# The Fluid Mosaic Model of the Structure of Cell Membranes

Cell membranes are viewed as two-dimensional solutions of oriented globular proteins and lipids.

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Biological membranes play a crucial role in almost all cellular phenomena, yet our understanding of the molecular organization of membranes is still rudimentary. Experience has taught us, however, that in order to achieve a satisfactory understanding of how any biological system functions, the detailed molecular composition and structure of that system must be known. While we are still a long way from such knowledge about membranes in general, progress at both the theoretical and experimental levels in recent years has brought us to a stage where at least the gross aspects of the organization of the proteins and lipids of membranes can be discerned. There are some investigators, however, who, impressed with the great diversity of membrane compositions and functions, do not think there are any useful generalizations to be made even about the gross structure of cell membranes. We do not share that view. We suggest that an analogy exists between the problems of the structure of membranes and the structure of proteins. The latter are tremendously diverse in composition, function, and *detailed* structure. Each kind of protein molecule is structurally unique. Nevertheless, generalizations about protein structure have been very useful in understanding the properties and functions of protein molecules. Similarly, valid generalizations may exist about the ways in which the proteins and lipids are organized in an intact membrane. The ultimate test of such generalizations, or models, is whether they are useful to explain old experiments and suggest new ones.

Singer (*1*) has recently examined in considerable detail several models of the gross structural organization of membranes, in terms of the thermodynamics of macromolecular systems and in the light of the then available experimental evidence. From this analysis, it was concluded that a mosaic structure of alternating globular proteins and phospholipid bilayer was the only membrane model among those analyzed that was simultaneously consistent with thermodynamic restrictions and with all the experimental data available. Since that article was written, much new evidence has been published that strongly supports and extends this mosaic model. In particular, the mosaic appears to be a fluid or dynamic one and, for many purposes, is best thought of as a two-dimensional oriented viscous solution. In this article, we therefore present and discuss a fluid mosaic model of membrane structure, and propose that it is applicable to most biological membranes, such as plasmalemmal and intracellular membranes, including the membranes of different cell organelles, such as mitochondria and chloroplasts. These membranes are henceforth referred to as functional membranes. There may be some other membrane-like systems, such as myelin, or the lipoprotein membranes of small animal viruses, which we suggest may be rigid, rather than fluid, mosaic structures, but such membrane systems are not a primary concern of this article.

Our objectives are (i) to review briefly some of the thermodynamics of macromolecular, and particularly membrane, systems in an aqueous environment; (ii) to discuss some of the properties of the proteins and lipids of functional membranes; (iii) to describe the fluid mosaic model in detail; (iv) to analyze some of the recent and more direct experimental evidence in terms of the model; and (v) to show that the fluid mosaic model suggests new ways of thinking about membrane functions and membrane phenomena.

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## Thermodynamics and Membrane Structure

The fluid mosaic model has evolved by a series of stages from earlier versions (*1–4*). Thermodynamic considerations about membranes and membrane components initiated, and are still central to, these developments. These considerations derived from two decades of intensive studies of protein and nucleic acid structures; the thermodynamic principles involved, however, are perfectly general and apply to any macromolecular system in an aqueous environment. These principles and their application to membrane systems have been examined in detail elsewhere (*1*) and are only summarized here. For our present purposes, two kinds of noncovalent interactions are most important, *hydrophobic* (*5*) and *hydrophilic* (*1*). By hydrophobic interactions is meant a set of thermodynamic factors that are responsible for the sequestering of hydrophobic or nonpolar groups away from water, as, for example, the immiscibility of hydrocarbons and water. To be specific, it requires the expenditure of 2.6 kilocalories of free energy to transfer a mole of methane from a nonpolar medium to water at 25°C (*5*). Free energy contributions of this magnitude, summed over the many nonpolar amino acid residues of soluble proteins, are no doubt of primary importance in determining the conformations that protein molecules adopt in aqueous solution (*6*), in which the nonpolar residues are predominantly sequestered in the interior of the molecules away from contact with water. By hydrophilic interactions is meant a set of thermodynamic factors that are responsible for the preference of ionic and polar groups for an aqueous rather than a nonpolar environment. For example, the free energy required to transfer a mole of zwitterionic glycine from water to acetone is about 6.0 kcal at 25°C, showing that ion pairs strongly prefer to be in water than in a nonpolar medium (*1*). These and related free energy terms no doubt provide the reasons why essentially all the ionic residues of protein molecules are observed to be in contact with water,

usually on the outer surface of the molecule, according to x-ray crystallographic studies. Similar thermodynamic arguments apply to saccharide residues (*1*). It requires the expenditure of substantial free energy to transfer a simple saccharide from water to a nonpolar solvent, and such residues will therefore be in a lower free energy state in contact with water than in a less polar environment.

There are other noncovalent interactions, such as hydrogen bonding and electrostatic interactions, which also contribute to determine macromolecular structure. However, with respect to gross structure, with which we are now concerned, these are very likely of secondary magnitude compared to hydrophobic and hydrophilic interactions.

The familiar phospholipid bilayer structure illustrates the combined effects of hydrophobic and hydrophilic interactions. In this structure (Fig. 1) the nonpolar fatty acid chains of the phospholipids are sequestered together away from contact with water, thereby maximizing hydrophobic interactions. Furthermore, the ionic and zwitterionic groups are in direct contact with the aqueous phase at the exterior surfaces of the bilayer, thereby maximizing hydrophilic interactions. In the case of zwitterionic phospholipids such as phosphatidylcholine, dipole-dipole interactions between ion pairs at the surface of the bilayer may also contribute to the stabilization of the bilayer structure.

In applying these thermodynamic principles to membranes, we recognize first that of the three major classes of membrane components—proteins, lipids, and oligosaccharides—the proteins are predominant. The ratio by weight of proteins to lipids ranges from about 1.5 to 4 for those functional membranes which have been well characterized [compare (*7*)]. A substantial fraction of this protein most probably plays an important role in determining the structure of membranes, and the structural properties of these proteins are therefore of first-order importance. Membrane proteins are considered in some detail in the following section. At this juncture, the significant point is that if hydrophobic and hydrophilic interactions are to be maximized and the lowest free energy state is to be attained for the intact membrane in an aqueous environment, the nonpolar amino acid residues of the proteins—along with the fatty acid chains of the phospholipids—should be sequestered

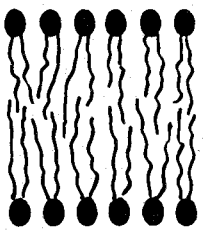

Fig. 1. A phospholipid bilayer: schematic cross-sectional view. The filled circles represent the ionic and polar head groups of the phospholipid molecules, which make contact with water; the wavy lines represent the fatty acid chains.

(to the maximum extent feasible) from contact with water, while the ionic and polar groups of the proteins—along with those of the lipids and the oligosaccharides—should be in contact with the aqueous solvent. These requirements place restrictions on models of membrane structure; in particular, they render highly unlikely the classical model of a trilaminar arrangement of a continuous lipid bilayer sandwiched between two monolayers of protein. The latter model is thermodynamically unstable because not only are the nonpolar amino acid residues of the membrane proteins in this model perforce largely exposed to water but the ionic and polar groups of the lipid are sequestered by a layer of protein from contact with water. Therefore, neither hydrophobic nor hydrophilic interactions are maximized in the classical model.

## Some Properties of Membrane Components

*Peripheral and integral proteins.* It seems both reasonable and important to discriminate between two categories of proteins bound to membranes, which we have termed *peripheral* and *integral* proteins (*1*). Peripheral proteins may be characterized by the following criteria. (i) They require only mild treatments, such as an increase in the ionic strength of the medium or the addition of a chelating agent, to dissociate them molecularly intact from the membrane; (ii) they dissociate free of lipids; and (iii) in the dissociated state they are relatively soluble in neutral aqueous buffers. These criteria suggest that a peripheral protein is held to the membrane only by rather weak noncovalent (perhaps mainly electrostatic) interactions and is not strongly associated with membrane lipid. The cytochrome c of mitochondrial membranes, which can be dissociated free of lipids by high salt concentrations, and the protein spectrin (*8*) of erythrocyte membranes, which can be removed by chelating agents under mild conditions, are examples of membrane proteins that satisfy the criteria for peripheral proteins. On the other hand, the major portion ($>$ 70 percent) of the proteins of most membranes have different characteristics, which may be assigned to integral proteins: (i) they require much more drastic treatments, with reagents such as detergents, bile acids, protein denaturants, or organic solvents, to dissociate them from membranes; (ii) in many instances, they remain associated with lipids when isolated; (iii) if completely freed of lipids, they are usually highly insoluble or aggregated in neutral aqueous buffers (*9*).

The distinction between peripheral and integral proteins may be useful in several regards. It is assumed that only the integral proteins are critical to the structural integrity of membranes. Therefore, the properties and interactions of peripheral proteins, while interesting in their own right, may not be directly relevant to the central problems of membrane structure. The properties of cytochrome c, for example, may not be typical of mitochondrial membrane proteins. Furthermore, the biosynthesis of peripheral and integral proteins and their attachment to the membrane may be very different processes. This is not the appropriate occasion to discuss membrane biogenesis in any detail, but it may be significant that, although cytochrome c is a mitochondrial protein, it is synthesized on cytoplasmic rather than mitochondrial ribosomes; in fact only a small fraction of the total mitochondrial protein (perhaps only the integral proteins of the inner mitochondrial membrane?) appears to be synthesized on mitochondrial ribosomes (*10*). In any event, because of the relatively unimportant membrane structural role assigned to the peripheral proteins, they are not a primary concern of this article.

*Properties of integral proteins.* Since the proteins we have classified as integral, according to the criteria specified, constitute the major fraction of membrane proteins, we assume that the properties to be discussed apply to the integral proteins.

1) For several well-characterized membrane systems, including erythrocyte and other plasma membranes, and mitochondrial membranes, the proteins have been shown to be grossly heterogeneous with respect to molecular

weights (*11*). There is no convincing evidence that there exists one predominant type of membrane protein that is specifically a structural protein; recent reports to the contrary have been withdrawn. We consider this heterogeneity to be more significant for a general model of membrane structure than the fact that in a few specialized instances, as in the case of disk membranes of retinal rod outer segments (*12, 13*), a single protein species predominates. A satisfactory membrane model must be capable of explaining the heterogeneity of the integral membrane proteins.

2) The proteins of a variety of intact membranes, on the average, show appreciable amounts of the $\alpha$-helical conformation, as was first shown by Ke (*14*), Wallach and Zahler (*4*), and Lenard and Singer (*3*). For example, circular dichroism measurements of aqueous suspensions of intact and mechanically fragmented human erythrocyte membranes (provided that we take into account certain optical anomalies of these measurements) reveal that about 40 percent of the protein is in the right-handed $\alpha$-helical conformation (*15*). Most soluble globular proteins whose circular dichroism spectra have been obtained exhibit a smaller fraction of $\alpha$-helix in their native structures. This suggests that the integral proteins in intact membranes are largely globular in shape rather than spread out as monolayers. On the other hand, a membrane model in which such globular proteins are attached to the *outer* surfaces of a lipid bilayer (*16*) would not be satisfactory because, among other reasons, it would require membrane thicknesses much larger than the 75 to 90 angstroms generally observed. A model in which globular protein molecules are intercalated within the membrane would, however, meet these restrictions.

*The phospholipids of membranes.* There is now substantial evidence that the major portion of the phospholipids is in bilayer form in a variety of intact membranes. For example, differential calorimetry of intact mycoplasma membranes shows that they undergo a phase transition in a temperature range very similar to that of aqueous dispersions of the phospholipids extracted from the membranes (*16, 17*). Thus the structures of the lipid in the membrane and of the lipid in isolated aqueous dispersion are closely similar; presumably the latter is the bilayer form. This conclusion is supported by x-ray diffraction (*18*) and spin-label studies (*19*) on similar membrane preparations.

The bilayer character of membrane lipids rules out models such as that of Benson (*20*) in which the proteins and lipids form a single-phase lipoprotein subunit that is repeated indefinitely in two dimensions to constitute the membrane. In such a model, most of the lipids would be expected to have distinctly different properties from those of a bilayer.

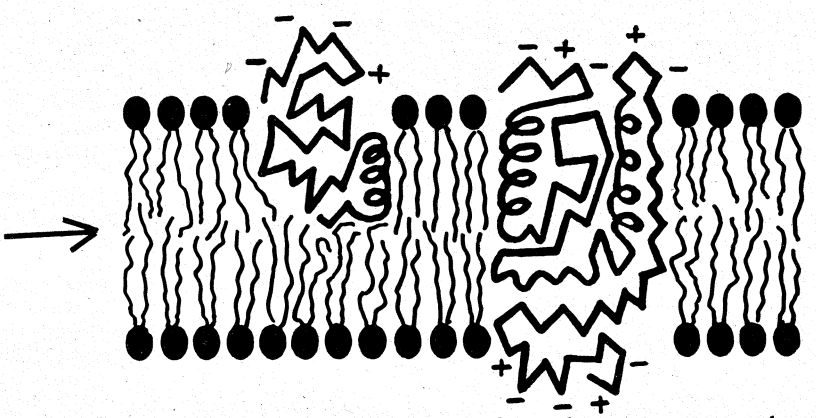

Fig. 2. The lipid-globular protein mosaic model of membrane structure: schematic cross-sectional view. The phospholipids are depicted as in Fig. 1, and are arranged as a discontinuous bilayer with their ionic and polar heads in contact with water. Some lipid may be structurally differentiated from the bulk of the lipid (see text), but this is not explicitly shown in the figure. The integral proteins, with the heavy lines representing the folded polypeptide chains, are shown as globular molecules partially embedded in, and partially protruding from, the membrane. The protruding parts have on their surfaces the ionic residues (— and +) of the protein, while the nonpolar residues are largely in the embedded parts; accordingly, the protein molecules are amphipathic. The degree to which the integral proteins are embedded and, in particular, whether they span the entire membrane thickness depend on the size and structure of the molecules. The arrow marks the plane of cleavage to be expected in freeze-etching experiments (see text). [From Lenard and Singer (*3*) and Singer (*1*)]

Two qualifications should be stressed, however, concerning the bilayer form of membrane lipids. (i) None of the evidence so far obtained for the bilayer form permits us to say whether the bilayer is *continuous* or *interrupted* (*1*). The calorimetrically observed phase transitions, for example, occur over a broad temperature interval, allowing the possibility that the cooperative unit involved in the phase transition is quite small, consisting perhaps of only 100 lipid molecules on the average. (ii) None of the experiments mentioned above is sufficiently sensitive and quantitative to prove whether 100 percent of the phospholipid is in the bilayer form. It is therefore not excluded that some significant fraction of the phospholipid (perhaps as much as 30 percent) is physically in a different state from the rest of the lipid.

*Protein-lipid interactions in membranes.* Several kinds of experiments indicate that protein-lipid interactions play a direct role in a variety of membrane functions. Many membrane-bound enzymes and antigens require lipids, often specific phospholipids, for the expression of their activities [see table 2 in (*21*)]. Furthermore, the nature of the fatty acids incorporated into phospholipids affects the function of certain membrane-bound proteins in bacterial membranes (*22*).

On the other hand, the calorimetric data discussed above give no significant indication that the association of proteins with the phospholipids of intact membranes affects the phase transitions of the phospholipids themselves. Experiments with phospholipase C and membranes have shown that the enzymic release of 70 percent of the phosphorylated amines from intact erythrocyte membranes profoundly perturbs the physical state of the residual fatty acid chains, but has no detectable effect (as measured by circular dichroism spectra) on the average conformation of the membrane proteins (*2*). Such results therefore suggest that the phospholipids and proteins of membranes do not interact strongly; in fact, they appear to be largely independent.

This paradox, that different types of experiments suggest strong protein-lipid interactions on the one hand, and weak or no interactions on the other, can be resolved in a manner consistent with all the data if it is proposed that, while the largest portion of the phospholipid is in bilayer form and not strongly coupled to proteins in the membrane,

a small fraction of the lipid is more tightly coupled to protein. With any one membrane protein, the tightly coupled lipid might be specific; that is, the interaction might require that the phospholipid contain specific fatty acid chains or particular polar head groups. There is at present, however, no satisfactory direct evidence for such a distinctive lipid fraction. This problem is considered again in connection with a discussion of the experiments of Wilson and Fox (*23*).

## Fluid Mosaic Model

*Mosaic structure of the proteins and lipids of membranes.* The thermodynamic considerations and experimental results so far discussed fit in with the idea of a mosaic structure for membranes (*1–3, 24*) in which globular molecules of the integral proteins (perhaps in particular instances attached to oligosaccharides to form glycoproteins, or interacting strongly with specific lipids to form lipoproteins) alternate with sections of phospholipid bilayer in the cross section of the membrane (Fig. 2). The globular protein molecules are postulated to be amphipathic (*3, 4*) as are the phospholipids. That is, they are structurally asymmetric, with one highly polar end and one nonpolar end. The highly polar region is one in which the ionic amino acid residues and any covalently bound saccharide residues are clustered, and which is in contact with the aqueous phase in the intact membrane; the nonpolar region is devoid of ionic and saccharide residues, contains many of the nonpolar residues, and is embedded in the hydrophobic interior of the membrane. The amphipathic structure adopted by a particular integral protein (or lipoprotein) molecule, and therefore the extent to which it is embedded in the membrane, are under thermodynamic control; that is, they are determined by the amino acid sequence and covalent structure of the protein, and by its interactions with its molecular environment, so that the free energy of the system as a whole is at a minimum. An integral protein molecule with the appropriate size and structure, or a suitable aggregate of integral proteins (below) may transverse the entire membrane (*3*); that is, they have regions in contact with the aqueous solvent on both sides of the membrane.

It is clear from these considerations that different proteins, if they have the appropriate amino acid sequence to adopt an amphipathic structure, can be integral proteins of membranes; in this manner, the heterogeneity of the proteins of most functional membranes can be rationalized.

The same considerations may also explain why some proteins are membrane-bound and others are freely soluble in the cytoplasm. The difference may be that either the amino acid sequence of the particular protein allows it to adopt an amphipathic structure or, on the contrary, to adopt a structure in which the distribution of ionic groups is nearly spherically symmetrical, in the lowest free energy state of the system. If the ionic distribution on the protein surface were symmetrical, the protein would be capable of interacting strongly with water all over its exterior surface, that is, it would be a monodisperse soluble protein.

The mosaic structure can be readily diversified in several ways. Although the nature of this diversification is a matter of speculation, it is important to recognize that the mosaic structure need not be restricted by the schematic representation in Fig. 2. Protein-protein interactions that are not explicitly considered in Fig. 2 may be important in determining the properties of the membrane. Such interactions may result either in the specific binding of a peripheral protein to the exterior exposed surface of a particular integral protein or in the association of two or more integral protein subunits to form a specific aggregate within the membrane. These features can be accommodated in Fig. 2 without any changes in the basic structure.

The phospholipids of the mosaic structure are predominantly arranged as an interrupted bilayer, with their ionic and polar head groups in contact with the aqueous phase. As has been discussed, however, a small portion of the lipid may be more intimately associated with the integral proteins. This feature is not explicitly indicated in Fig. 2. The thickness of a mosaic membrane would vary along the surface from that across a phospholipid bilayer region to that across a protein region, with an *average* value that could be expected to correspond reasonably well to experimentally measured membrane thicknesses.

*Matrix of the mosaic: lipid or protein?* In the cross section of the mosaic structure represented in Fig. 2, it is not indicated whether it is the protein or the phospholipid that provides the matrix of the mosaic. In other words, which component is the mortar, which the bricks? This question must be answered when the third dimension of the mosaic structure is specified. These two types of mosaic structure may be expected to have very different structural and functional properties, and the question is therefore a critical one. It is our hy-

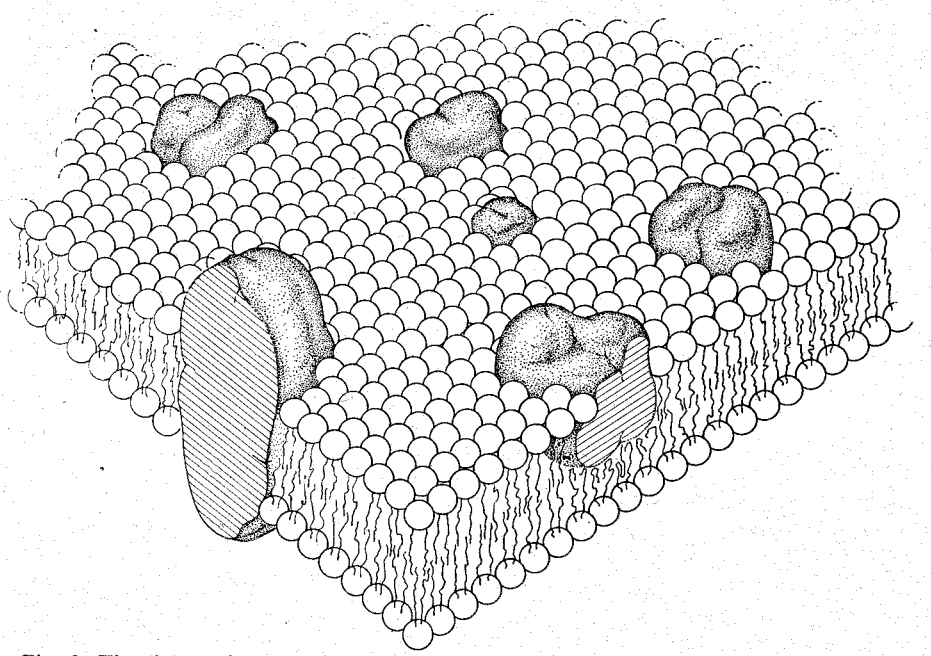

Fig. 3. The lipid-globular protein mosaic model with a lipid matrix (the fluid mosaic model); schematic three-dimensional and cross-sectional views. The solid bodies with stippled surfaces represent the globular integral proteins, which at long range are randomly distributed in the plane of the membrane. At short range, some may form specific aggregates, as shown. In cross section and in other details, the legend of Fig. 2 applies.

pothesis that functional cell membranes have a long-range mosaic structure with the *lipids* constituting the matrix, as is shown in Fig. 3. Supporting evidence is discussed later. At this point, let us consider some of the consequences of this hypothesis.

1) There should generally be no long-range order in a mosaic membrane with a lipid matrix. By long range, we mean over distances of the order of a few tenths of a micrometer and greater. Suppose we have a membrane preparation containing many different protein species, and suppose further that 10,000 molecules of protein *A* are present in the membrane of a single cell or organelle. How is protein *A* distributed over the membrane surface? If the membrane proteins formed the matrix of the mosaic, defined by specific contacts between the molecules of different integral proteins, protein *A* might be distributed in a highly ordered, two-dimensional array on the surface. On the other hand, if lipid formed the matrix of the mosaic, there would be no long-range interactions *intrinsic* to the membrane influencing the distribution of *A* molecules, and they should therefore be distributed in an aperiodic random arrangement on the membrane surface.

The absence of long-range order should not be taken to imply an absence of short-range order in the membrane. It is very likely that such short-range order does exist, as, for example, among at least some components of the electron transport chain in the mitochondrial inner membrane. Such short-range order is probably mediated by specific protein (and perhaps protein-lipid) interactions leading to the formation of stoichiometrically defined aggregates within the membrane. However, in a mosaic membrane with a lipid matrix, the long-range distribution of such aggregates would be expected to be random over the entire surface of the membrane.

The objection may immediately be raised that long-range order clearly exists in certain cases where differentiated structures (for example, synapses) are found within a membrane. We suggest, in such special cases, either that short-range specific interactions among integral proteins result in the formation of an unusually large two-dimensional aggregate or that some agent extrinsic to the membrane (either inside or outside the cell) interacts multiply with specific integral proteins to produce a clustering of those proteins in a limited area of the membrane surface. In other words, we suggest that long-range random arrangements in membranes are the norm; wherever nonrandom distributions are found, mechanisms must exist which are responsible for them.

2) It has been shown that, under physiological conditions, the lipids of functional cell membranes are in a fluid rather than a crystalline state. (This is not true of myelin, however.) This evidence comes from a variety of sources, such as spin-labeling experiments (*25*), x-ray diffraction studies (*18*), and differential calorimetry (*16, 17*). If a membrane consisted of integral proteins dispersed in a fluid lipid matrix, the membrane would in effect be a two-dimensional liquid-like solution of monomeric or aggregated integral proteins (or lipoproteins) dissolved in the lipid bilayer. The mosaic structure would be a dynamic rather than a static one. The integral proteins would be expected to undergo translational diffusion within the membrane, at rates determined in part by the effective viscosity of the lipid, unless they were tied down by some specific interactions intrinsic or extrinsic to the membrane. However, because of their amphipathic structures, the integral proteins would maintain their molecular orientation and their degree of intercalation in the membrane while undergoing translational diffusion in the plane of the membrane (as discussed below).

In contrast, if the matrix of the mosaic were constituted of integral proteins, the long-range structure of the membrane would be essentially static. Large energies of activation would be required for a protein component to diffuse in the plane of the membrane from one region to a distant one because of the many noncovalent bonds between the proteins that would have to be simultaneously broken for exchange to take place. Therefore, a mosaic membrane with a protein matrix should make for a relatively rigid structure with essentially no translational diffusion of its protein components within the membrane.

From the discussion in this and the previous section, it is clear that the fluid mosaic model suggests a set of structural properties for functional membranes at least some of which can be tested experimentally. In an earlier article (*1*), a large body of experimental evidence was examined for its relevance to models of membrane structure. It was concluded that a mosaic structure was most consistent with the available evidence. Some more recent results, however, bear even more directly on the problem, and only this evidence is discussed below.

## Some Recent Experimental Evidence

*Evidence for proteins embedded in membranes.* One proposal of the fluid mosaic model is that an integral protein is a globular molecule having a significant fraction of its volume embedded in the membrane. The results of recent freeze-etching experiments with membranes strongly suggest that a substantial amount of protein is deeply embedded in many functional membranes. In this technique (*26*) a frozen specimen is fractured with a microtome knife; some of the frozen water is sublimed (etched) from the fractured surface if desired; the surface is then shadow cast with metal, and the surface replica is examined in the electron microscope. By this method the topography of the cleaved surface is revealed. A characteristic feature of the exposed surface of most functional membranes examined by this technique, including plasmalemmal, vacuolar, nuclear, chloroplast, mitochondrial, and bacterial membranes (*27, 28*), is a mosaic-like structure consisting of a smooth matrix interrupted by a large number of particles. These particles have a fairly characteristic uniform size for a particular membrane, for example, about 85-Å diameter for erythrocyte membranes. Such surfaces result from the cleavage of a membrane along its *interior* hydrophobic face (*29*). This interior face (Fig. 2) corresponds to the plane indicated by the arrow. If cleavage were to occur smoothly between the two layers of phospholipid in the bilayer regions, but were to circumvent the protein molecules penetrating the mid-plane of the membrane, then the alternating smooth and particulate regions observed on the freeze-etch surfaces can be readily explained by a mosaic structure for the membrane (Fig. 2), provided that the particles can be shown to be protein in nature. That the particles are indeed protein has been suggested by recent experiments (*30*).

Another consequence of the mosaic model, suggested from its inception (*3*), is that certain integral proteins possessing the appropriate size and structure may span the entire thickness of the membrane and be exposed at both membrane surfaces. Chemical evidence

that a trans-membrane protein, whose molecular weight is about 100,000, is present in large amounts in the human erythrocyte membrane has been obtained by two independent methods—one involving proteolysis of normal compared to everted membranes (*31*), and the other specific chemical labeling of the membrane proteins (*32*).

*Distribution of components in the plane of the membrane.* A prediction of the fluid mosaic model is that the two-dimensional long-range distribution of any integral protein in the plane of the membrane is essentially random. To test this prediction, we have developed and applied electron microscopic techniques to visualize the distribution of specific membrane antigens over large areas of their membrane surfaces (*33*) and have so far studied the distribution of the $Rh_o(D)$ antigen on human erythrocyte membranes (*34*), and of H-2 histocompatibility alloantigens on mouse erythrocyte membranes (*35*).

In the case of the $Rh_o(D)$ antigen, for example, cells of O, Rh-positive type were reacted with a saturating amount of $^{125}I$-labeled purified human antibody to $Rh_o(D)$ [anti-$Rh_o(D)$], and the treated (sensitized) cells were lysed at an air-water interface, so that the cell membranes were spread out flat. The flattened membranes, after being picked up on an electron microscope grid, were treated with the specific "indirect stain," ferritin-conjugated goat antibodies specific for human γ-globulin. Thus, wherever the human anti-$Rh_o$ (D) molecules were bound to the $Rh_o$ (D) antigen on the membrane surface, the ferritin-labeled goat antibodies became specifically attached. In other words, the human γ-globulin antibody now functioned as an antigen for the goat antibodies (Fig. 4). The ferritin was distributed in discrete clusters, each containing two to eight ferritin molecules within a circle of radius about 300 Å. The numbers of such clusters per unit area of the membrane surface corresponded to the number of $^{125}I$-labeled human anti-$Rh_o(D)$ molecules bound per unit area. This indicates that each ferritin cluster was bound to a single anti-$Rh_o(D)$ molecule, and a cluster represents the number of goat antibody molecules bound to a single human γ-globulin molecule. Each cluster therefore corresponds to a single $Rh_o(D)$ antigen site (*36*) on the membrane. Since the clusters were distributed in a random array, we conclude that the $Rh_o(D)$ antigen, which exhibits properties of an integral protein (*37*), is molecularly dispersed and is distributed in a random two-dimensional array on the human erythrocyte membrane.

Similar experiments were carried out with the H-2 alloantigenic sites on mouse erythrocyte membranes. In this case (Fig. 5) the clusters of ferritin molecules of the indirect stain were not isolated, as in the case of the $Rh_o(D)$ antigen, but instead occurred in patches. The patchy distribution of the H-2 histocompatibility alloantigenic sites had earlier been observed by different techniques (*38*), but the two-dimensional distribution of the patches could not be ascertained. In our experiments, the patches contained variable numbers of clusters, and were arranged in an irregular two-dimensional array on the membrane surface. The histocompatibility antigen appears to be glycoprotein in nature (*39*). The long-range distribution of both the $Rh_o(D)$ and H-2 histocompatibility antigens on their respective membrane surfaces, therefore,

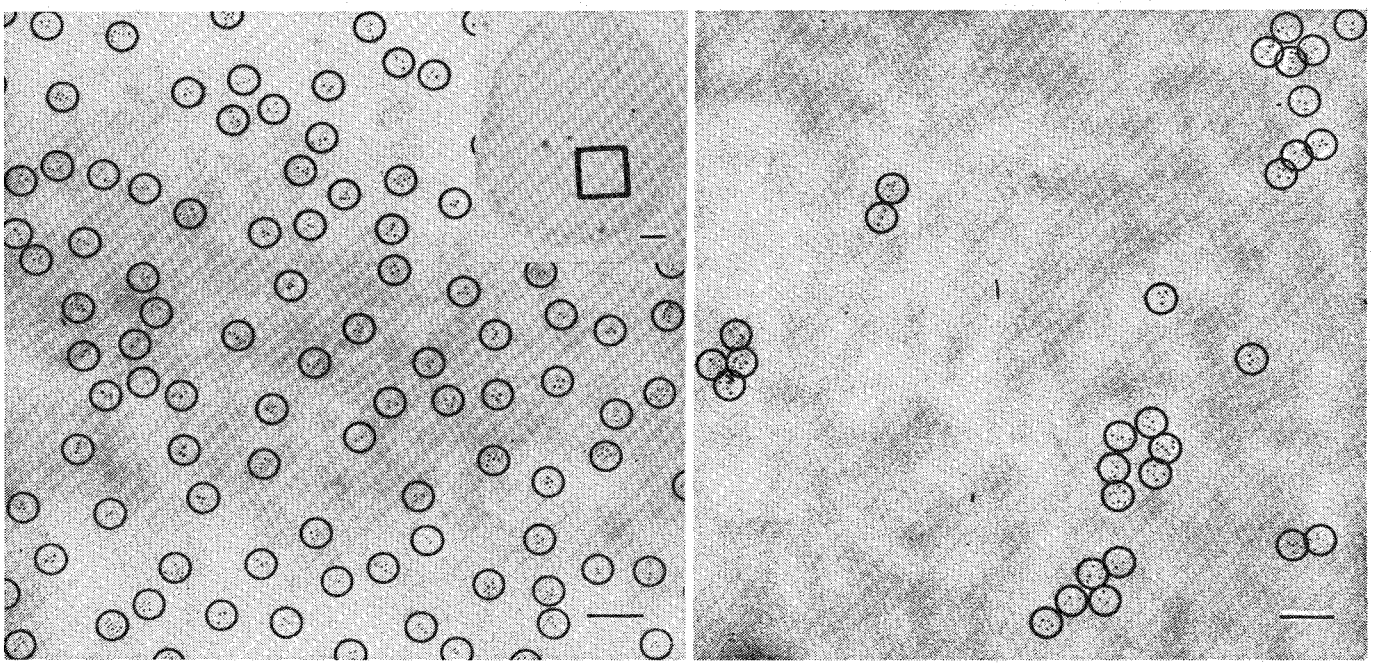

Fig. 4 (left). The outer membrane surface of an Rh-positive human erythrocyte sensitized with human anti-$Rh_o(D)$ and stained with ferritin-conjugated goat antibody to human γ-globulin. The cells were first labeled to saturation with purified $^{125}I$-labeled human antibody to $Rh_o(D)$ and then lysed at an air-water interface. The erythrocyte membrane ghosts, flattened by surface forces (inset, low magnification) were picked up on a coated, electron microscope grid and indirectly stained with ferritin-conjugated goat antibodies to human γ-globulin. The ferritin appears bound to the membrane in discrete clusters of two to eight ferritin-conjugates; each cluster is circumscribed by a circle of radius 300 Å. The number of such clusters per cell (9300) is equal within experimental error to the number of $^{125}I$-labeled human antibody to $Rh_o(D)$ molecules bound per cell (10,200). Each cluster therefore corresponds to an individual $Rh_o(D)$ antigenic site. Scale is 0.1 μm; inset scale is 1 μm. [From Nicolson, Masouredis, and Singer (*34*)] Fig. 5 (right). The outer membrane surface on a mouse erythrocyte ($H\text{-}2^b$) sensitized with alloantibodies against $H\text{-}2^b$ histocompatibility antigens and stained with ferritin-conjugated antibodies against 7*S* mouse γ-globulin. The procedures are the same as listed in the legend to Fig. 4. The ferritin-antibody clusters are present in randomly spaced "patches" of variable size on the membrane surface. Scale is 0.1 μm. [From Nicolson, Hyman, and Singer (*35*)]

are in accord with the prediction of the fluid mosaic model that the integral proteins of membranes are randomly arranged in two dimensions.

The particles on the inner membrane faces revealed by freeze-etching experiments, which (as discussed above) are probably protein in nature, are generally also relatively randomly distributed in two dimensions.

*Evidence that proteins are in a fluid state in intact membranes.* An important series of experiments has been carried out (*12*, *40–44*) with receptor disk membranes from the retina of the frog. This membrane system is unusual in that it contains as its predominant, if not only, protein component the pigment rhodopsin. In electron microscopy of the negatively stained surfaces of the dried membranes, a somewhat tightly packed and ordered array of particles (about 40 Å) was observed. These particles are the individual rhodopsin molecules. Although the earlier studies suggested that there was a long-range order in the distribution of the particles (*40*), more recent x-ray diffraction data (*42*) on pellets of wet, receptor disk membranes showed that only a few orders of reflection were observed corresponding to the spacings of the rhodopsin molecules in the plane of the membrane. This indicated that a noncrystalline, aperiodic arrangement of the particles existed in the plane of the membrane. Furthermore, the temperature dependence of the characteristics of the x-ray diffraction maxima were consistent with the suggestion that the particles were in a planar liquid-like state in the intact membrane. Additional support for the existence of this liquid-like state was the observation that the absorption of a foreign protein (bovine serum albumin) to the membrane could definitely alter the x-ray spacings due to the rhodopsin particles; that is, the distribution of the rhodopsin molecules in the plane of the membrane was radically altered by the weak binding of the albumin. This alteration would not be expected if a rigid lattice structure of the rhodopsin molecules, or aggregates, were present in the plane of the membrane.

These studies are particularly noteworthy because they involved a membrane which, by conventional electron microscopic techniques, appears to show long-range periodicity over its surface. Other specialized membranes have also exhibited ordered electron micrographic images of their surfaces [compare (*43*)]. However, it is likely that a very concentrated two-dimensional fluid solution of identical protein molecules will appear, when dried, to be arranged in an ordered array, particularly when optical tricks are used to enhance the apparent order (*43*). What is really a fluid phase may therefore artifactually be made to appear as a crystalline solid. This appears to be the situation with the retinal receptor disk membranes.

A major contribution to membrane studies has been made by Frye and Edidin (*44*), who investigated the membrane properties of some cell fusion heterokaryons. Human and mouse cells in culture were induced to fuse with one another, with Sendai virus as the fusing agent. The distribution of human and mouse antigenic components of the fused cell membranes was then determined by immunofluorescence, with the use of rabbit antibodies directed to the whole human cells, mouse antibodies directed against the H-2 alloantigen on the mouse cell membranes, and, as indirect stains, goat antiserum to rabbit $\gamma$-globulin and goat antiserum to mouse $\gamma$-globulin labeled with two different fluorescent dyes. Shortly after cell fusion, the mouse and human antigenic components were largely segregated in different halves of the fused cell membranes; but after about 40 minutes at 37°C the components were essentially completely intermixed. Inhibitors of protein synthesis, of adenosine triphosphate (ATP) formation, and of glutamine-dependent synthetic pathways, applied before or after cell fusion, had no effect on the rate of this intermixing process, but lowering the temperature below 15°C sharply decreased it.

Frye and Edidin (*44*) suggest that the intermixing of membrane components is due to diffusion of these components within the membrane, rather than to their removal and reinsertion, or to the synthesis and insertion of new copies of these components, into the heterokaryon membrane. An unexplained finding of these experiments was the fairly frequent occurrence, at early and intermediate times after cell fusion, of heterokaryon membranes in which the human antigenic components were uniformly distributed over the membrane surface but the mouse components were still largely segregated to about half the membrane ($M_{1/2}$-$H_1$ cells). On the other hand, the reverse situation, with the mouse antigenic components uniformly spread out over the membrane and the human components segregated ($M_1$-$H_{1/2}$), was only rarely observed. This result can now be explained by a diffusion mechanism for the intermixing process, as follows. The antibodies to the human cell membrane were no doubt directed to a heterogeneous set of antigens, whereas the antibodies to the mouse cell were directed specifically to the histocompatibility alloantigen. However, the histocompatibility antigens occur as large aggregates in the membrane (Fig. 5), and might therefore be expected to diffuse more slowly than a complex mixture of largely unaggregated human antigens in the membrane. Thus, at appropriate intermediate times after cell fusion, significant numbers of ($M_{1/2}$-$H_1$) but not of ($M_1$-$H_{1/2}$) fused cells might appear, to be converted at longer times to cells with completely intermixed components.

A rough estimate may be made of the average effective diffusion constant required of the membrane components to account for the kinetics of intermixing in the Frye-Edidin experiments. Taking the average distance of migration, $x$, to have been about 5 micrometers in a time, $t$, of 40 minutes gives an apparent diffusion constant, $D = x^2/2t$, of $5 \times 10^{-11}$ cm²/sec. For comparison, the diffusion constant of hemoglobin in aqueous solutions is about $7 \times 10^{-7}$ cm²/sec. The apparent effective viscosity of the membrane fluid phase is therefore about $10^3$ to $10^4$ times that of water.

The Frye-Edidin experiments can be rationalized by the fluid mosaic model of membrane structure as being the result of the free diffusion and intermixing of the lipids and the proteins (or lipoproteins) within the fluid lipid matrix.

Some experiments, however, appear to suggest that the lipids of membranes are not readily interchangeable within the membrane and are therefore not free to diffuse independently. For example, Wilson and Fox (*23*) have studied the induction of $\beta$-galactoside and $\beta$-glucoside transport systems in mutants of *Escherichia coli* that cannot synthesize unsaturated fatty acids. Such fatty acids can be incorporated into phospholipids, however, if they are supplied in the growth medium. When cells were grown in particular fatty acid supplements and induced for the synthesis of the transport systems, the effect of temperature on the transport rate was characteristic of that fatty acid. If, then, the cells were first grown in medium containing oleic acid and then shifted to growth in a medium supplemented with linoleic acid during a brief period of induction of either of the transport systems, the effect of temperature on trans-

port was said to be characteristic of cells grown continually in the linoleic acid medium. In other words, although most of the phopholipids of the membrane contained oleic acid chains, these did not appear to exchange with the newly synthesized small amounts of phospholipids containing linoleic acid chains. These experiments, however, do not necessarily contradict the thesis that most of the phospholipids of membranes are freely diffusible and, hence, exchangeable. For example, each of the two transport systems might be organized in the membrane as a specific protein aggregate containing intercalated and strongly bound phospholipid components. If such lipoprotein aggregates had first to be assembled in order to be incorporated into the bulk lipid matrix of the membrane, the results of Wilson and Fox would be anticipated. In particular, the small fraction of the membrane phospholipid that was strongly bound, and perhaps segregated in such aggregates from the bulk of the membrane lipid, might not exchange rapidly with the bulk lipid. The Wilson-Fox experiments therefore do not require that the major part of the membrane phospholipid be static, but only that a small fraction of the lipids be structurally differentiated from the rest. The structural differentiation of some of the membrane lipid by strong binding to integral proteins is a possibility that was discussed above.

The observations of Wilson and Fox, that there is a significant coupling of lipid and protein incorporation into membranes, appear to be a special case. The experiments of Mindich (*45*) demonstrate that more generally lipid and protein incorporation into bacterial membranes can occur independently, and that quite wide variations in the ratio of lipids and proteins in the membrane can be produced in vivo, as might be expected from the fluid mosaic model of membrane structure.

*The asymmetry of membranes.* A substantial amount of evidence has accumulated showing that the two surfaces of membranes are not identical in composition or structure. One aspect of this asymmetry is the distribution of oligosaccharides on the two surfaces of membranes. There exist plant proteins, called lectins or plant agglutinins, which bind to specific sugar residues, and, as a result, can cause the agglutination of cells bearing the sugar residues on their surfaces. By conjugating several such agglutinins to ferritin, we have been able to visualize the distribution of oligosaccharides on membranes in the electron microscope (*33*). For example, the ferritin conjugate of concanavalin A, a protein agglutinin that binds specifically to terminal α-D-glucopyranosyl or α-D-mannopyranosyl residues (*46*), attaches specifically to the outer surface of erythrocyte membranes and not at all to the inner cytoplasmic surface (*33*). A similar, completely asymmetric distribution of ferritin conjugates of ricin (a protein agglutinin) on the membranes of rabbit erythrocytes is shown in Fig. 6. Ricin binds specifically to terminal β-D-galactopyranosyl and sterically related sugar residues (*47*). Such asymmetry has now been observed with several ferritin-conjugated agglutinins and a number of different mammalian cell plasma membranes (*48*). These findings extend earlier results obtained by different methods (*49*).

The foregoing observations bear on many problems, including cell-cell interactions and membrane biogenesis (*50*). In the context of this article, however, the absence of oligosaccharides on inner membrane surfaces indicates that rotational transitions of the glycoproteins of erythrocyte and other plasma membranes from the outer to the inner surfaces must occur at only negligibly slow rates. This conclusion probably applies to membrane proteins other than glycoproteins; for example, the Na,K-dependent and Mg-dependent adenosine triphosphatase activities of erythrocyte membranes are exclusively localized to the inner cytoplasmic surfaces (*51*). Individual molecules of spin-labeled zwitterionic and anionic phospholipids also exhibit very slow inside-outside transitions in synthetic vesicles of phospholipid bilayers (*52*). The very slow or negligible rates of such transitions can be explained by the mosaic model and the thermodynamic arguments already discussed. If the integral proteins (including the glycoproteins) in intact membranes have, like the phospholipids, an amphipathic structure, a large free energy of activation would be required to rotate the ionic and polar regions of the proteins through the hydrophobic interior of the membrane to the other side.

To accommodate the fluid mosaic model to these conclusions concerning asymmetry, we specify that, while the two-dimensional translational diffusion of the integral proteins and the phospholipids of membranes occurs freely,

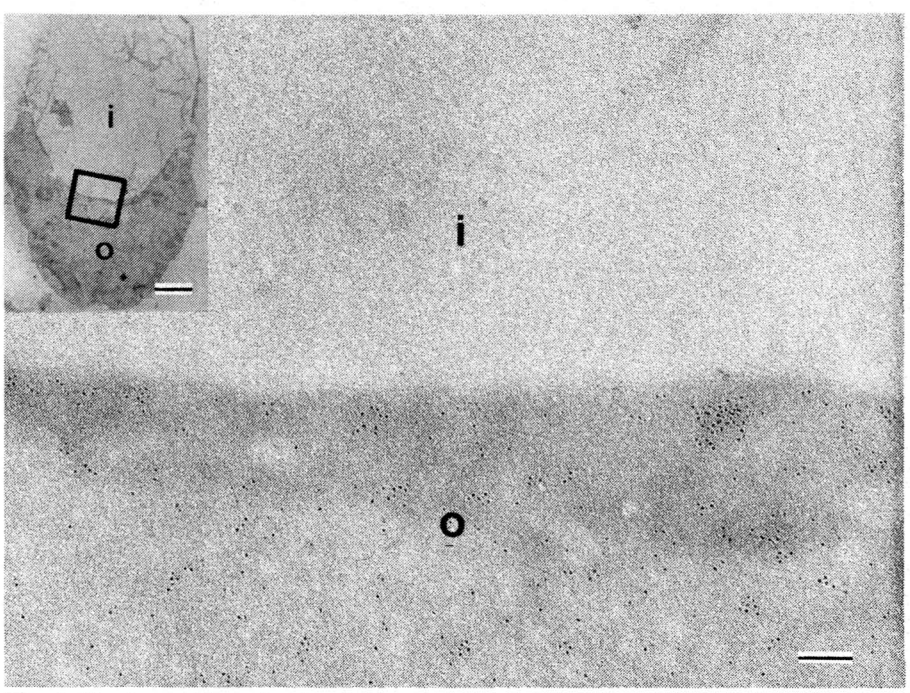


Fig. 6. The inner (*i*) and outer (*o*) membrane surfaces of a rabbit erythrocyte membrane that has been stained with ferritin-conjugated ricin. In preparing membrane specimens such as are shown in Figs. 4 and 5, occasionally a cell lyses with membrane rupture such that both inner and outer surfaces of the membrane are exposed. In this case the mounted membrane was stained with ferritin conjugated to ricin, a plant agglutinin that specifically binds to terminal β-D-galactopyranosyl and sterically related terminal sugar residues in oligosaccharides. The ferritin-agglutinin is found on the outer membrane surface only. The scale is equivalent to 0.1 μm; the insert scale is equivalent to 1 μm.

the rotational diffusion of these components is generally restricted to axes perpendicular to the plane of the membrane; that is, in general, molecular tumbling does not occur at significant rates within the membrane. The asymmetry of the membrane introduces another factor into the problem of translational diffusion of membrane components. In the experiments of Frye and Edidin (*44*) only those membrane antigens exposed at the outer surface of the membrane were labeled by fluorescent antibodies, and the conclusion that these particular antigens were mobile in the plane of the membranes therefore, strictly speaking, applies only to those components accessible at the outer surface. Whether components confined to the inner surfaces also intermix and diffuse should be separately established.

Thus, recent evidence obtained with many experimental methods and different kinds of functional membrane systems is entirely consistent with the predictions of the fluid mosaic model of membrane structure and provides strong support for the model. It seems amply justified, therefore, to speculate about how a fluid mosaic structure might carry out various membrane functions, and to suggest specific mechanisms for various functions that can be subjected to experimental tests.

## The Fluid Mosaic Model and Membrane Functions

The hypothesis that a membrane is an oriented, two-dimensional, viscous solution of amphipathic proteins (or lipoproteins) and lipids in instantaneous thermodynamic equilibrum, leads to many specific predictions about the mechanisms of membrane functions. Rather than catalog a large number of these, we suggest some directions that such speculations may usefully take. Among these problems are nerve impulse transmission, transport through membranes, and the effects of specific drugs and hormones on membranes (*1*). The fluidity of the mosaic structure, which introduces a new factor into such speculations, is emphasized here. This new factor may be stated in general form as follows. The physical or chemical perturbation of a membrane may affect or alter a particular membrane component or set of components; a redistribution of membrane components can then occur by translational diffusion through the viscous two-dimensional solution, thereby allowing new thermodynamic interactions among the altered components to take effect. This general mechanism may play an important role in various membrane-mediated cellular phenomena that occur on a time scale of minutes or longer. Much more rapidly occurring phenomena, such as nerve impulse transmission, would find the mosaic structure to be a static one, insofar as translational diffusion of the membrane components is concerned. In order to illustrate the concepts involved, we discuss two specific membrane phenomena.

*Malignant transformation of cells and the "exposure of cryptic sites."* Normal mammalian cells grown in monolayer culture generally exhibit "contact inhibition"; that is, they divide until they form a confluent monolayer and they then stop dividing. Cells that have become transformed to malignancy by oncogenic viruses or by chemical carcinogens lose the property of contact inhibition; that is, they overgrow the monolayer. For some time, this experimental finding has been thought to reflect the difference between the normal and the malignant states in vivo, and to be due to differences in the surface properties of normal and malignant cells. Much excitement and investigative activity therefore attended the demonstration (*53*, *54*) that malignant transformation is closely correlated with a greatly increased capacity for the transformed cells to be agglutinated by several saccharide-binding plant agglutinins. Furthermore, mild treatment of normal cells with proteolytic enzymes can render them also more readily agglutinable by these protein agglutinins. Burger (*54*) has suggested, therefore, that the agglutinin-binding sites are present on the membrane surfaces of normal cells but are "cryptic" (Fig. 7A) (that is, they are shielded by some other membrane components from effectively participating in the agglutination process), and that proteolytic digestion of normal cells or the processes of malignant transformation "exposes" these cryptic sites on the membrane surface. In some cases, quantitative binding studies have indeed indicated that no significant change in the numbers of agglutinin-binding sites on the membrane accompanies either mild proteolysis of normal cells or malignant transformation (*55*).

An alternative explanation of these phenomena (Fig. 7B), based on the fluid mosaic model of membrane structure, may be proposed. Consider first the proteolysis experiments with normal cells. Suppose that the integral glycoproteins in the normal cell membrane are molecularly dispersed in the fluid mosaic structure. It is likely that mild proteolysis would preferentially release a small amount of glycopeptides and other polar peptides from these proteins because these are the most exposed portions of the integral proteins at the outer surface of the membrane (Figs. 2 and 3). The remaining portions of these proteins may still contain a large fraction of their original oligosaccharide chains after the limited proteolysis, but the release of some of the more polar structures would make the remaining portions more hydrophobic. As these more hydrophobic glycoproteins diffused in the membrane, they might then aggregate in the plane of the membrane. The result would be a *clustering* of the agglutinin-binding sites on the enzyme-treated cell surface, as compared to the normal untreated surface. Such clustering (with no increase, or perhaps even a decrease in the total numbers of sites because of digestion) could enhance the agglutination of the treated cells, as compared to that of normal cells, because it would increase the probability of agglutinin bridges forming between the surfaces of two cells.

In malignant transformation, distinct chemical changes in the glycolipids and the glycoproteins of the cell membrane are known to occur (*56*), and the enhanced agglutinability of the transformed cells may be much more complicated than is the case in the proteolysis of normal cells. If, however, the two phenomena do have a basic feature in common, it could be a similar clustering of saccharide-binding sites on the transformed and the enzyme-treated normal cells. In malignant transformation, such clustering could be the result of the chemical changes in the membrane mentioned above; or some virus-induced gene product (*57*) may be incorporated into the cell membrane and serve as a nucleus for the aggregation of the agglutinin-binding glycoproteins within the membrane.

These suggestions can be tested experimentally by the use of ferritin-conjugated agglutinins (*33*) as already discussed (Fig. 6). The prediction is that with normal cells subjected to mild proteolysis, and also with malignant transformed cells, the total number of ferritin-agglutinin particles specifically

bound to the outer surfaces of the cells might not be greatly different from those of normal cells, but larger clusters of ferritin particles would be found.

*Cooperative phenomena in membranes.* By a cooperative phenomenon we mean an effect which is initiated at one site on a complex structure and transmitted to another remote site by some structural coupling between the two sites. A number of important membrane phenomena may fall into this category. However, before enumerating them, we should first discriminate between two types of cooperative effects that may occur. These can be termed *trans* and *cis*. *Trans* effects refer to cooperative (allosteric) changes that have been postulated to operate at some localized region on the membrane surface, to transmit an effect from one side of the membrane to the other (*58*). For example, an integral protein may exist in the membrane as an aggregate of two (or more) subunits, one of which is exposed to the aqueous solution at the outer surface of the membrane, and the other is exposed to the cytoplasm at the inner surface. The specific binding of a drug or hormone molecule to the active site of the outward-oriented subunit may induce a conformational rearrangement within the aggregate, and thereby change some functional property of the aggregate or of its inward-oriented subunit. By *cis* effects, on the other hand, we refer to cooperative changes that may be produced over the *entire* membrane, or at least large areas of it, as a consequence of some event or events occurring at only one or a few localized points on the membrane surface. For example, the killing effects of certain bacteriocins on bacteria (*59*), the lysis of the cortical granules of egg cells upon fertilization of eggs by sperm (*60*), and the interaction of growth hormone with erythrocyte membranes (*61*) are cases which may involve transmission and amplification of localized events over the entire surface of a membrane. These phenomena may not all occur by the same or related mechanisms, but in at least two experimental studies, that involving the interaction of colicin $E_1$ with intact *Escherichia coli* cells (*62*), and that of human growth hormone and isolated human erythrocyte membranes (*61*), there is substantial evidence that long-range *cis*-type cooperative effects intrinsic to the membranes are involved.

The question we now address is, How might such *cis* effects work? Changeux and his co-workers (*63*) have proposed an extension to membranes of the Monod-Wyman-Changeux allosteric model of protein cooperative phenomena, using as a model of membrane structure an infinite two-dimensional aggregate of identical lipoprotein subunits [as, for example, the model described by Benson (*20*)]. In this theoretical treatment, the individual subunits are capable of existing in either of two conformational states, one of which has a much larger binding affinity for a specific ligand than does the other. The binding of a single ligand molecule to any one subunit then triggers the cooperative conversion of many of the subunits to the ligand-bound conformation, in order to maximize the interactions among the subunits.

This theory as presented relies on the membrane model used. If, however, the membrane is not a two-dimensional aggregate of lipoprotein subunits, but is instead a fluid mosaic of proteins and lipids, the physical situation would be quite different. The basic theory of Changeux *et al.* (*63*) might still be formally applicable, but with important changes in physical significance. It is possible, for example, that a particular integral protein can exist in either of two conformational states, one of which is favored by ligand binding; in its normal unbound conformation the integral protein is monomolecularly dispersed within the membrane, but in the conformation promoted by ligand binding, its aggregation is thermodynamically favored. The binding of a ligand molecule at one integral protein site, followed by diffusion of the nonliganded protein molecules to it, might then lead to an aggregation and simultaneous change in conformation of the aggregated protein within the membrane. This mechanism could result in a long-range *cis*-type cooperative phenomenon, if the eventual aggregate size was very large and if its presence produced local perturbations in the properties of the membrane. However,

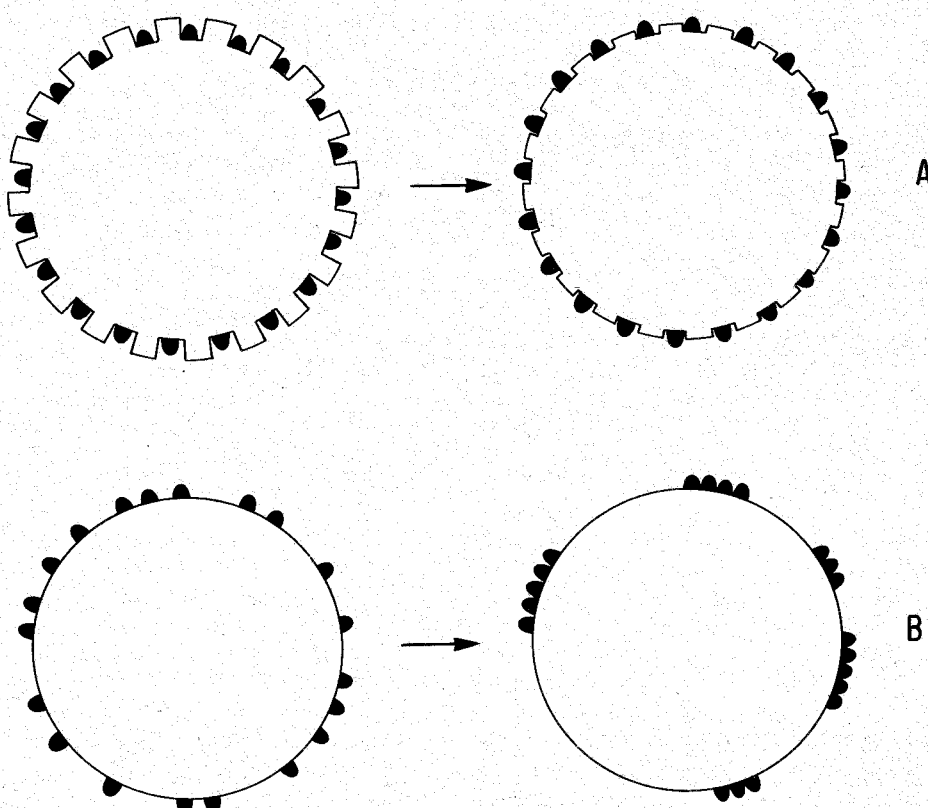


Fig. 7. Two different mechanisms to explain the findings that either malignantly transformed cells or normal cells that are subjected to mild proteolysis become much more readily agglutinable by several plant agglutinins. (A) The mechanism of Burger (*54*): agglutinin-binding sites that are present on the surfaces of normal cells, but are obstructed ("cryptic sites"), are exposed by proteolysis or the processes of malignant transformation. (B) The redistribution mechanism (see text): the agglutinin sites on normal cell surfaces are largely monomolecularly dispersed in the fluid mosaic structure, but on proteolysis or malignant transformation, they diffuse and aggregate in clusters. The probability of agglutination of two such modified cells is enhanced by the clustering of binding sites.

the transition would occur at a rate and over a time period determined by the rate of diffusion of the molecules of the integral protein in the fluid mosaic membrane. This time period is likely to be relatively long, of the order of minutes (*44*), as already mentioned. On the other hand, if *cis*-type cooperative effects occurred in a lipoprotein subunit model according to the mechanism postulated by Changeux *et al.* (*63*), one would expect the cooperative change to be much faster. Conformation changes in the soluble allosteric protein aspartyltranscarbamylase, for example, have half-times of the order of 10 milliseconds (*64*). It is therefore of some interest that in the studies of the interaction of colicin $E_1$ and *E. coli* the fluorescence changes that marked the apparent *cis*-type cooperative transitions in the cell membrane occurred over intervals of one to several minutes (*62*). If this suggested mechanism for the colicin effect is valid, one would predict that (i) freeze-etching experiments on the colicin-treated bacteria (*28*) might reveal an aggregation of normally dispersed particles at the inner membrane face, or (ii) changes in membrane fluidity, such as would be produced by suitable changes in temperature or by different compositions of membrane phospholipids (*65*), might markedly affect the kinetics of the fluorescence changes that are observed on addition of the colicin to the bacteria.

In this discussion of membrane functions, some detailed mechanisms to account for two membrane phenomena have been presented. It may well turn out that these mechanisms are incorrect. Our object has been not so much to argue for these specific mechanisms, as to illustrate that the fluid mosaic model of membrane structure can suggest novel ways of thinking about membrane functions—ways that are amenable to experimental tests. Other membrane phenomena may be influenced by similar diffusional mechanisms: for example, cell-cell and cell-substrate interactions, where the apposition of intense local electric fields to a cell membrane may affect the distribution of electrically charged integral proteins within the membranes; or the specific binding of multivalent antibody to cell surface antigens, where the simultaneous binding of one antibody molecule to several molecules of the antigen may induce rearrangements of the distribution of the antigen in the plane of the membrane, an effect that may be involved in the phenomenon of antigenic modulation (*66*). There are other specific examples as well.

It may well be that a number of critical metabolic functions performed by cell membranes may require the translational mobility of some important integral proteins. This could be the ultimate significance of the long-standing observation (*67*) that the membrane lipids of poikilothermic organisms contain a larger fraction of unsaturated fatty acids the lower their temperature of growth. Appropriate enzymes apparently carry out the necessary biochemical adjustment (*68*) that keeps the membrane lipids in a fluid state at the particular temperature of growth; if these enzymes are not functional, for example, because of mutations, the organism—to grow at the lower temperature (*65*)—must be supplied with the unsaturated fatty acid exogenously. While it has been suggested before that the maintenance of lipid fluidity may be important to carrier mechanisms operating across a functional membrane, it is also possible that the real purpose of fluidity is to permit some critical integral proteins to retain their translational mobility in the plane of the membrane, as an obligatory step in their function.

## Summary

A fluid mosaic model is presented for the gross organization and structure of the proteins and lipids of biological membranes. The model is consistent with the restrictions imposed by thermodynamics. In this model, the proteins that are integral to the membrane are a heterogeneous set of globular molecules, each arranged in an *amphipathic* structure, that is, with the ionic and highly polar groups protruding from the membrane into the aqueous phase, and the nonpolar groups largely buried in the hydrophobic interior of the membrane. These globular molecules are partially embedded in a matrix of phospholipid. The bulk of the phospholipid is organized as a discontinuous, fluid bilayer, although a small fraction of the lipid may interact specifically with the membrane proteins. The fluid mosaic structure is therefore formally analogous to a two-dimensional oriented solution of integral proteins (or lipoproteins) in the viscous phospholipid bilayer solvent. Recent experiments with a wide variety of techniques and several different membrane systems are described, all of which are consistent with, and add much detail to, the fluid mosaic model. It therefore seems appropriate to suggest possible mechanisms for various membrane functions and membrane-mediated phenomena in the light of the model. As examples, experimentally testable mechanisms are suggested for cell surface changes in malignant transformation, and for cooperative effects exhibited in the interactions of membranes with some specific ligands.

*Note added in proof*: Since this article was written, we have obtained electron microscopic evidence (*69*) that the concanavalin A binding sites on the membranes of SV40 virus–transformed mouse fibroblasts (3T3 cells) are more clustered than the sites on the membranes of normal cells, as predicted by the hypothesis represented in Fig. 7B. There has also appeared a study by Taylor *et al.* (*70*) showing the remarkable effects produced on lymphocytes by the addition of antibodies directed to their surface immunoglobulin molecules. The antibodies induce a redistribution and pinocytosis of these surface immunoglobulins, so that within about 30 minutes at 37°C the surface immunoglobulins are completely swept out of the membrane. These effects do not occur, however, if the bivalent antibodies are replaced by their univalent Fab fragments or if the antibody experiments are carried out at 0°C instead of 37°C. These and related results strongly indicate that the bivalent antibodies produce an aggregation of the surface immunoglobulin molecules in the plane of the membrane, which can occur only if the immunoglobulin molecules are free to diffuse in the membrane. This aggregation then appears to trigger off the pinocytosis of the membrane components by some unknown mechanism. Such membrane transformations may be of crucial importance in the induction of an antibody response to an antigen, as well as in other processes of cell differentiation.

### References and Notes

1. S. J. Singer, in *Structure and Function of Biological Membranes*, L. I. Rothfield, Ed. (Academic Press, New York, 1971), p. 145.
2. M. Glaser, H. Simpkins, S. J. Singer, M. Sheetz, S. I. Chan, *Proc. Nat. Acad. Sci. U.S.* **65**, 721 (1970).
3. J. Lenard and S. J. Singer, *ibid.* **56**, 1828 (1966).
4. D. F. H. Wallach and P. H. Zahler, *ibid.*, p. 1552.
5. W. Kauzmann, *Advan. Protein Chem.* **14**, 1 (1959).

6. C. Tanford, *J. Amer. Chem. Soc.* **84**, 4240 (1962).
7. E. D. Korn, *Annu. Rev. Biochem.* **38**, 263 (1969).
8. V. T. Marchesi and E. Steers, Jr., *Science* **159**, 203 (1968).
9. S. H. Richardson, H. O. Hultin, D. E. Green, *Proc. Nat. Acad. Sci. U.S.* **50**, 821 (1963).
10. M. Ashwell and T. S. Work, *Annu. Rev. Biochem.* **39**, 251 (1970).
11. D. Haldar, K. Freeman, T. S. Work, *Nature* **211**, 9 (1966); E. D. Kiehn and J. J. Holland, *Proc. Nat. Acad. Sci. U.S.* **61**, 1370 (1968); D. E. Green, N. F. Haard, G. Lenaz, H. I. Silman, *ibid.* **60**, 277 (1968); S. A. Rosenberg and G. Guidotti, in *Red Cell Membrane*, G. A. Jamieson and T. J. Greenwalt, Eds. (Lippincott, Philadelphia, 1969), p. 93; J. Lenard, *Biochemistry* **9**, 1129 (1970).
12. J. K. Blasie, C. R. Worthington, M. M. Dewey, *J. Mol. Biol.* **39**, 407 (1969).
13. D. Bownds and A. C. Gaide-Huguenin, *Nature* **225**, 870 (1970).
14. B. Ke, *Arch. Biochem. Biophys.* **112**, 554 (1965).
15. M. Glaser and S. J. Singer, *Biochemistry* **10**, 1780 (1971).
16. J. M. Steim, M. E. Tourtellotte, J. C. Reinert, R. N. McElhaney, R. L. Rader, *Proc. Nat. Acad. Sci. U.S.* **63**, 104 (1969).
17. D. L. Melchoir, H. J. Morowitz, J. M. Sturtevant, T. Y. Tsong, *Biochim. Biophys. Acta* **219**, 114 (1970).
18. D. M. Engelman, *J. Mol. Biol.* **47**, 115 (1970); M. H. F. Wilkins, A. E. Blaurock, D. M. Engelman, *Nature* **230**, 72 (1971).
19. M. E. Tourtellotte, D. Branton, A. Keith, *Proc. Nat. Acad. Sci. U.S.* **66**, 909 (1970).
20. A. A. Benson, *J. Amer. Oil Chem. Soc.* **43**, 265 (1966).
21. D. Triggle, *Recent Progr. Surface Sci.* **3**, 273 (1970).
22. H. U. Schairer and P. Overath, *J. Mol. Biol.* **44**, 209 (1969).
23. G. Wilson and C. F. Fox, *ibid.* **55**, 49 (1971).
24. J. Lenard and S. J. Singer, *Science* **159**, 738 (1968); G. Vanderkooi and D. E. Green, *Proc. Nat. Acad. Sci. U.S.* **66**, 615 (1970).
25. W. L. Hubbell and H M. McConnell, *Proc. Nat. Acad. Sci. U.S.* **61**, 12 (1968); A. D. Kieth, A. S. Waggoner, O. H. Griffith, *ibid.*, p. 819.
26. R. L. Steere, *J. Biophys. Biochem. Cytol.* **3**, 45 (1957); H. Moor, K. Mühlethaler, H. Waldner, A. Frey-Wyssling, *ibid.* **10**, 1 (1961).
27. D. Branton, *Annu. Rev. Plant Physiol.* **20**, 209 (1969). J. M. Wrigglesworth, L. Packer, D. Branton [*Biochim. Biophys. Acta* **205**, 125 (1970)] find no evidence by freeze-etching for the existence of the stalked-knobs on mitochondrial inner membranes that are observed with negatively stained preparations.
28. M. E. Bayer and C. C. Remsen, *J. Bacteriol.* **101**, 304 (1970).
29. P. Pinto da Silva and D. Branton, *J. Cell Biol.* **45**, 598 (1970); T. W. Tillack and V. T. Marchesi, *ibid.*, p. 649.
30. P. Pinto da Silva, S. D. Douglas, D. Branton, *Abstracts of the 10th Meeting of the American Society for Cell Biology, San Diego, Calif., November 1970* (1970), p. 159; T. W. Tillack, R. E. Scott, V. T. Marchesi, *ibid.*, p. 213.
31. T. L. Steck, G. Fairbanks, D. F. H. Wallach, *Biochemistry* **10**, 2617 (1971).
32. M. S. Bretscher, *Nature* **231**, 225 (1971); *J. Mol. Biol.* **59**, 351 (1971).
33. G. L. Nicolson and S. J. Singer, *Proc. Nat. Acad. Sci. U.S.* **68**, 942 (1971).
34. G. L. Nicolson, S. P. Masouredis, S. J. Singer, *ibid.*, p. 1416.
35. G. L. Nicolson, R. Hyman, S. J. Singer, *J. Cell Biol.* **50**, 905 (1971).
36. R. E. Lee and J. D. Feldman, *ibid.* **23**, 396 (1964).
37. F. A. Green, *Immunochemistry* **4**, 247 (1967); *J. Biol. Chem.* **243**, 5519 (1968).
38. W. C. Davis and L. Silverman, *Transplantation* **6**, 536 (1968); T. Aoki, U. Hämmerling, E. de Harven, E. A. Boyse, L. J. Old, *J. Exp. Med.* **130**, 979 (1969).
39. A. Shimada and S. G. Nathenson, *Biochemistry* **8**, 4048 (1969); T. Muramatsu and S. G. Nathenson, *ibid.* **9**, 4875 (1970).
40. J. K. Blasie, M. M. Dewey, A. E. Blaurock, C. R. Worthington, *J. Mol. Biol.* **14**, 143 (1965).
41. M. M. Dewey, P. K. Davis, J. K. Blasie, L. Barr, *ibid.* **39**, 395 (1969).
42. J. K. Blasie and C. R. Worthington, *ibid.*, p. 417.
43. R. C. Warren and R. M. Hicks, *Nature* **227**, 280 (1970).
44. C. D. Frye and M. Edidin, *J. Cell Sci.* **7**, 313 (1970).
45. L. Mindich, *J. Mol. Biol.* **49**, 415, 433 (1971).
46. R. D. Poretz and I. J. Goldstein, *Biochemistry* **9**, 2870 (1970).
47. R. G. Drysdale, P. R. Herrick, D. Franks, *Vox Sang.* **15**, 194 (1968).
48. G. L. Nicolson and S. J. Singer, in preparation.
49. E. H. Eylar, M. A. Madoff, O. V. Brody, J. L. Oncley, *J. Biol. Chem.* **237**, 1962 (1962); E. L. Benedetti and P. Emmelot, *J. Cell Sci.* **2**, 499 (1967).
50. G. L. Nicolson and S. J. Singer, in preparation.
51. V. T. Marchesi and G. E. Palade, *J. Cell Biol.* **35**, 385 (1967).
52. R. K. Kornberg and H. M. McConnell, *Biochemistry* **10**, 1111 (1971).
53. M. M. Burger and A. R. Goldberg, *Proc. Nat. Acad. Sci. U.S.* **57**, 359 (1967); M. Inbar and L. Sachs, *ibid.* **63**, 1418 (1969).
54. M. M. Burger, *ibid.* **62**, 994 (1969).
55. B. Sela, H. Lis, N. Sharon, L. Sachs, *Biochim. Biophys. Acta*, in press; B. Ozanne and J. Sambrook, *Nature* **232**, 156 (1971).
56. S. Hakamori and W. T. Murakami, *Proc. Nat. Acad. Sci. U.S.* **59**, 254 (1968); P. T. Mora, R. O. Brady, R. M. Bradley, V. M. McFarland, *ibid.* **63**, 1290 (1969); C. A. Buck, M. C. Glick, L. Warren, *Biochemistry* **9**, 4567 (1970); H. C. Wu, E. Meezan, P. H. Black, P. W. Robbins, *ibid.* **8**, 2509 (1969).
57. T. L. Benjamin and M. M. Burger, *Proc. Nat. Acad. Sci. U.S.* **67**, 929 (1970).
58. T. R. Podleski and J.-P. Changeux, in *Fundamental Concepts in Drug-Receptor Interactions*, D. J. Triggle, J. F. Danielli, J. F. Moran, Eds. (Academic Press, New York, 1969), p. 93.
59. M. Nomura, *Proc. Nat. Acad. Sci. U.S.* **52**, 1514 (1964).
60. D. Epel, B. C. Pressman, S. Elsaesser, A. M. Weaver, in *The Cell Cycle: Gene-Enzyme Interactions*, G. N. Padilla, G. L. Whitson, I. L. Camerson, Eds. (Academic Press, New York, 1969), p. 279.
61. M. Sonenberg, *Biochem. Biophys. Res. Commun.* **36**, 450 (1969); *Proc. Nat. Acad. Sci. U.S.* **68**, 1051 (1971).
62. W. A. Cramer and S. K. Phillips, *J. Bacteriol.* **104**, 819 (1970).
63. J. P. Changeux, J. Thiéry, Y. Tung, C. Kittel, *Proc. Nat. Acad. Sci. U.S.* **57**, 335 (1967).
64. J. Eckfeldt, G. G. Hammes, S. C. Mohr, C. W. Wu, *Biochemistry* **9**, 3353 (1970).
65. D. F. Silbert and P. R. Vagelos, *Proc. Nat. Acad. Sci. U.S.* **58**, 1579 (1967).
66. E. A. Boyse and L. J. Old, *Annu. Rev. Genet.* **3**, 269 (1969).
67. E. F. Terroine, C. Hofferer, P. Roehrig, *Bull. Soc. Chim. Biol.* **12**, 657 (1930); G. Frankel and A. S. Hopf, *Biochem. J.* **34**, 1085 (1940).
68. M. Sinensky, *J. Bacteriol.* **106**, 449 (1971).
69. G. L. Nicolson, *Nature* **233**, 244 (1971).
70. R. B. Taylor, W. P. H. Duffus, M. C. Raff, S. dePetris, *ibid.*, p. 225.
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**NEWS AND COMMENT**

# The Soviet Space Program: Effort Said to Surpass Peak U.S. Level

A new and authoritative study of the Soviet space program indicates that, while American space efforts continue winding down toward the last Apollo flight this year, the overall Soviet space program remains "a strong and growing enterprise," its ambitions unhindered by budgetary strain and undimmed by the deaths of three cosmonauts last year.

The study,* produced for the Senate Committee on Aeronautical and Space Sciences by analysts in three divisions of the Library of Congress, concludes that the current level of Soviet space activity exceeds that of the United States at its peak in 1966. The space study also indicates that the Soviet Union is almost certainly pressing ahead —cautiously but intently—with a manned lunar program that may be expected to put cosmonauts on the moon in the mid-1970's and possibly as early as 1973. A related conclusion, perhaps the most surprising of the 670-page study, is that the Russians may end up spending the equivalent of $49 billion to land men on the moon, far more than the cost of the Apollo program.

Whether or not the Soviets actually carry through with their evident intentions, the study goes on, "it is not possible to establish that the Russians have invested smaller total resources in lunar exploration than the United States" even though the Soviet effort "has not produced the visible result in this regard which the United States has achieved." These and other findings stand in direct contradiction of assertions by Soviet

* " Soviet Space Programs, 1966-70" Report of the Committee on Aeronautical and Space Sciences, prepared by the Science Policy Research Division, Foreign Affairs Division, and the European Law Division, Library of Congress; available from the Government Printing Office, Washington, D.C. 20402, $3; stock number 5271-0263.