 24.3 (right). Nerve net system of the fresh-water coelenterate *Hydra*. Note the absence of a centralized nerve tract and ganglia. The majority of *Hydra* behavior is limited to feeding and defensive contractile responses.



of the synaptic cleft, and the synapse seems to be a two-way connection.

There are two nerve nets spread over the swimming bell of the young medusa (jellyfish stage) of *Aurelia* (Figure 24.4). One nerve is composed of large, bipolar cells with straight processes. This *giant fiber net* lies over the ring of muscle that extends outward from the ring toward the eight rhopalia, or tentaculocysts, which are sensory structures extending from the edge of the bell. The other nerve net is composed of smaller, multipolar cells with shorter processes. This *diffuse net* covers the entire surface of the medusa, and synapses connect the two nets only at the rhopalia. The rhopalia contain equilibrium receptors, or statocysts, and light receptors. They also contain several kinds of neurons whose processes apparently remain within the rhopalia, where they synapse with each other, with receptor cells, and with elements of the two nerve nets. Each of the eight rhopalia serves as a point of interaction among the two nerve nets, the sensory receptors, and the neuronal network of the rhopalium itself. In structure and function, the rhopalia can be considered the prototype of a brainlike structure.

The medusa shows two major kinds of motor activity: (1) a rhythmic, stereotyped, rapid twitch contraction of the bell muscles as it swims and (2) localized, variable, slow contractions associated with feeding or avoidance behaviors and modification of swimming behavior in response to stimuli. Each of the two nerve nets plays a specific role in the total behavior.

If the giant fiber net is cut in such a way that a segment of muscle is isolated from all contact with rhopalia through that net, the isolated segment ceases to show regular swimming contractions. Apparently, then, the signals that drive the coordinated swimming beat originate in the rhopalia and travel to the muscles through the giant fiber net. If cuts are made in such a way that each rhopalium is connected to an area of muscle but is isolated from contact with other rhopalia through the giant fiber net, each segment contracts at an independent rhythm. In the intact animal, the most active or strongest of the eight rhopalia initiates a beat and simultaneously drives the remaining seven via the giant fiber net.

The diffuse net apparently plays no role in the swimming twitch, except perhaps as an indirect inhibitory effect. This net is thought to control the slower contractions involved in feeding or in spastic avoidance reaction. Swimming contractions are slowed, weakened, or absent when slow contractions are occurring.

The coelenterate nervous system exhibits several principles of organization also found in the more complex nervous systems of higher animals. First, the nervous system is composed of more than one anatomically and functionally separate subsystem. These subsystems interact only at particular centers that also receive sensory information from nearby sense organs. These centers contain pacemakers that initiate rhythmic impulses. External stimuli apparently are not necessary to trigger these impulses. Second, the coordinated activity of the organism involves neural inhibition as well as excitation, and the inhibition may serve to switch the organism from one behavior pattern to another as various subsystems are turned off or on. The qualitative differences in behavior patterns depend largely upon which part of the system is active, not upon changes in the nature of the activity of a single subsystem.

Coelenterates possess complex and specialized receptor organs. Ocelli are cup-shape patches of pigment-containing, light-sensitive cells covered with a transparent, lenslike layer of cells (Figure 24.5). Statocysts contain a

Figure 24.4. Oral view (above) of *Aurelia aurita* showing canal development and location of sensory apparatus (tentaculocysts). Below is shown the life cycle of the scyphozoan medusae (jellyfish) *Aurelia*.

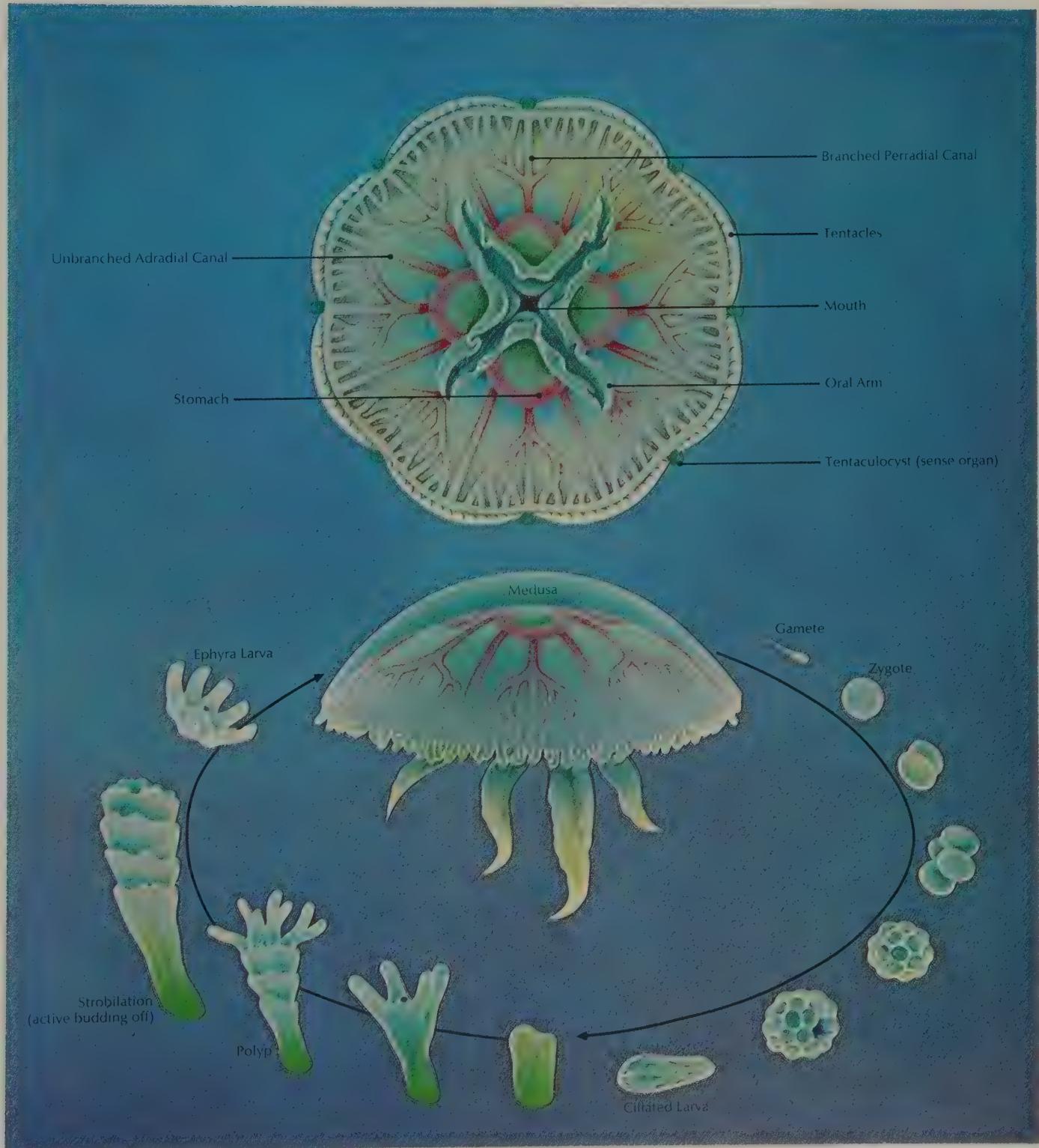
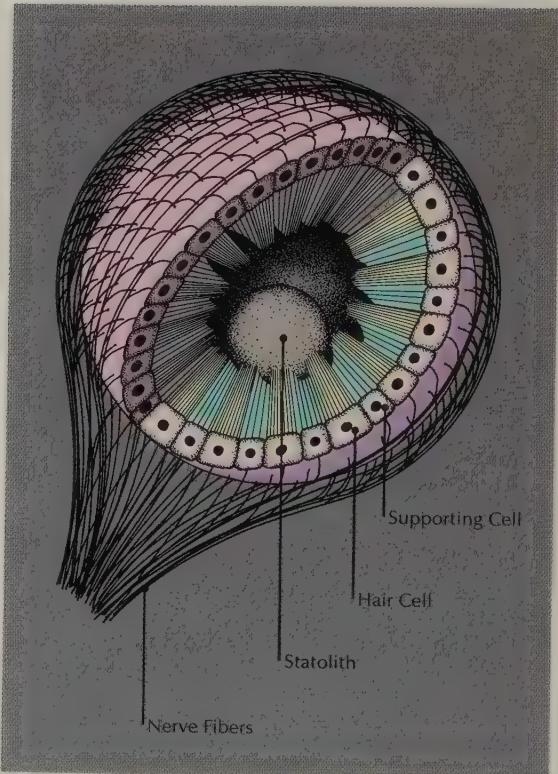
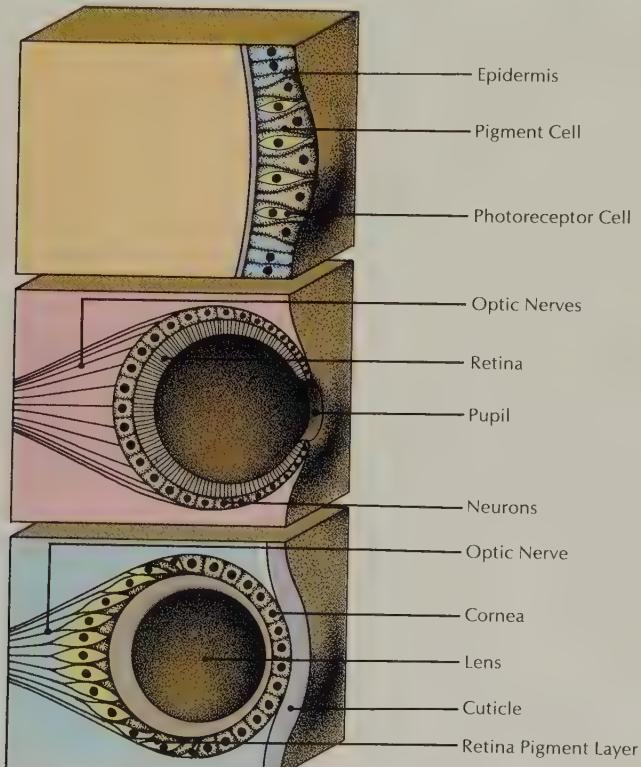


Figure 24.5 (left). Three types of light receptor organs (ocelli) typically found in the tentaculocysts, or rhopalia, of jellyfish.

Figure 24.6 (right). Schematic diagrams of a statocyst receptor organ of a jellyfish. These organs orient the jellyfish with respect to gravity and are housed in the rhopalia. If the bell tilts, the statolith is displaced and touches the hair cells. This action stimulates the nerve fibers and signals the animal to correct its position.



round, hard object made up of organic materials and calcium carbonate (Figure 24.6). This object rolls against various sensory cells as the orientation of the organism changes.

The behavior exhibited by a coelenterate involves a series of interactions among the various sensory, conducting, and effector cells. These interactions permit relatively few variations from a limited repertoire of standardized behavior patterns. A coelenterate's response to a given set of stimuli depends on its internal state and its nervous system. Repeated stimulation by touch, for example, leads to a gradual decrease in the strength of avoidance behaviors elicited by the touch, and repeated feeding leads to a lessening of feeding behaviors, even in the presence of potential food. Overall, this group of animals exhibits a remarkable number of phenomena that higher animals depend on in their nervous systems.

Echinoderms possess more complex nervous systems than those of coelenterates. In addition to nerve nets, bundles of nerves are located well inside the body of the organism. Greater numbers of interneurons increase the number of nerve cells between receptor and effector. Many neurons are closely associated with glial cells (which are absent in coelenterates), and conduction along axons and across synapses occurs only in one direction.

In a starfish, a ring of nerves surrounds the mouth, with radial nerve tracts extending into the arms; a complicated nerve net, or *plexus*, lies beneath the skin. The outer layer of the plexus has a network similar to that of coelenterates, but the inner layer is organized into nerve bundles, or tracts. The nerves of the outer layer form synapses with receptor cells,

which are present by the thousands in each square millimeter of skin. The nerve ring and radial nerves are located inside the hard structures of the body wall, as are the muscles. Synaptic connections between the outer and inner nervous systems occur at various thin spots in the body wall.

The interactions of the various parts of the nervous system may be illustrated by the response of a starfish to a light touch at one spot on the lower side of an arm. Nerve impulses spreading out from the stimulated sensory cells through the dorsal plexus cause the spines in the immediate vicinity to bend toward the touched spot. Next, an impulse travels through a chain of nerves within the arm segment and stimulates retraction of the tube foot nearest the touch. Nerve impulses also move along the radial nerve away from the stimulated point, causing a slightly delayed retraction of adjacent tube feet. Finally, the nerve impulses reach the nerve ring and control centers at the base of each arm. The control centers send out impulses along the radial nerve fibers, causing coordinated motions of the arms and tube feet that result in locomotion away from the touched spot. When a starfish is moving, the control center at the base of the leading arm tends to dominate the movement of all tube feet by impulses sent via the nerve ring and radial nerves.

BILATERAL NERVOUS SYSTEMS

Most other animals that have highly differentiated, complex nervous systems are bilaterally symmetrical. Locomotion is usually in a direction such that the head leads, and most major sensory receptors are located on or about the head. Because most of these bilateral organisms are segmented, various elements of the nervous system tend to be repeated in each segment.

In bilaterally symmetrical organisms of increasing complexity, several trends in nervous system organization can be detected. First, there is a trend toward increased cephalization, or development of the size and complexity of the brain. Second, there is an increase in the number of interneurons, or neurons whose processes synapse only with other neurons and not with sensory or effector cells. Third, there is an increased variety of structurally different kinds of neurons and glial cells. Fourth, there is an increase in the variety and differentiation of synaptic regions within the brain. In the more complex animals, the brain has more neurons and a far more complex organization of subsystems than any other part of the nervous system.

There are two major anatomical patterns of organization. In the evolutionary branch containing the molluscs, annelids, and arthropods, the central nervous system is organized into ganglia, or knots of nervous tissue, and each body segment often has one pair of ganglia. Each ganglion contains an outer rind of neuron cell bodies and an interior region of synaptic areas (*neuropil*) and nerve fiber tracts, which connect the various ganglia. In general, each ganglion controls half of one body segment, but in most species the ganglia of several anterior segments are fused to form the brain.

In the chordate branch, the primary organization consists of a continuous tube of nervous tissue extending the length of the organism rather than segmented neuron clumps. Neuron cell bodies, glia, and neuropil (designated *gray matter*) are mixed together in the wall of the tube—not separated as in the ganglia of the arthropod branch. Bundles of axons (or fiber tracts) are separate from the gray matter. They are called the *white matter* because

of the glistening appearance of the myelin sheaths formed by Schwann cells that surround each axon.

The nervous system of the earthworm is an example of a simple ganglionated system. In addition to the central nervous system, it has a subepidermal nerve net, but this net apparently functions only in localized and minor responses. Locomotion is accomplished by peristaltic waves of contraction that pass along the length of the body. The contraction of muscles in one segment stimulates receptor cells in the next segment, which trigger the contraction of that segment. An inhibitory mechanism that keeps the wave moving in one direction along the body is apparently built into the nervous system. The cerebral ganglion above the gut in the front segments is well developed and regulates behavior on the basis of input from the sensory organs of the anterior region. The next set of ganglia, located below the gut, is also well developed and appears to initiate many control impulses regulated by the cerebral ganglion. Removal of the cerebral ganglion does not prevent the worm from exhibiting normal burrowing and eating behaviors, but it does limit the worm's ability to modify its behavior in response to changing conditions. The behavior of an earthworm consists of a fairly stereotyped repertoire of responses, which may be modified under varying environmental conditions.

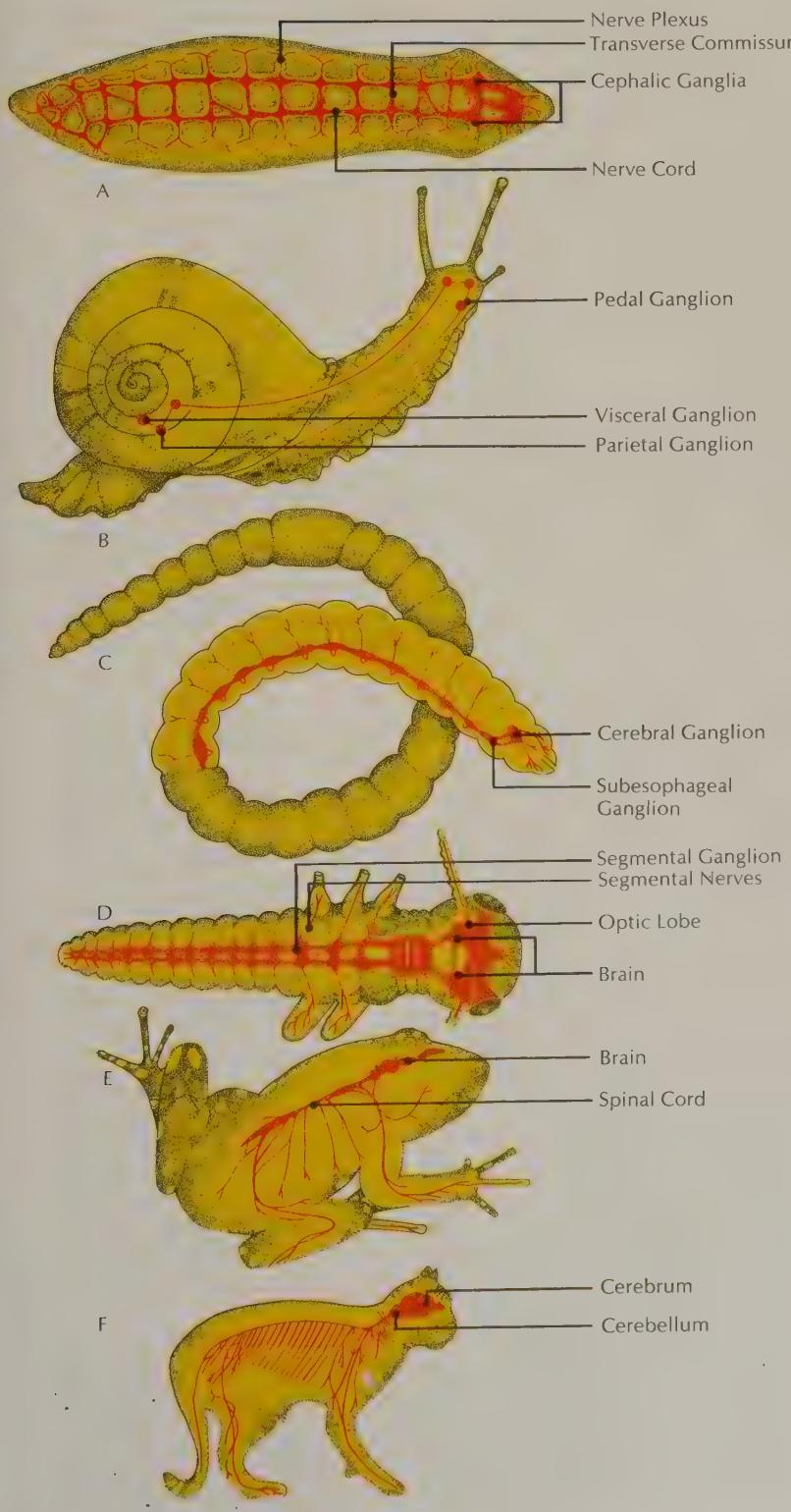
Molluscan nervous systems vary widely in complexity. A chiton has a simple ganglionated and segmented nervous system. In most molluscs, the nervous system has five major ganglia, each of which controls important organs. By studying these simple nerve centers, researchers have made some fundamental discoveries. Many gastropods (slugs, snails, limpets) have giant nerve cell bodies, often over a millimeter in diameter. The pacemaker properties of single neurons and synaptic interactions between neurons have been studied in *Aplysia* (a sea slug). In this animal, simple neural examples of conditioning and training have been studied. Recently, workers have discovered that the large cells in many gastropod ganglia can be named or numbered, and the same cells are present in every individual examined. Each cell seems to have a specific function and anatomy, with even some detail of its complex branching processes repeated in different individuals.

Snails and limpets are capable of limited behavioral modification, but the octopus, a cephalopod mollusc, is better at learning than many vertebrates. Cephalopods have the greatest degree of cephalization of any mollusc, and perhaps of any invertebrate. Their brains are very similar to those of mammals in organization and complexity of structures. They have fiber tracts and layered arrangements of cells and neuropil that are reminiscent of cerebral cortex. It is perhaps because of the complexity of their brains that they are such good learners. They can be taught to discriminate between objects on the basis of touch or sight (Boycott, 1965). Some researchers feel that the mechanism of memory may be more easily discovered in cephalopods than in mammals. The anatomy of their nervous systems has received much attention, but as yet few neurophysiological studies have been done.

Arthropods have highly developed ganglionated nervous systems with advanced cephalization. The nervous systems of crustaceans are distinguished by the relatively small number of neurons (less than 100,000 in the crayfish, for example). Interneurons with numerous and complex processes make possible intricate interconnections despite the small number of cells.

Figure 24.7. Representative invertebrate and vertebrate animals selected to portray the phylogenetic progression in the evolution of nervous systems. The diagram in A shows the bilateral ladderlike nervous system of a triclad flatworm. Note the beginning of cephalization, or brain development, as denoted by the cephalic ganglia. The molluscan nervous system in B, represented by a gastropod, shows placement of ganglia in strategic locations. In C, the ventral solid nerve tract of the earthworm is shown with its segmental arrangement of ganglia. The arthropod nervous system

in D also shows the effect of segmentation. Advanced cephalization is also characteristic of this phylum. In E, the primitive vertebrate nervous system is represented by an amphibian, and the advanced vertebrate system is represented by a mammal in F. The central nervous system of all chordates includes a dorsal, hollow, fluid-filled nerve cord.



Insects possess extremely specialized sensory organs and complex brains. The study of the genetics and development of nervous systems, a new and exciting field, makes good use of the diversity of experimental subjects presented by the insect world. Many species are capable of complex behavior patterns similar to those of vertebrates. Communication activities in honeybees have been studied extensively and are a good example of complex behavior mediated by a ganglionated nervous system (Frisch, 1967).

VERTEBRATE NERVOUS SYSTEMS

In vertebrates, there is a continuous, hollow, dorsal nerve cord rather than the ganglionated, ventral cord of invertebrates. Centralization of the nervous tissues, decreasing autonomy of outlying ganglia and increasing numbers of neurons, and the complexities of their interconnections characterize the organization of the vertebrate nervous system. The division into sensory, associative, and motor/effectuator subsystems is probably the most useful way of subdividing any nervous system. But anatomists and physiologists traditionally have divided the vertebrate nervous system into a *central nervous system* (CNS) and a *peripheral nervous system* (PNS). The CNS comprises those neural structures encased in the bony protection of the skull and vertebral column and is composed of interneurons. The PNS includes all nerve processes and neurons that lie outside the CNS. It also forms functional and anatomical links between the sensory receptor system and the CNS and between the CNS and effector organs and glands.

The Somatic Nervous System

Another useful way of dividing the vertebrate nervous system involves consideration of two major parts: a somatic nervous system involved in control of voluntary responses to stimuli and an autonomic nervous system that regulates involuntary activities. Both of these systems include parts of the CNS and PNS, although these divisions are most frequently used in discussions of the PNS.

The central nervous system, together with the elements of the peripheral nervous system that connect it to receptors and effectors, makes up a complete neural network capable of initiating behavioral responses to particular stimuli. The sensory portion of this somatic nervous system includes neural circuits that carry input from each of the sensory systems to specific areas of the brain, where they are processed independently. Other neural circuits respond more generally to arouse or to depress the organism as a result of the interactions of various kinds of stimuli. Similarly, the motor portion of the system includes major nerves that run directly from specific regions of the motor cortex to particular groups of muscles and other nerve systems that function as feedback, stimulation, and inhibition controls over these direct motor responses. The sensory and motor portions of the system are not totally separate but interconnect in spinal reflex arcs as well as within the various parts of the brain.

The Autonomic Nervous System

Control of the involuntary, or vegetative, functions of the body by centers in the CNS is accomplished through the autonomic nervous system (Figure 24.8). This system consists of two divisions: *sympathetic* and *parasympathetic*. In both divisions, the nerve fibers exerting primary control over varied vegetative activities leave the brain stem or spinal cord and synapse