# The Molecules of the Cell Membrane

*They spontaneously form a simple, two-dimensional liquid controlling what enters and leaves the cell. Some cells internalize and then recycle a membrane area equivalent to their entire surface in less than an hour*

by Mark S. Bretscher

The organization of chemical activity in all higher cells depends in large part on the compartmentation afforded by biological membranes. The basic building blocks of membranes are a class of molecules called lipids, which by virtue of their interactions with one another in a watery medium form a closed and flexible compartment. Embedded in the lipid matrix are many different kinds of protein molecules, which give each kind of membrane its distinctive identity and carry out its specialized functions. The primary function of all membranes, then, is to separate what is inside the membrane compartment from the environment outside it. Within the cell, for example, membranes serve to isolate the chemical reactions that take place inside each intracellular organelle. The cell itself is encapsulated by its own cell membrane: the plasma membrane. The plasma membrane is the best-understood membrane, and most of this discussion will be devoted to it.

Evidently if nutrients are to enter the cell or if waste material is to leave it, the materials must somehow cross the barrier created by the lipid matrix of the plasma membrane. The crossing is usually effected by globular protein molecules that span the plasma membrane and catalyze the transfer of specific nutrients and waste molecules. Some of the nutrient molecules required by eukaryotic cells are too large to be transported across the membrane in this way, however. Instead certain protein receptor molecules, anchored by their tail in the plasma membrane, bind these nutrients from the surrounding medium. In a process called endocytosis, pits develop in the membrane and engulf many such receptor molecules and their bound nutrients, which at this stage are called ligands. The pits close up and bud off into the cell, forming vesicles in the cytoplasm, or internal fluid of the cell. At the same time other vesicles from the interior of the cell fuse with the plasma membrane and expel their contents into the surrounding medium. Such pitting and fusing circulates membrane from the surface of the cell to its interior and back again. An area of membrane equivalent to the area of the entire surface of the cell takes part in the cycle every 50 minutes.

The study of the plasma membrane has focused in recent years on the mechanisms that underlie this circulation and on its various effects. Although it has been accepted for some time that the primary function of the endocytic cycle is to bring specific nutrients into the cell, it is now increasingly clear that it can serve the cell in other ways too. For example, my own recent work (which I shall not describe here) suggests that the cell can exploit the endocytic cycle to move about on a substrate.

Another major issue is to understand how each kind of membrane, including the plasma membrane, gains its own unique set of proteins, which determines both its identity and its functions. The problem of membrane identity is complicated by the continual exchange of membrane among the various cellular organelles that takes place, for example, during the endocytic cycle. How, given such mixing of membrane, is the integrity of each set of membrane proteins maintained?

The basic framework of all membranes is a double layer of lipid molecules, an arrangement originally proposed by E. Gorter and F. Grendel of the University of Leiden in 1925. Nature has evolved a variety of lipid molecules all of which share a critical property: one end of the molecule is soluble in water and is chemically described as hydrophilic; the other end is a hydrocarbon, is therefore oily and insoluble in water and is chemically described as hydrophobic.

The commonest membrane lipids belong to a class called the phospholipids. They have a hydrophilic head group made up of a phosphate linked to a residue that can be either choline, ethanolamine, serine or inositol. The head group is attached to two hydrophobic tails, each of which is a fatty acid chain. The most abundant and most widely studied phospholipid is the one having a choline residue. It is called phosphatidylcholine. Like other phospholipids it has a remarkable property: when they are introduced into a watery environment, the individual molecules spontaneously arrange themselves into a bilayer. In the bilay-

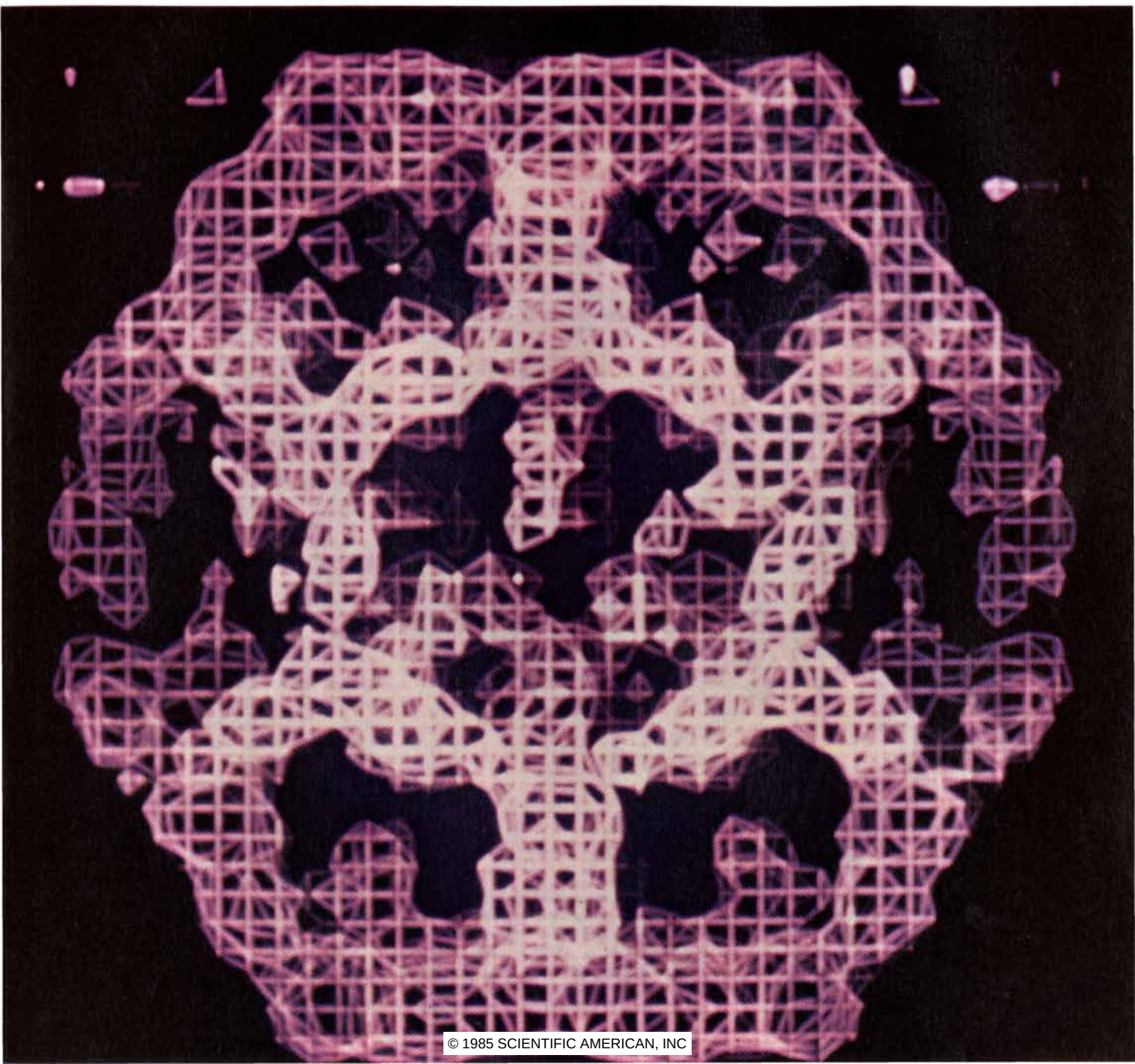
**BASKETLIKE NETWORK of protein molecules called clathrin coats a closed, spherical piece of membrane called a vesicle, which was isolated from a human placenta. The coated vesicle is derived from the plasma membrane of the cell through a dynamic process called receptor-mediated endocytosis, whereby large molecules are brought into the cell. When selected molecules outside the cell become attached to protein receptors in the membrane, a coat of clathrin begins to assemble itself on the side of the membrane facing the cell interior. Each molecule of clathrin is a chain of about 1,600 amino acids, and the coat is a honeycomb structure formed when the molecules become aligned in a regular pattern. As the coat grows, the region of membrane to which it is attached bulges into the cell in a way that resembles the formation of a drop of water on the lip of a faucet. The bulge pinches off from the surface of the cell and becomes a vesicle whose inside surface carries the receptors and their ligands and whose outside surface retains the honeycombed coat of clathrin seen in the image. The image was constructed by computer from a series of electron micrographs made at various tilt angles by Guy Vigers of the Medical Research Council's Laboratory of Molecular Biology in Cambridge. Enlargement is more than two million diameters.**

er the molecules in both layers align themselves in such a way that their longest axis is roughly perpendicular to the plane of the bilayer. The hydrophilic head groups face water on both sides of the bilayer, and the oily, hydrophobic tails sequester themselves in the middle of the bilayer, thereby excluding water from it. The arrangement is the state of lowest free energy for these molecules in water.

In 1965 Alec D. Bangham and his colleagues at the Agricultural Research Council's Institute of Animal Physiology in Cambridge showed that phospholipid bilayers in water form closed spherical vesicles having two separated compartments: the fluid inside the vesicle and the fluid outside. Such vesicles form because if a free edge on a bilayer were exposed, some of the hydrophobic regions of the phospholipid molecules would be in contact with water; that would be energetically unfavorable. It is this property of lipids that makes them so effective in biological systems: they spontaneously form a closed envelope with considerable mechanical strength.

Two general features of the bilayer are important in the formation of a biological membrane. First, because they have a hydrocarbon interior, they are essentially impermeable to most biological molecules, such as amino acids, sugars, proteins and nucleic acids, and to ions. All are highly soluble in water and insoluble in hydrocarbon solvents. It is this feature that enables the bilayer to function as a barrier.

Second, a bilayer formed from naturally existing phospholipids is a liquid. There is a double sense in which the bilayer exhibits the random motions characteristic of the liquid phase. The hydrocarbon tails of the phospholipid molecules wiggle about, and so the bilayer is soft and flexible—with the viscosity of, say, olive oil rather than paraffin wax. Furthermore, the molecules can diffuse sideways freely within their own monolayer, and so two neighboring phospholipids in the same monolayer can change places with each



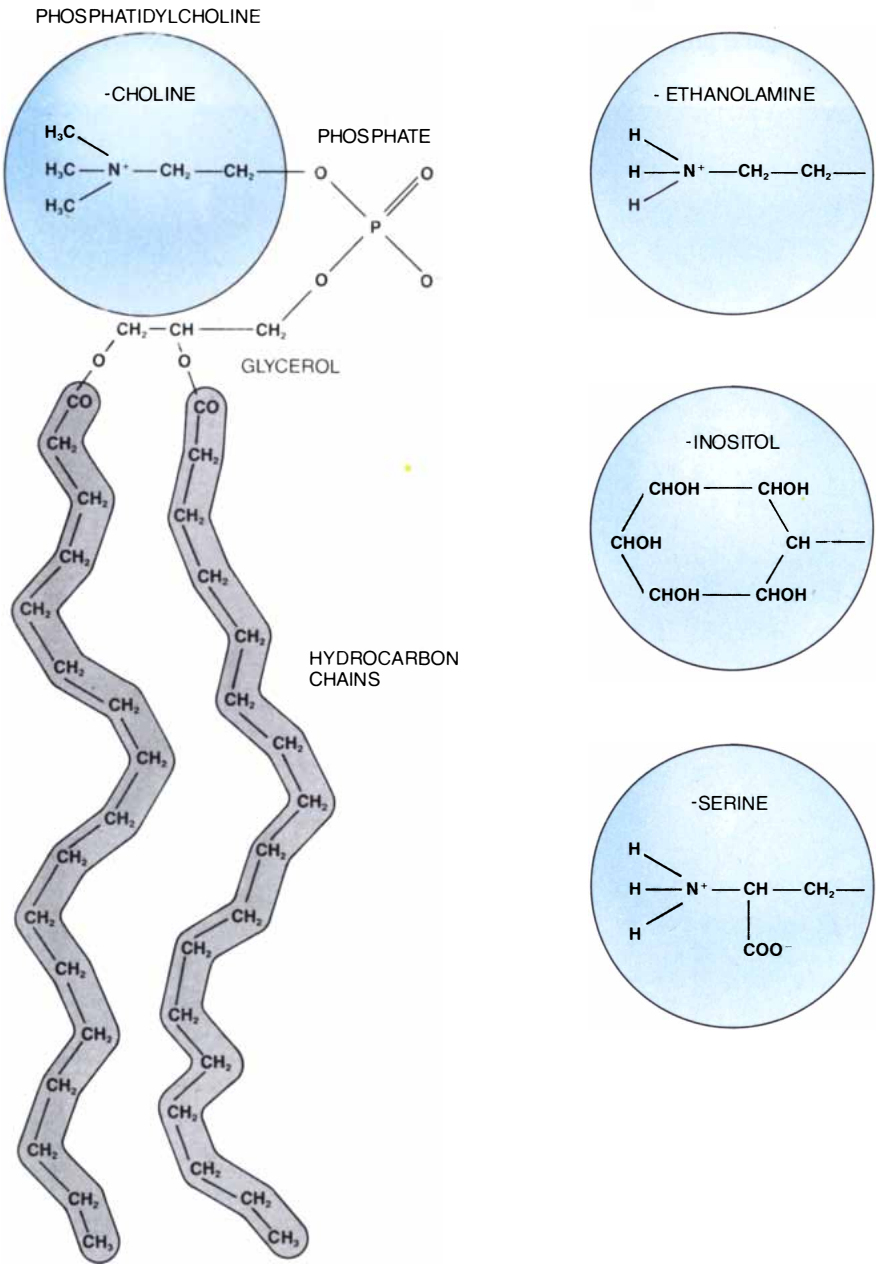
other about once every microsecond. The phospholipid molecules in opposite monolayers, however, almost never change places: such an exchange is made, on the average, only about once a year. Hence each monolayer is a two-dimensional liquid. Physiologically the liquid nature of bilayers is quite important. If the bilayer were a rigid structure, for example, the nerve cells in the neck would crack whenever a person nodded.

In a natural membrane one might expect to find the various kinds of phospholipid molecules randomly distributed on both sides of the bilayer, but in 1972 I discovered that the distribution is much more orderly. In the plasma membrane of the red blood cell I found the outer monolayer includes only phosphatidylcholine and its close relative, sphingomyelin, both of which contain choline. In contrast, the monolayer facing the cytoplasm has phosphatidylethanolamine and phosphatidylserine. It is thought that phosphatidylinositol also resides on the cytoplasmic side of the bilayer.

In addition to the phospholipids two other kinds of lipids are found in the membranes of animal cells: glycolipids and cholesterol. The glycolipid molecule has a hydrophobic tail similar to that of sphingomyelin. As its prefix implies (glyco- is from the Greek word for sweet), the glycolipid's hydrophilic end is composed of a variety of simple sugars joined to form a linear or branching structure called an oligosaccharide. Glycolipids make up only a small fraction of the lipids in the membrane, and they are confined to the outer monolayer.

Cholesterol, on the other hand, is (together with phospholipid) a major membrane lipid. It is a large, disk-shaped molecule with four carbon rings that are fused together, giving the molecule a rigid structure. One end of cholesterol is hydrophilic, but the rest of it is hydrophobic and embeds itself in the hydrophobic part of the plasma membrane. Roughly equal numbers of cholesterol and phospholipid molecules are in the plasma membrane of eukaryotic cells. The addition of cholesterol to the phospholipid matrix makes the membrane somewhat less flexible and even less permeable.

Several puzzles about the lipids in the plasma membrane are still unsolved. The biological role of the glycolipids, for example, is not yet known. Nor is there yet any convincing explanation for the distribution of phospholipids in the bilayers. Why are the bilayers of a eukaryotic cell made up of a variety of phospholipids rather than of only, say, phosphatidylcholine? What is the function of the phospholipids' asymmetric distribution? Finally, the geometry of the bilayer itself presents a problem. The two monolayers are essentially independent of each other, but of course they cover the same area. What are the lateral forces in each monolayer, then? Is one monolayer under compression and the other under tension, or is the lateral pressure the same in both?




**PHOSPHOLIPID MOLECULE is the primary structural element in all cell membranes. Four main kinds of phospholipid are found in animal-cell membranes. The one shown at the left in the diagram is phosphatidylcholine, but the other three differ from it and from one another only in the chemical structure of their head groups, which are diagrammed here as colored spheres. The electric charge in each head group makes the group hydrophilic. The head group is connected to a glycerol group, and two hydrocarbon chains are attached in turn to the glycerol. The hydrocarbon chains are oily and therefore hydrophobic.**

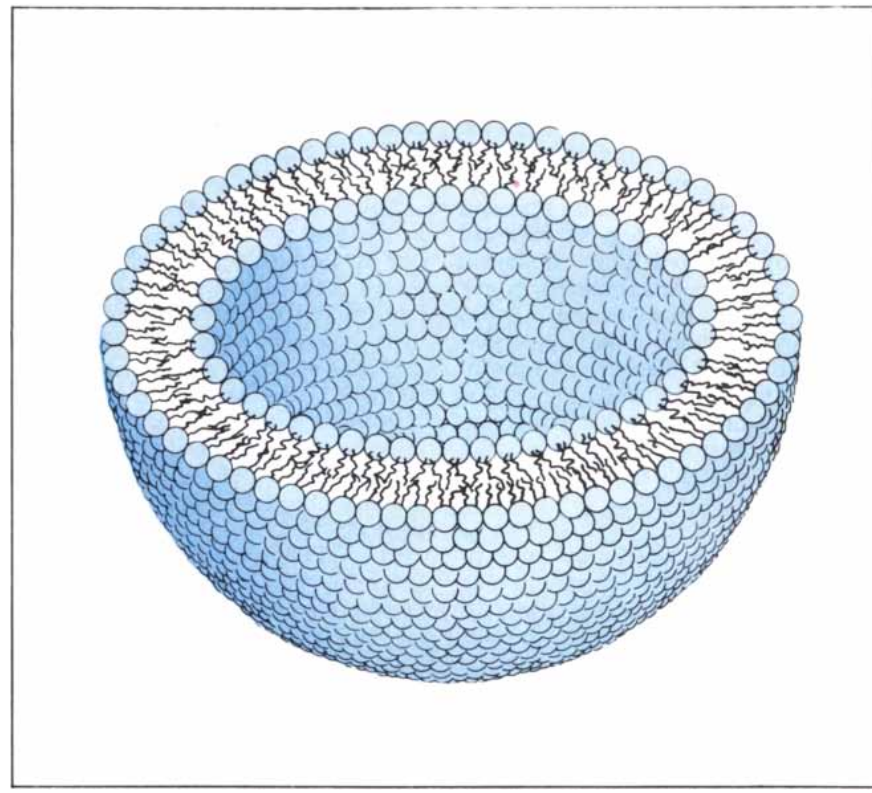
Whereas the lipids form the matrix of a membrane, the proteins carry out all its specific functions. The membrane proteins can be classified roughly into two general kinds according to their shape within the hydrocarbon core of the membrane. The shape of one kind is a rodlike, tightly coiled spiral called an alpha helix. In this structure the amino acids that make up the polypeptide chain are so arranged that the protein backbone is a helix and the amino acid side chains project outward from the helix. The second kind of membrane protein appears to have a substantial globular structure within the membrane's hydrophobic region.

One of the clearest examples of a membrane protein with the alpha-he-

lical structure is glycophorin, the major glycoprotein of the red blood cell. Although its function remains enigmatic, its structure is now quite well known. Most of the molecule resides on the outside of the cell. This extracellular region is a long sequence of amino acids to which hydrophilic oligosaccharide chains are attached.

In 1971 I showed that glycophorin spans the cell membrane. Two years later Vincent T. Marchesi, who was then at the National Institute of Arthritis, Metabolism, and Digestive Diseases, suggested the geometry of its intramembrane domain. He and his colleagues determined the amino acid sequence of the protein and found that its extracellular region is attached to a segment of 26 hydrophobic amino acids. The 26 amino acids are joined in turn to a short hydrophilic tail. The hydrophobic sequence is just the right length to span the bilayer as an alpha helix, and the short hydrophilic tail rests in the cytoplasm to anchor the protein in the bilayer.

Many other kinds of membrane protein are now known to be fixed to the cell surface by a single hydrophobic alpha helix and anchored in the cytoplasm by a hydrophilic tail. Typically they function as receptors for extracellular molecules or as highly specific markings (such as the major transplantation antigens *H*2 in mice and HLA in humans) that enable the immune system to distinguish foreign invaders from cells belonging to the organism. Other proteins in this class include the surface immunoglobulin receptors on *B* lymphocytes and the spike proteins of many membrane viruses. Since the functioning of such proteins depends primarily on their extracellular domain, the intramembrane structure need not be extensive.



**MOLECULAR ARCHITECTURE of the animal-cell membrane is determined primarily by the interactions of phospholipid molecules in water. Phospholipids can minimize their energy in water by forming a bilayer about 40 angstrom units thick. The hydrophobic tails of the molecules sequester themselves on the inside of the bilayer and the hydrophilic heads (*blue*) face the water on both sides of the bilayer. If any edge of the bilayer were open to the water, hydrophobic tails along the edge would be exposed; hence the bilayer closes to form a vesicle, effectively segregating fluid inside the vesicle from fluid surrounding it.**

Perhaps not surprisingly, the globular structure of the second kind of membrane protein is associated with functions requiring a substantial structure within the plane of the lipid bilayer. For example, one of the most abundant proteins in the membrane of the red blood cell is a globular transport protein called the anion channel. As its name implies, the protein catalyzes the passive exchange of negatively charged ions such as chloride or bicarbonate between the blood plasma and the cytoplasm of the cell. How does such a protein function? One early scheme suggested the protein might bind the ion or molecule to be transported on one side of the membrane, diffuse across the membrane and release it on the other side. Another scheme proposed that the protein molecule might rotate within the membrane, thereby bringing the binding site and its attached substrate from one side of the membrane to the other.

Neither view turned out to be correct. In 1971 I showed that what is now known to be the anion channel spans the membrane bilayer and has a fixed and unique orientation in it. It is now thought there is a small passageway for anions through the protein, which enables them to cross the bilayer.

One of the best-understood globular membrane proteins is bacteriorhodopsin, which straddles the membrane of the bacterium *Halobacterium halobium*. The halobacterium, or salt-loving bacterium, lives in the salt beds of San Francisco Bay. The bacteriorhodopsin in the bacterial membrane is a proton pump: it captures photons from sunlight and exploits their energy to pump protons across the membrane against an energy gradient. The proton gradient generated by the pumping represents potential energy, which later serves to drive the synthesis of adenosine triphosphate (ATP). The breakdown of ATP provides energy for the bacterium's biosynthetic pathways.

The structure of bacteriorhodopsin was determined in 1975 by Nigel Unwin and Richard Henderson, who were then at the Medical Research Council's Laboratory of Molecular Biology in Cambridge. Their model shows that the polypeptide chain zigzags seven times across the bilayer. Each transmembrane segment is an alpha helix and the helixes are packed together to form a globular structure. The photon is captured by a molecule called retinal (a relative of vitamin A), which is attached to the protein by a covalent bond. The mechanism whereby the energy of the photon is directed to the transport of protons is still not known.

In eukaryotic cells it is a general rule that all membrane proteins carry an oligosaccharide chain (or several chains) on their extracellular domains, just as glycophorin does. The function of the oligosaccharide chains is obscure, as it is in the case of the glycolipids. In addition all membrane proteins, both globular and alpha-helical, are held in place in the bilayer by the same kinds of forces that hold the lipid molecules there: the amino acid side chains of the protein in contact with the hydrophobic lipid chains are also hydrophobic, whereas the other parts of such proteins are hydrophilic. The hydro-

philic parts are exposed to water on each side of the bilayer.

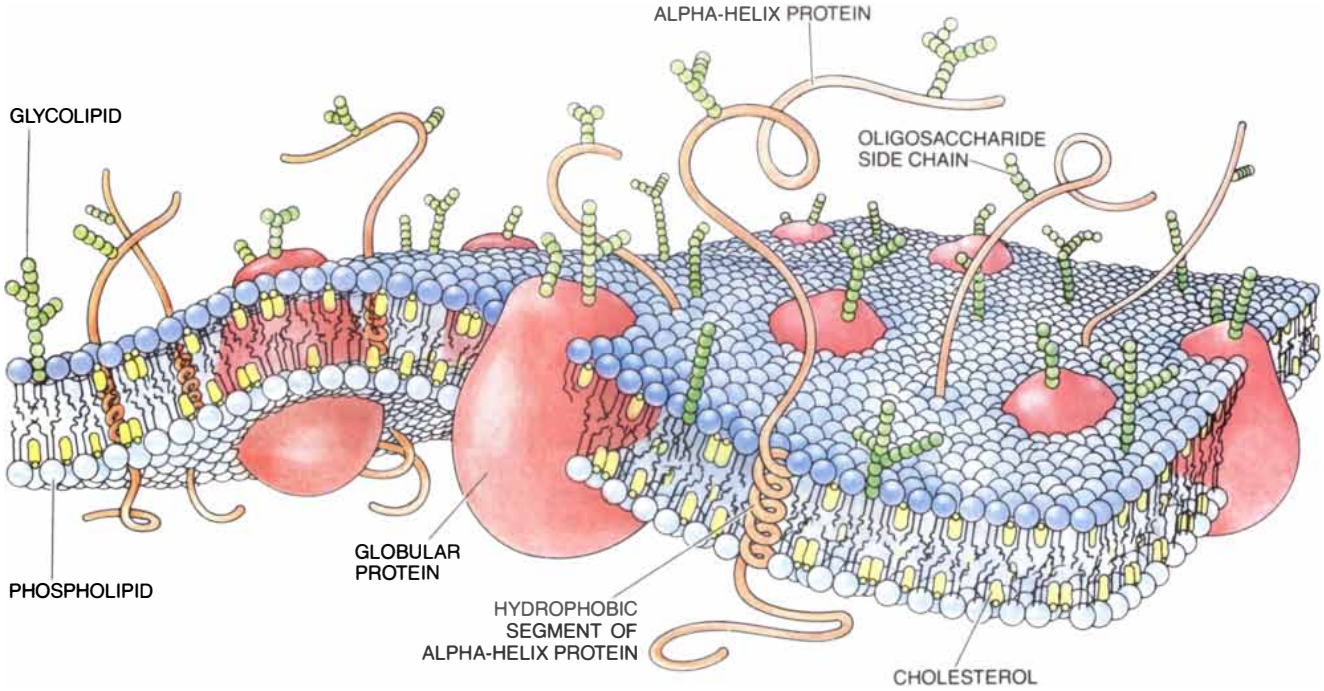
Because all membrane proteins reside in a liquid bilayer, they can diffuse sideways just as the lipid molecules do. How fast they diffuse is determined in part by how liquid the phospholipid matrix is. In 1974 Mu-ming Poo and Richard A. Cone, then at Harvard University, showed that rhodopsin diffuses about 10 micrometers in one minute. It seems likely that most other membrane proteins diffuse at about the same rate. Unless they are constrained from doing so, membrane proteins in most eukaryotic cells can therefore diffuse, on the average, from one end of the cell to the other in a few minutes.

The constraints on the diffusion of molecules across the membrane are probably best exemplified in cells that are joined to one another to form an epithelial sheet. They include the cells that line the gut, the dividing cells of the skin and the cells of internal organs such as the liver, kidney and pancreas. Epithelial sheets are only one cell thick; often they are folded extensively to form a compact organ.

Epithelial sheets have two surfaces. In the gut, for example, one surface (the apical surface) faces the digestive tract and the other (the basolateral surface) faces the blood. Because the epithelial gut cells must transport useful materials—and only useful materials—from the intestine to the blood, the cells making up the epithelial sheet must be held together tightly, with no spaces between them. The cells are therefore joined by a set of so-called tight junctions.

The tight junction can be pictured as a circular belt or gasket that lies in the plasma membrane. The belt not only prevents leaks (even leaks of ions) but also separates the cell's plasma membrane into two domains: the apical surface and the basolateral surface. Membrane proteins can wander at random within their own domain, but the tight junction keeps them from moving from one domain to the other.

The separation between the two parts of the membrane maintains the functional asymmetry needed to transport material in only one direction. For example, on the apical surface of the epithelial sheet in the gut each cell carries proteins that channel sodium from the gut into the cell. On the basolateral membrane there is a different set of proteins that pump the sodium out of the cell into the blood. The net result is an extremely selective transfer of sodium ions across the epithelial sheet, and it is accomplished because the specific proteins needed for each step in the transfer are concentrated on the part of the membrane sur-

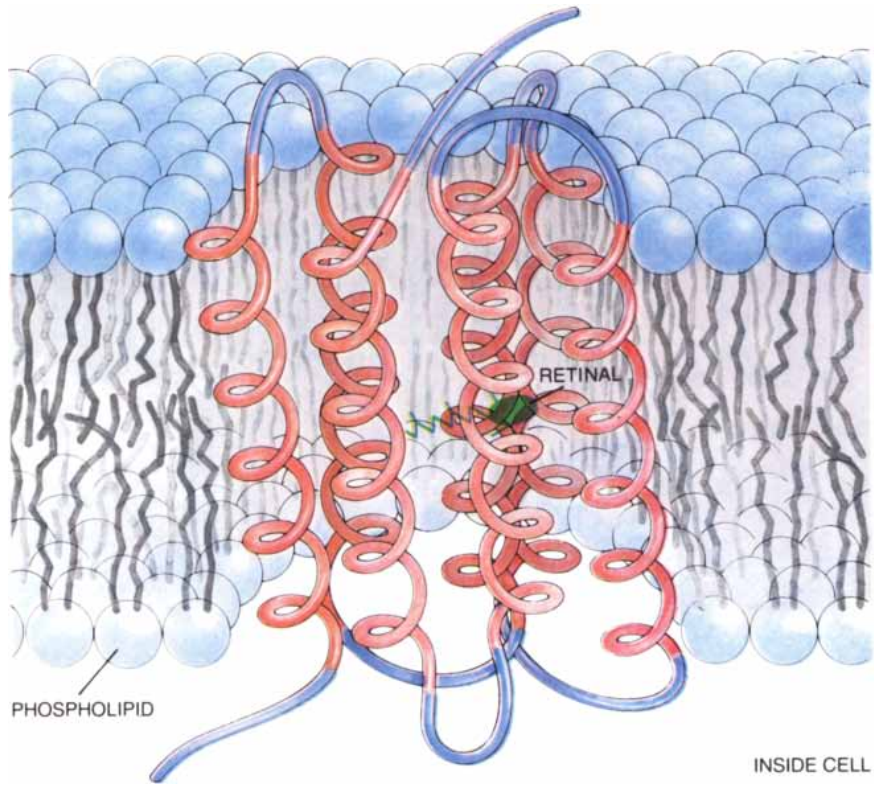



**PLASMA MEMBRANE is a phospholipid bilayer in which cholesterol and various kinds of protein molecules are embedded. In this schematic diagram of the membrane the phospholipid molecules in the top layer, which faces the external medium, are shown as dark blue spheres each having two wiggly tails. The chaotic Brownian motion of the molecules within the monolayers is indicated by the diagram at the left; the fluidity of the hydrocarbon interior is suggested by the random configurations of the tails. The bottom layer, which faces the cytoplasm inside the cell, has a different phospholipid composition and is shown in light blue. Although a random exchange of phospholipid molecules also takes place across the bilayer, the event is extremely rare. Two main kinds of protein in the membrane traverse the bilayer. One kind makes the crossing as a single chain of amino acids that is coiled into a so-called alpha helix (*orange*); the intramembrane portion of the second kind of protein is globular in structure (*red*). For clarity the ratio of phospholipid to protein is much larger here than it is in a natural membrane. Rigid cholesterol molecules (*yellow*) tend to keep the tails of the phospholipids relatively fixed and orderly in the regions closest to the hydrophilic heads; the parts of the tails closer to the core of the membrane move about freely. Side chains of sugar molecules attached to proteins and lipids are green.**

face where they can properly carry out their role.

How are tight junctions formed, and how do epithelial cells sort their membrane proteins into two domains? The first question is still unanswered, but the second is beginning to yield to attacks based on a discovery by Enrique Rodriguez Boulan and David D. Sabatini of the New York University School of Medicine in 1978. They found that when an epithelial sheet growing in culture is infected with influenza virus, the progeny viruses emerge only from the apical surface of the sheet. On the other hand, a virus called vesicular stomatitis virus (VSV), which causes a mild disease in cattle, emerges only from the basolateral surface. In order to leave its host cell a virus must assemble a protective coat, and so Rodriguez Boulan and Sabatini concluded that the cell directs the coat proteins of the influenza virus to the apical surface and the coat proteins of VSV to the basolateral surface. The two viruses therefore constitute an experimental system in which the development of asymmetry in epithelial cells can readily be studied.

The cells that make up an epithelial sheet can also be joined to one another by a so-called gap junction. The gap junction is rather like two studs pressed together with a hole through their middles [*see bottom illustration on next page*]. The hole allows neighboring cells to communicate and coordinate their activities. Small molecules whose diameter is less than about 20 angstroms can pass freely from the cytoplasm of one cell through the pipe formed by the gap junction and into the cytoplasm of an adjoining cell.




**GLOBULAR MEMBRANE PROTEIN bacteriorhodopsin is made up of seven largely hydrophobic sequences of amino acids, joined by short hydrophilic ones. On the basis of findings by Nigel Unwin and Richard Henderson, who were at the Laboratory of Molecular Biology when the work was done, it is now thought that each of the seven hydrophobic sequences is an alpha helix (*red*) embedded in the hydrocarbon core of the membrane and that the hydrophilic sequences link the helixes to one another on each side of the membrane (*blue*). A molecule called retinal (*green*) is attached to the middle of one helix. Retinal captures solar photons, triggering the protein to pump protons across the membrane of certain salt-loving bacteria. The process sets in motion an unusual kind of photosynthesis. The blue spheres and their tails represent the phospholipid bilayer of the bacterial membrane.**

The structure of gap junctions has been elucidated by Unwin, now at Stanford University, and his colleagues. Their work shows that each junction is made up of 12 protein subunits, six from each cell. Each group of six is arranged in a hexagon in the plasma membrane of each apposed cell; the two hexagons lock into each other to form a channel between the cells. The channel can be held open or closed, but precisely how such control is achieved is not known. Gap junctions often interact with one another to form a raft, or a large group of junctions, on the cell surface. The aggregate size of the rafts and their confinement to the membrane regions between two cells make it likely that gap junctions are relatively motionless within the liquid bilayer.

Until this point I have carefully avoided any detailed discussion of the many membranes in addition to the plasma membrane that are found in the eukaryotic cell. Recall, nonetheless, that many intracellular organelles are defined by a limiting membrane and that such membranes play an essential role in the transport, communication and orderly processing of chemical substances and information within the cell.

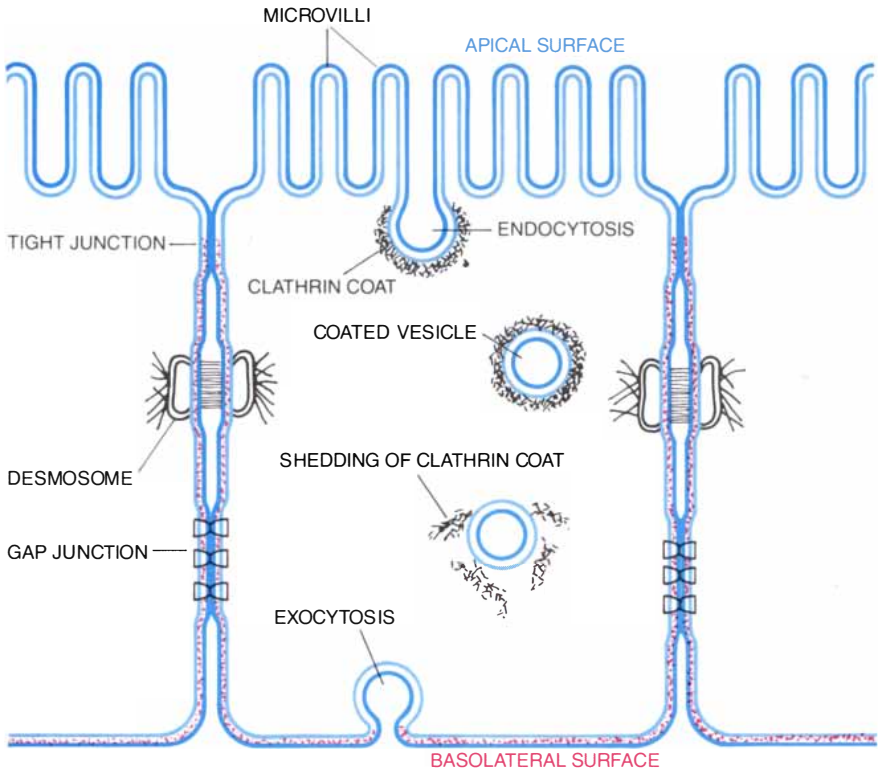
Some of the main intracellular organelles take part in the manufacture of membrane components. Membranes are assembled in the endoplasmic reticulum, and oligosaccharides are added to membrane proteins in the Golgi apparatus. Hence the relations among many of the organelles are far from static. For example, there is a continual transfer of membrane from the endoplasmic reticulum to the Golgi apparatus and from there to the plasma membrane. The transfers are probably always mediated by phospholipid vesicles.

Such continual movements of membrane material, as well as the mergers and dissociations of vesicular membranes that accompany the movement, raise anew the question of membrane integrity. How can specific membrane proteins, destined for or belonging to a specific organelle, avoid mixing and homogenizing during a transfer? A general and precise answer to the question is not yet available, but there is no doubt that what is transferred is not a random sample of the donor membrane. There is one process of material transfer involving two membranes for which the way this is accomplished is beginning to come into focus. That process is endocytosis.

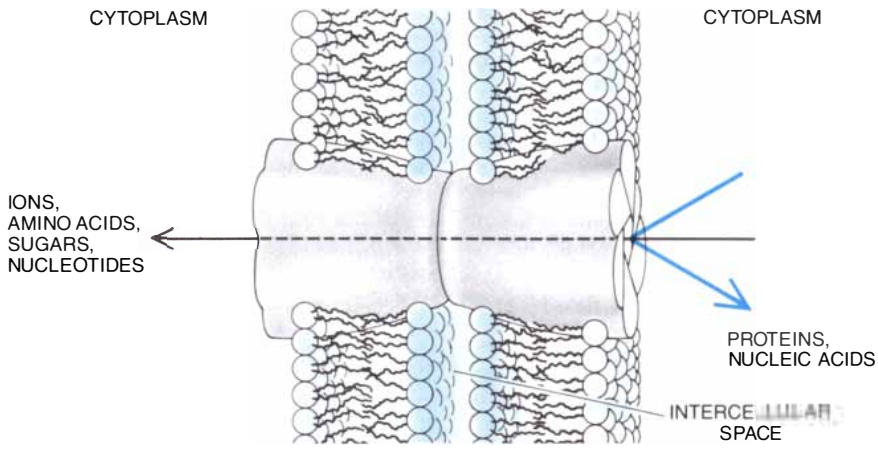
Animal cells obtain most of the small molecules they need for growth either by synthesizing them or by importing them from the blood. The imported molecules are usually transferred across the plasma membrane by specific protein channels or pumps. There are some essential nutrients, however, that for one reason or another cannot be so easily absorbed.

For example, cholesterol (which is needed for the synthesis of membranes) and the ferric ion (an iron atom carrying three positive charges, which

is needed for the synthesis of the large, pigmented molecules called cytochromes) both circulate in the blood as large complexes. Cholesterol circulates in the form of cholesteryl esters, which make up the hydrophobic core of a particle called low-density lipoprotein (LDL) that is some 200 angstroms across. Ferric ions in blood are bound inside a large carrier protein called transferrin. Both LDL and transferrin are much too large to pass through a small channel or pump, and so the cell must adopt a radically different strategy to obtain the nutrients it requires.




**EPITHELIAL CELL and the adjacent parts of its two nearest neighbors are depicted schematically. Such cells line the gut and form dividing layers in other internal organs; in the gut they form a leakproof barrier between the gut and the blood. The seal is effected by the tight junction, which also separates the apical surface facing the gut from the basolateral surface facing the blood. Below the tight junction is a desmosome, which welds the two adjacent cells together, and below the desmosome is a gap junction, which allows small molecules to pass from the cytoplasm of one cell directly into the cytoplasm of the adjacent cell. Nutrient material in the gut can cross the epithelial sheet only if it is first absorbed into an epithelial cell. Some macromolecules can be taken up by endocytosis. The endocytic vesicle can then discharge its contents into the bloodstream by exocytosis on the cell's basolateral surface. The apical membrane and basolateral membrane have different sets of proteins.**



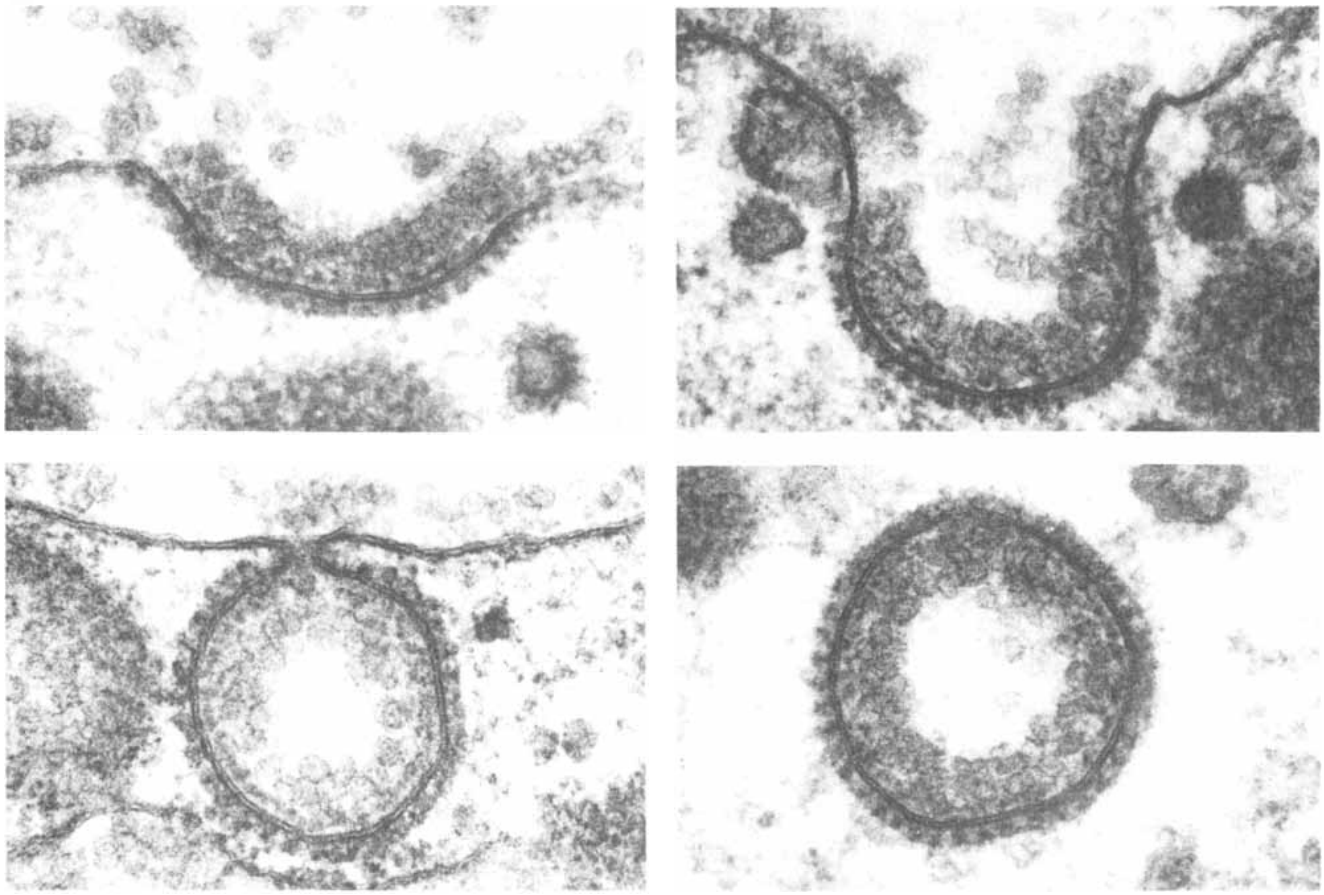

**GAP JUNCTION between two apposed epithelial cells is made up of two hexagonal studs (*gray*), each embedded in the membrane bilayer of one cell (*blue spheres with tails attached*); the two studs are pressed together in the gap between the cells. Ions, amino acids, sugars, nucleotides and other molecules smaller than about 20 angstroms in diameter can pass through the junction, but proteins, nucleic acids and other larger molecules cannot.**

The current picture of how such nutrients enter the cell began to emerge in 1964 with the work of Thomas F. Roth and Keith R. Porter, who were then at Harvard. They were studying how growing oocytes (egg cells) of the mosquito build up the oocyte's yolk. Examining thin sections of oocytes under the electron microscope, they found that the yolk precursor is bound to the plasma membrane of the oocyte at sites where the membrane is indented and appears to have a thick, dark coat of material on the side facing the cytoplasm. The sites are called coated pits. In the same thin sections of the oocyte Roth and Porter also saw vesicles inside the cell that were full of yolk precursor and had thick coats on their outer surface. They called these structures coated vesicles. The coated vesicles arise when coated pits bud into the cell; they are intermediates, inside the oocyte's cytoplasm, in the transfer of yolk precursor from the cell exterior to the large yolk granules stored inside the oocyte.

More recent work by Richard G. W. Anderson, Michael S. Brown and Joseph L. Goldstein of the University of Texas Health Science Center at Dallas on the uptake of LDL and by many other groups, including my own, on the uptake of transferrin and other large molecules has by now drawn a fairly coherent picture of the early stages of endocytosis initiated by coated pits. On the outer surface of most growing animal cells there are specific protein receptors for LDL, for transferrin and for other large imported molecules. As the receptors diffuse across the surface of the cell they can bind LDL or transferrin.

When an LDL or transferrin receptor encounters a coated pit, it enters the pit. Other proteins in the plasma membrane, however, are excluded from the pit, which thereby acts as a molecular sorting device. In about a minute the coated pit has reached its full diameter of about .3 micrometer. The pit then invaginates and breaks away from the plasma membrane into the cytoplasm, where it forms a coated vesicle. The mechanical force driving the process is assumed to be provided by the coat on the cytoplasmic side of the pit.

Once the coated vesicle has formed in the cytoplasm it sheds its coat in a few seconds. Then two things happen. The vesicle fuses with an intracellular organelle called an endosome and the acidity inside the endosome is then increased to a *p*H of about 5. The acidic environment causes LDL to fall



**SUCCESSIVE STAGES in the formation of a coated vesicle are shown in a series of electron micrographs. The shallow indentation in the plasma membrane of a developing chicken oocyte (*top left*) is a coated pit; it holds many particles of a lipoprotein gathered from the external environment of the cell. A coat of clathrin molecules can be seen just under the pit, on the cytoplasmic side of the membrane. The pit deepens (*top right*), the outer membrane of the cell closes behind the pit (*bottom left*) and the pit buds off to form a coated vesicle that carries the lipoprotein molecules into the cell (*bottom right*). The micrographs were made by M. M. Perry and A. B. Gilbert of the Agricultural Research Council's Poultry Research Centre in Edinburgh. The enlargement is 135,000 diameters.**

off its receptor and the ferric ions to pop out of transferrin. By unknown processes the receptors for LDL and for transferrin (the latter with its ligand, transferrin, still attached) are recycled to the plasma membrane. At the same time the LDL, the ferric ions and other contents of the endosome are transferred to lysosomes, again by vesicular transport. The lysosome is a primitive digestive organelle, and it degrades the LDL, thereby liberating cholesterol to serve the needs of the cell. Note that at this stage both the cholesterol and the ferric ions must still be transported across at least one membrane, namely the lysosomal membrane, in order to reach their destinations within the cell.

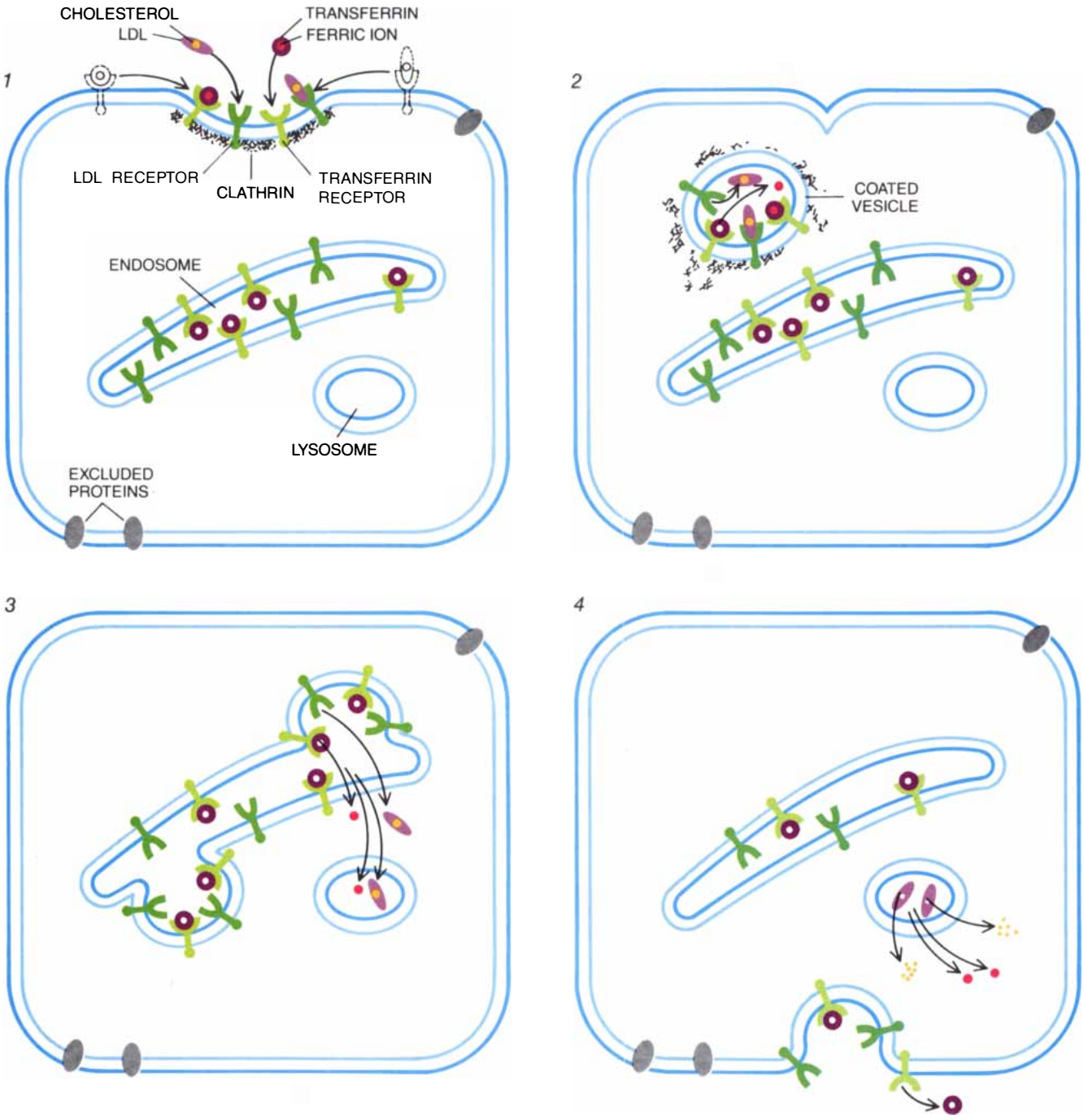
The endocytic cycle initiated by coated pits gives a dynamic picture of a cell. At any instant about 2 percent of the surface of a cell growing in culture is taken up by deepening coated pits. Given such a large flux of membrane from the plasma membrane through the endosomal compartment and back again, one might expect that the protein components of the two membranes would quickly become identical. Such mixing does not take place, however, because the coated pits select only certain proteins from the plasma membrane for transfer into the cell. It is thought the same set of membrane proteins, now residing in the endosomal membrane, is cycled back to the plasma membrane by a similar selective process. This selective transfer of membrane by coated pits may explain how the integrity of numerous distinct membrane compartments can be maintained in spite of continual traffic among them. Note also that during the endocytic cycle the topology and asymmetry of the membrane are always maintained.

The understanding of how the coated pit selects proteins from the plasma membrane is admittedly incomplete. Nevertheless, it has been greatly advanced by structural studies of the closely related coated vesicles. In 1976 Barbara M. F. Pearse of the Laboratory of Molecular Biology isolated coated vesicles and showed that the coat is a lattice of a large, fibrous protein she named clathrin. Such coated vesicles also carry receptors, the ligand molecules attached to the receptors and a variety of other proteins that could mediate the interaction of clathrin with the receptors. Coated vesicles are generated not only by the plasma membrane but also by intracellular organelles such as the Golgi apparatus. Hence there is hope that the sorting mechanism may soon be clarified.

The picture of the plasma membrane emerging from this work is that of a lipid bilayer spanned by a host of different proteins. Some of them simply catalyze the transfer of small molecules across the bilayer, and they have a globular structure. Others have only a single hydrophobic helical segment that holds them in the membrane; some of the helical proteins are receptors that bring large molecules into the cell. Whereas all, or almost all, of these molecules are free to diffuse around in the liquid bilayer, there are other structures such as the gap junc-

tions and the tight junctions that remain relatively static. In contrast there is also the highly dynamic movement of the membrane during the endocytic cycle.

Plasma membranes take part in many cellular functions I have not discussed. Since they make up the interface between a cell and the rest of the organism, they must be involved in the movement of cells and in how the movement is directed during growth and development. The plasma membrane also plays a role in cancerous growth, in which cell multiplication and migration can become uncontrolled. Although a molecular understanding of such processes is yet to be achieved, current knowledge of the structure of membranes is a major step in that direction.




**GENERALIZED CYCLE of endocytosis and exocytosis is shown in four stages. Ferric ions in transferrin molecules and cholesterol in particles of low-density lipoprotein (LDL) bind to receptors in the plasma membrane. The receptors bearing transferrin and LDL diffuse into a coated pit, which somehow blocks the entry of other kinds of membrane proteins (*1*). After a pit buds into the cell and becomes a coated vesicle the clathrin coat is shed, and increasingly acidic conditions begin to release LDL from its receptor and the ferric ions from the transferrin (*2*). The liberated vesicle then fuses with an endosome already bearing receptors from previous cycles of endocytosis. The released ferric ions and LDL are transferred to a lysosome. A vesicle is also shown budding away from the endosome (*3*). The vesicle, bearing empty LDL receptors and iron-free transferrin still attached to its receptor, then fuses again with the plasma membrane, and the receptors enter another cycle of endocytosis. In the lysosome cholesterol is released from the LDL; ferric ions and cholesterol are transported to other parts of the cell (*4*). The asymmetry of the membrane is maintained throughout the cycle.**