Growth of the Concept of the Paucimolecular Membrane

By Hugh Davson, Sc.D.

The concept of the paucimolecular membrane derived in the first place from studies, such as those of Gorter and Grendel, on the amount of fatty material that could be extracted from the cell, based on the assumption that this was mainly concentrated at its surface. These studies, and other ones, led to the concept of the bimolecular leaflet of lipid as the fundamental basis of membrane structure. Various considerations demanded, also, the presence of protein as an intimate part of the structure. The simple structure put forward can account for many of the features of cell permeability, but, where facilitated diffusion is concerned, we must invoke the presence of specialized regions, perhaps pores, that have specific structures into which favored molecules and ions are able to fit. If this is true, variations in the gross analysis of cell membranes are unlikely to be correlated with gross variations in permeability characteristics; instead we must seek, by indirect methods, to lay bare the chemical features of these specialized regions, which doubtless share many of the characteristics of enzymes.

My task is essentially one of relating history; in later papers we shall be taken into the secrets of membrane structure, revealed by the most modern techniques of resolving morphological detail. We have already seen how the kinetic studies, notably those of Overton and, more recently and more quantitatively, of Collander and Jacobs and their colleagues, demand the presence of a layer of fatty material on the surface of the cell, separating its internal from its external environment. An analysis of these kinetic phenomena has revealed, moreover, that they could be accounted for on the basis of a thin layer of relatively undifferentiated lipid, and in this paper I propose to put myself back to those pre-World War II days when we were speculating as to the nature of this layer. As we shall see, even then the phenomena demanded that the membrane should not be completely undifferentiated, but that certain regions should have special characteristics that gave the membrane as a whole a selectivity that would have been beyond the capabilities of a uniform layer of lipid.

Thickness of the Membrane

The most fundamental problem in determining the possible character of the cell membrane was, in the early days, the assessment of its thickness, since that would tell us just what amount of material we had at our disposal in constructing any hypothetical membrane; this was possible because roughly, at any rate, the dimensions of the likely constituent molecules were known. The membrane was not resolvable by the light microscope, so this meant that it could not be much thinner than 0.12 µ, i.e., about 1200 Å, but between this and the length of a single fatty molecule, about 30 Å, there was left a lot of room for speculation.

The classical contribution to this problem is that of Gorter and Grendel,1,2 and we may consider it first, because their arguments and their experimental technique lead at once to considerations of the possible structure of the fatty layer. Gorter and Grendel extracted the lipid from red blood cells by means of large quantities of acetone; the evaporated residue was dissolved in a little benzene, and the lipid was then spread as a unimolecular film on the surface of a Langmuir trough. The area of this film of lipid was slowly reduced by sliding a glass barrier across the

*We may note in this context that Gough,3 in his classical paper describing the disk-sphere transformation in the erythrocyte, calculated, from lipid analyses of erythrocytes, that the thickness of the lipid layer on the cell surface was 2–3 µ, i.e., 20–30 Å, and suggested that this might be spread as a single layer.

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surface, forcing the lipid against an opposite retaining barrier, and as soon as the film started to exert a measurable resistance to compression the actual area occupied by it was measured (fig. 1). At this area the individual molecules were packed together to give a coherent film, presumably with the polar, or water-soluble, endings of the molecules attached to the water and the nonpolar, or hydrocarbon, chains sticking out, as illustrated schematically in figure 2. This measurement thus gives the total area that the lipids were capable of covering, supposing they were able to spread out in this manner to give a cohesive film. Gorter and Grendel next measured the dimensions of the red blood cells; these have a rather unfortunate shape (fig. 3), being biconcave discs, so that the assessment of their area is not easy; however, by measuring the thickness and diameter and applying Knoll's formula, they were able to make what was a reasonably approximate measure. Some results for various species are shown in table 1, where the ratio indicates the
area of lipid divided by the area of the cells, and it will be seen that this ratio comes out at approximately 2.

Gorter and Grendel concluded from their study that the cell membrane probably consisted of a bimolecular leaflet, as illustrated by figure 4, or at any rate, that the lipid constituents were probably arranged in this way. They enquired, also,\(^2\)\(^,\)\(^5\) into the nature of the lipid material present; earlier analyses\(^6\) had indicated that the cells of 1 litre of blood contained 0.5 Gr. sphingomyelin, 0.5 Gr. of cephalin and lecithin, and 0.5 Gr. of cholesterol. The formulae of these substances are illustrated in figure 5, and it is evident that they can, indeed, be regarded as so-called head-and-tail molecules, the hydroxyl group of cholesterol, for example, acting as the polar end-group and the phenanthrene ring structure, the nonpolar hydrocarbon end; again, the phosphocholine part of lecithin or sphingomyelin would act as the polar group, and the two long hydrocarbon chains as the nonpolar tail. Grendel\(^5\) analyzed the total extracted lipids, and, from measurements of the surface areas occupied by the individual constituents, he concluded that the relative contributions, in terms of spread area, were as follows: cholesterol, 36 per cent; cephalin and lecithin, 50 per cent; and sphingomyelin, 13 per cent. He computed that the double layer of lipid would have an average thickness of 31 Å,\(^*\) but more recent studies\(^7\)\(^,\)\(^8\) based on x-ray analysis of orientated lipids, suggest that the thickness of orientated lipids in a double layer would be considerably higher, namely 54 Å.

**Surface Films**

Gorter and Grendel were employing a technique for the study of lipids that had been developed by Langmuir and later by Adam and Rideal and their collaborators, and it is necessary to digress for a moment, to give an account of the elementary theory of surface films. As indicated by figure 2, substances like long-chain fatty acids, long-chain alcohols, etc., will spread on the surface of a tank of water, and when this spread film is compressed a point is reached, namely when the film is said to be coherent, where a lateral surface pressure is exerted resisting the compression. By increasing the compression beyond this point the area is reduced, and we may plot curves of surface pressure F, against area of film. According to the nature of the substance and the physical conditions of temperature, pH, etc., the curves fall into certain categories, and those that interest us are the condensed and the liquid-expanded films; examples are shown in figure 6. Essentially, the condensed film has the characteristics of a two-dimensional solid; the molecules

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*The thicknesses of single layers were computed from the area-per-molecule occupied by the films and an assumed specific gravity of unity. Since lecithin and cephalin gave expanded films with high limiting areas per molecule (155 Å\(^2\)) the relative thinness of the layers so computed is understandable.*
Figure 5
Structure of certain lipids.

preserve fixed positions, the polar heads being attached to the water phase and the nonpolar tails sticking up into the air, not necessarily vertically. When compressed, the molecules show that there is some possibility of rearrangement, but usually this does not lead to great changes of area, with the result that the F-A curve is very steep. At zero pressure the films tend to a stable area, determined by the cross-sectional area of the molecule, and it depends on the relative bulks, among other things, as to whether the polar group or the hydrocarbon tail determines this limiting area. The expanded film partakes more of the characteristics of a two-dimensional liquid,
the individual molecules having independent motion and sliding round one another while the tails are in vigorous vibratory movement. Obviously, a condensed film may often be made to expand to the liquid form by raising the temperature. The average area occupied per molecule will be much larger than in the case of the condensed films.

Gorter and Grendel\(^2\) pointed out that cephalin and lecithin would form expanded films at 37 C., while cholesterol and sphingomyelin would form condensed films, and they suggested that the mutual interaction of the various constituents might lead to a film with intermediate characteristics. At any rate, a variety of studies on mixed films, notably those of Leathes\(^9\) and of Schulman,\(^10-12\) have shown that the combined film is not necessarily the algebraic sum of the characteristics of its constituents, so that the interaction may sometimes amount to an apparent complex formation between the constituents. Outstanding in its ability to increase the stability of films is cholesterol, which may act by virtue of its bulk, preventing or slowing down the translational motions of the film molecules.

Gorter and Grendel’s study thus leaves us with a highly plausible basis for membrane structure; the membrane they proposed was indeed very thin; in fact it is difficult to conceive of anything thinner so far as the lipid constituent is concerned, although a monomolecular film, rigidly attached to perhaps a protein framework, might well fill the bill. There are obvious uncertainties in their estimate of thickness—which on the basis of two molecules of lipid with the hydrocarbon tails orientated radially from the surface would be some 60 Å—notably the estimate of the surface area of the cells, the efficiency of extraction of the lipid, and the question whether some of the lipid might not be in the form of fat globules within the cytoplasm of the cell. Furthermore, of course, the membrane could also have a protein constituent, which might act as a framework on which the lipid film was deposited, the two being held together either by intermolecular attractive forces or by true complex formation to give lipoproteins; again, the protein might, besides acting as a scaffolding, also cover the lipid surface. Before describing further work relevant to these problems, we must consider independent estimates of the membrane thickness.

### The Cell as a Condenser

The classical studies of Höber\(^13,14\) on the electrical conductivity of cell suspensions made it clear that the cell could be considered, electrically, as a conducting medium—cytoplasm—separated by a nonconducting dielectric—the membrane—from the outside suspension medium, which was also conducting. Fricke in a series of papers\(^15-18\) developed the theoretical treatment of Clark Maxwell, applied it to suspensions of cells, and showed how the static capacity per sq. cm. of surface of the cell could be calculated from measurements on specific capacity of suspensions; it came out at 0.81 μf./cm. By assuming a value of 3 for the dielectric constant of the membrane and substituting in the formula

\[ C_0 = \frac{3}{4}\pi \cdot x \cdot 9 \cdot 10^5 \text{ microfarads} \]

we obtain the value of x, the membrane thickness, of 3·3·10⁻⁷ cm., i.e., 33 Å, or about one molecule thick of lipid. Here there are, once again, a number of uncertainties due partly to the application of simple theory to a rather complex situation and also the use of a value of 3 for the dielectric constant for the membrane, a value chosen by Fricke on the basis of the dielectric constants of solid lipids. With such a thin layer it becomes questionable whether the value for the solid or the liquid should be chosen. In fact, however, the value of 0.8 μf./cm. found by Fricke for the erythrocyte agrees remarkably well with the capacity of many other membranes, e.g., the giant axon of the squid, where measurements were easier to interpret. Danielli\(^19\) moreover, pointed out that the behavior of a lipid in an oscillating electric field that determines its dielectric constant depends on the dipole of the molecules, which, with the types of lipid with which we are concerned, resides almost exclusively in the polar water-soluble region, and it is this region that is anchored in the
water phase. The dipole moments of many lipids are known, and they are quite simply related to the surface potentials of films, i.e., the potential across the film due to the orientated layer of dipoles. The relationship is

$$\Delta V = \frac{\mu \sin \alpha \cdot n \cdot 4\pi}{D}$$

where \(\Delta V\) is the potential; \(D\), the dielectric constant; \(n\) is the number of molecules; \(\mu\) is the dipole moment; and \(\alpha\) is the angle at which the dipoles are orientated. Only \(\alpha\) is unknown here, but the maximum value of \(\sin \alpha\) is unity, hence maximum values for \(D\), the dielectric constant, can be derived from measurements of surface potential and dipole moment. The values found were in the region of 4, showing that Fricke's choice was probably correct, the molecules of the surface film partaking of the character of a solid, by virtue of the anchoring of the polar groups that restricts the rotations of the dipoles in the electric field.

### The Analytical Leptoscope

Another attempt at determining the thickness of the cell membrane is that of Waugh and Schmitt. The principle on which their instrument—called an analytical leptoscope—was based consists in the comparison of the intensities of light reflected from a dried cell ghost with that reflected from a lipid film of known thickness. According to elementary optical theory, the intensities of reflected light will be equal when the two have the same thickness. The comparison films of graded thickness were prepared by dipping a microscope slide into a solution of barium stearate. Each time the slide was dipped and removed a double layer of stearate was deposited; thus films of thickness, which were multiples of the known length of a stearate chain (25 Å), could be prepared. The cell ghost consists of the red cell after it has been caused to burst and lose all its soluble contents; when dried it is a circular disc and consists of its two layers of cell membrane, as illustrated by figure 7, plus any other material of which the cytoplasm of the cell may be composed. The estimated thickness of this dried ghost will be greater than the thickness of two membranes, by the thickness of this dried residual material. As figure 8 shows, the result depends on the pH at which the ghosts are formed, suggesting that at pH greater or less than 6 material is extracted. The results show (upper curve) a maximum thickness of 200 Å. When the ghosts were extracted with lipid solvents (lower curve), the thickness of a single membrane decreased by about 100 Å, so that the results suggest that the lipid com-

![Figure 7](image1)

Illustrating dried erythrocyte ghosts.

![Figure 8](image2)

Corrected thickness of the rabbit erythrocyte envelope as a function of the pH of the hemolyzing solution. The upper and lower curves represent respectively the thickness of the whole envelopes and of the envelopes after extraction in organic solvents.
ponent is thicker than the two layers of Gorter and Grendel and might consist of four layers, as illustrated in figure 9.

![Diagram](https://example.com/diagram.png)

**Figure 9**

*Suggested four layers of lipid component.*

The studies on thickness, then, tell us that, so far as the erythrocyte is concerned, the envelope consists of a maximum of four layers of lipid, although the smaller value of two layers is consistent with the measurements of Gorter and Grendel and Fricke. We may ask next about the contribution of protein to the envelope. So far as the erythrocyte is concerned, the amount of protein material is almost twice as great as the amount of lipid, but the amount that is in the wall of the cell, as opposed to that being distributed throughout the bulk of the cell as a "stroma," is not known. We may consider here the likely contribution of protein to the stability of the lipid membrane and its possible contribution to its permeability characteristics.

At an air-water interface, proteins will spread out to give stable films which, on compression, may acquire a gel-like character. The thickness of such a film may be very small, 7.5 Å or less, so that the large molecules must unwrap themselves at the surface and, according to the degree of compression to which they are subjected, the side chains will lie in the surface or be forced out into the air. In the latter case the adhesion between the packed side chains will lead to the cohesion that Hughes and Rideal described as giving the "gel" state. The nature of a mixed film of protein and lipid was studied by Schulman and Rideal, just as surface-active substances, such as saponin, could penetrate a film of ergosterol, changing its character from that of a condensed to an expanded film, or as sodium cetyl sulfate could penetrate a film of sphingomyelin, converting it from an expanded to a condensed film, so the addition of gliadin to cholesterol converts it from its normal liquid-type to a gel-type, provided the film is compressed sufficiently to permit this. Perhaps more relevant to the problem is the behavior of films at a lipid-water interface, rather than at an air-water interface. The experimental difficulties inherent in the study of the lipid-water interface were overcome by Askew and Danielli, and the behavior of proteins was described by Danielli. Just as at an air-water interface, the spreading out was great, so that the proteins must have unrolled, and at low compression the films so formed were of the expanded liquid type, becoming gels on compression. The surface activity of many protein solutions, i.e., the extent to which they concentrated in the interface, was remarkable, so that at a brombenzene-water interface proteins are about a thousand times more active in reducing surface tension than, say, un-ionized oleic acid.

To return to the picture of the cell membrane, we may note that the tension at the surface of many cells and at the surface of oil drops in the interior of cells is very low (for review, see Harvey and Danielli), so that the presence of a protein on the surfaces of the cell must be postulated. On the analogy with the mixed protein-lipid films considered above, the protein may be unwrapped with its side chains projecting into the hydrocarbon chains of the lipid and its polar linkages projecting into the water phase, as illustrated by figure 10, from Danielli, contributing materially to the stability of the system and presumably to its permeability characteristics. Whether or not there are unrolled globular protein molecules on the surfaces, in addition to those that belong to the structure, as illus-
trated by an early hypothetical picture\textsuperscript{28} (fig. 11), it remains to be seen. Such a layer might well be responsible for the antigenic character of the cell surface.

**Polarization-Optical Analysis**

If the lipid membrane is only two molecules thick, it will be very difficult to establish its presence and analyze the orientation of its molecules by measurements on the retardation of polarized light, since this phenomenon requires, for its unequivocal interpretation, the presence of several repeating layers. The erythrocyte ghost does, in fact, show some birefringence (Schmitt, Bear, and Ponder\textsuperscript{29, 30}), and this was interpreted on the basis of the presence of a membrane with the characteristics of a Wiener mixed body, the lipids having an intrinsic birefringence due to their radial orientation, and the proteins giving a form birefringence by their tangential arrangement in leaflets. That is, the arrangement that could be established with some certainty in the much thicker myelin sheath seemed to be consistent with the observations on the red cell. As Mitchison\textsuperscript{31} has pointed out, however, this interpretation goes far beyond the facts. A detailed description of his own measurements and their ingenious interpretation will take me beyond the scope of this review. Suffice it to say that his analysis of the variation of retardation of rays passing through the edge of the cell and thus through its membrane indicates a thick structure 5,000 Å deep, which could only be made up of the stroma protein, dispersed as a heavily hydrated gel; on the surface of this is the bimolecular layer of orientated lipid. According to this view, the erythrocyte would have the appearance of figure 12 in cross-section, so that a very large part of its "membrane" or wall would really be a cortical gel, analogous with that which undoubtedly exists in *Amoeba*.

**Figure 10**

*A detail of the structure of the cell membrane (Danielli).* The polar groups of the protein are in roughly the same plane as those of the fatty molecules, and the hydrocarbon parts of the protein extend into the fatty layer.\textsuperscript{27}

**Figure 11**

*Sketch of globular protein molecules adsorbed onto a film of unrolled protein molecules previously adsorbed at an oil-water interface.*\textsuperscript{27}

**Figure 12**

*Suggested dimensions of erythrocyte based on polarization-optical study.*\textsuperscript{31}
**Hemolysis**

Any picture of the cell membrane must be able to account for a number of phenomena in a general fashion, and it must be emphasized that our ideas as to membrane structure have developed just as much as a result of these direct studies on surface films as from the studies on permeability and related phenomena. Let us, therefore, turn aside from the direct studies and consider hemolysis and related phenomena. A variety of surface-active substances, such as saponin or bile salts and soaps, will cause the cell to lose its character as an individual unit with an internal environment different from its suspending medium, e.g., plasma in the case of the red blood cell. The membrane may be said to be damaged, so that its selectivity is lost, and this is seen obviously as the escape of hemoglobin from the red cell, but much lesser degrees are observed earlier, in which merely an accelerated mobility of certain ions through the membrane occurs.

It would seem from the studies of Davson and Danielli\textsuperscript{32} and Davson and Ponder\textsuperscript{33} that the damage caused by these lytic agents is not so severe as was at first thought. On the classical basis, they ruptured the membrane to make holes large enough for hemoglobin molecules to escape (diameter 60 Å); in fact, however, it would seem that they simply increase the permeability of the membrane to Na and K, so that colloid osmotic forces come into play and make the cell take up more water than it can hold. The escape of hemoglobin is thus secondary to a primary change in cation permeability.

Presumably the hemolytic agents enter the cell membrane and, by their presence, induce sufficient instability to produce a generalized increase in permeability. This point was studied by Schulman and Rideal,\textsuperscript{12} who drew attention to analogies between the penetration of protein-lipid films of gliadin and cholesterol by surface-active substances and their ability to cause hemolysis; thus palmitic acid penetrates these films very rapidly and is strongly lytic, while pelargonic acid penetrates slowly and is only weakly lytic. Surface-active substances are not the only ones that cause lysis; thus fat-soluble substances, such as the narcotics, ether, chloroform, etc., are also efficient; they also will enter the lipid layer and, being relatively small and mobile molecules, may also be expected to induce instability. Heavy metals, such as copper, mercury, silver, are also effective, and it was considered by Davson and Danielli\textsuperscript{32} that here we were dealing with a packing effect of the lipid molecules; thus, we considered (Danielli and Davson\textsuperscript{28}) that calcium, which usually decreased the permeability of the cell membrane, acted by forming divalent links between adjacent lipid molecules, tending to pack them more tightly together; we considered that the heavy metals, like copper, were overdoing this, tending to form islands of highly compressed membrane material with gaps between. Thus, in a general way, many of the outstanding permeability phenomena might be accounted for on the basis of our simple scheme of a lipid-protein film. To some extent, however, this correspondence may have been misleading. For example, many of the narcotics, such as ether and chloroform, would inhibit permeability of certain molecules, while having the opposite effect so far as others were concerned, and it was just these exceptions that led us to modify our conception of a relatively homogeneous membrane and to introduce the concept of specific regions of unusual character. Before coming to this, however, we may consider a few more points regarding the general character of the membrane.

Lysis of the cell may be caused simply by creating conditions where it must take up water, as for example, by placing it in a hypotonic medium. The difference of osmolarity causes the penetration of water, and at a certain point the cell is unable to expand further to accommodate this; as a result the membrane ruptures and the contents escape. With an egg that is spherical, any increase in volume must be accompanied by an increase in surface area, and this must mean a thinning of the surface membrane or, alternatively, the migration of membrane material from the bulk of the cell into the surface. There is
certainly no evidence, from a study of impedance, that the membrane changes in thickness when the cell swells, and, if our concept of a bimolecular leaflet of lipid plus a layer of protein is correct, it seems most unlikely that such a film would expand to perhaps 1.6 times its area and remain stable. Thus, so far as spherical cells of the type of Arbacia egg are concerned, we must consider the possibility of reversible additions and subtractions from the membrane. In the case of the red blood cell, which has been studied so much more exhaustively so far as membrane composition is concerned, we do not have to invoke this additional factor, since the normal shape of this cell is a biconcave disc (fig. 13 A). On taking up water the surface is merely rearranged until the cell becomes a sphere (fig. 13 B and C) and the careful measurements of Ponder and Castle and Daland have shown that once the spherical form is reached further uptake of water causes bursting of the membrane. The erythrocyte is thus a cell with a membrane of limited area that apparently cannot expand.

The maintenance of the biconcave shape is still something of a puzzle. The biconcave disc may change to a sphere without increase in volume of the cell by various treatments, including simply placing the cells, washed free of plasma, between slide and coverslip (Gough). In this case, of course, the membrane becomes too big for the cell, and it goes into wrinkles or crenations. The presence of hemoglobin in the cell seems not to be necessary for the shape, since ghosts may exist as biconcave discs and undergo the change to spherical shape. The claim that the surface was covered by an antisphering factor (Furchgott and Ponder), loss of which allowed the cell to become a sphere, has not been substantiated by later work (Brown). If the cell wall is really a thick structure, as suggested by figure 13, the disc shape is easy to understand.

Just before bursting, there is some evidence that the permeability of the red cell changes, since the ability to maintain a high concentration of K in the cell is impaired with the resultant escape into the surrounding medium.

It is the purpose of this paper to illustrate not only the state of our knowledge regarding membrane structure towards the close of the thirties of this century, but also to show the range of techniques and the types of evidence on which such knowledge and speculation were based. It is therefore worth digressing, once again, this time to consider some direct, microscopical studies bearing on the nature of the surface separating the intact cell from its environment.

**Extraneous Coats**

Most, if not all, cells are separated from their environment, not only by the plasma membrane, but by one or more coats, which have been called extraneous because, by contrast with the plasma membrane, they may be removed or injured without prejudicing the life of the cell, which apparently maintains its normal permeability relations with its environment. These extraneous coats, moreover, are highly permeable to large water-soluble molecules, such as sucrose, so that the permeability characteristics of the cell are not usually affected by the removal. The most commonly studied cells, from this aspect, are the eggs of many invertebrates, such as the
sea-urchin Arbacia, and various protozoa, such as Amoeba. The study of the egg has been most profitable, since changes in its coats may be induced by the natural process of fertilization. This egg is surrounded by quite a thick layer of jelly, which can be easily removed by simply shaking the cells; its presence is concerned with the process of fertilization—its substance being called fertilizin, and removal leaves the egg otherwise normal and capable of development when fertilized. Another layer, to which this jelly adheres, is the vitelline membrane, an elastic pellicle that, on fertilization of the egg, lifts away from the underlying protoplasm to form a tough fertilization membrane within which the successive divisions of the egg take place. This membrane may be removed without impairing the viability of the cell by washing the eggs in KCl. This extraneous coat seems to be a universal feature of living cells: in the plant cell it is represented by the tough cellulose coat; in bacteria the coat may be of cellulose, protein, or mucopolysaccharide, and so on.

When the egg is fertilized, the vitelline membrane lifts away, and for a short time the protoplasm may be said to be stark naked; very soon, however, the egg secretes a hyaline plasma layer, a protein material that serves to bind the dividing blastomeres together and is essentially the forerunner of the intercellular cement that holds the individual cells of the tissue together. The presence of an adequate concentration of calcium in the medium surrounding the egg is necessary for the laying down of this layer; otherwise the material is secreted just the same but remains in solution.

An important quantitative method of studying the characteristics of the limiting layers on the surface of the cell was developed by Chambers and Kopac. They observed that minute droplets of oil, when brought into contact with eggs of several species, would, under appropriate conditions, coalesce with the cell, passing almost instantaneously from without inside, as in figure 13 from Chambers and Chambers. In a way, the cell and the oil droplet were behaving as liquids, the factor determining coalescence being whether the total surface energy of the system would be less when the drop was in contact with the outside of the cell or when it was inside. With ordinary liquid drops, however, minute differences of surface energy are adequate to cause coalescence, but, because of the structural features of the cell outer layers, a certain critical difference of energy would have to be present in order to get coalescence. Thus, in order to increase the chance of coalescence, we may raise the surface tension of the oil or we may increase the size of the drop. This provides a neat method for assessing the rigidity of the surface under different conditions or in different cells under the same conditions. For example, with unfertilized eggs with only their jelly coats removed, the diameter of the drop was some 40 μ.; if the egg was fertilized and its fertilization membrane (vitelline) immediately removed, the size decreased to some 25 μ.; as time progressed the coalescence decreased, revealed by the increasing size of oil drop necessary to get penetration, so that at 15 minutes it was some 125 μ., while after this coalescence did not occur. Clearly we are witnessing here the effects of the secretion of the hyaline plasma layer, and this is shown by keeping the fertilized eggs, without fertilization membrane, in KCl solution, in which case the size of the drop may be as small as 5 μ. Under these conditions, then, we have approximated as closely as possible to the naked egg so that the plasma membrane itself is exposed to the outside environment. Up to this point there is no evidence that we have affected the viability of the cell and, thus, the normal permeability characteristics of the membrane on which this viability depends. Thus we may inject into the cell one of the class of sulfonated dyes that are highly water soluble and do not penetrate cells to a measurable extent; the dye disperses throughout the cytoplasm but comes to a halt at the surface of the egg. In reverse, we may put the dye in the egg’s surrounding sea water, and it is seen to reach the surface of the egg and proceed no further.
Breakdown and Reformation of Plasma Membrane

If a needle is inserted into a naked egg and a sudden and extensive tear is made, the contents flow out and the cell disintegrates. If the tear is not too extensive, however, a healing process may be observed that may rescue the cell from complete destruction, and this consists of, apparently, the formation of new membrane material that effectually seals off the injured region. The decisive factor in the destruction of a cell by tearing is undoubtedly the injury to the plasma membrane, while the healing process represents the mobilization of new membrane material from the cytoplasm to form a new layer at the surface of the injured region. If this new film is punctured, it disintegrates in a wave up to the edges of the torn underlying cytoplasmic cortex; at this point disintegration of the surface ceases, so that it would seem that the underlying cortical gel acts as a support for the plasma membrane, significant damage to this membrane being only possible when the underlying cortex has been torn away. If the egg is torn in sea water from which the calcium has been removed, the formation of a new surface membrane does not take place, so that injury by tearing leads to complete destruction of the cell. According to Heilbrunn, the formation of new membrane material depends on a chemical reaction involving a substance, ovotrombin, formed from some precursor in the egg in the presence of calcium in rather the same way that blood clotting depends on a reaction involving thrombin, which is likewise formed from a precursor in the presence of calcium.

When the increased membrane area is brought about by simple swelling of the cell or by mitotic division, the extra membrane material appears at the surface, whether the medium contains calcium or not, so that these changes presumably involve the stretching of a pre-existing membrane or the addition, from the cytoplasm, of material that already has the required characteristics and needs no special reaction involving calcium.

The lipid character of the surface membrane in the egg of Arbacia, for example, was well demonstrated by Chambers, who, by choosing an oil drop of appropriate surface tension, made it form a cap on the surface of the cell, instead of coalescing; by appropriate manipulations the oil cap could be made to move over the surface of the egg, causing flowing movements in the surface.

The Lipid Composition

We may now return to the membrane of the erythrocyte and examine further into its composition and see whether this may be correlated with its permeability characteristics. Parpart and Dziemian have carried Gorter and Grendel's analysis a little further. They have shown that phospholipids and cholesterol constitute together some 87 to 96 per cent of the total lipids; thus some results for the rabbit, expressed as mg. lipid per mm.² of cell surface are as follows:

<table>
<thead>
<tr>
<th>Cephalin</th>
<th>Lecithin</th>
<th>Ether-Insol Residue</th>
<th>Cholesterol</th>
</tr>
</thead>
<tbody>
<tr>
<td>15</td>
<td>4</td>
<td>1.5</td>
<td>12</td>
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The fatty material that is not extracted by either is considered by Parpart and Dziemian to be lipoprotein complex.

Parpart and Dziemian attempted to correlate the variations in the lipid composition of different species of erythrocyte with variations in their general permeability characteristics; the attempt failed, and it is instructive to analyze the reason for this failure. Variations in permeability to a molecule like glycerol are very striking when different species are compared. Thus it might be said that the erythrocytes of monkey, rat, rabbit, and ox had orders of permeability in this order because the rates of penetration of glycerol into these erythrocytes are in decreasing order in the same series. Parpart and Dziemian found no correlation between differences in lipid composition in this series of species with the differences in permeability characteristics.

The error in this approach is to characterize a cell as being "highly permeable" and one as being "not so highly permeable" on the basis of the penetration of only one group of
compounds, in this case the polyalcohols, such as glycerol, erythritol, etc. An analysis of the permeability of certain erythrocytes to this type of molecule indicates the presence of some membrane specialization that gives an unusually high permeability to this class of molecules but does not affect appreciably the permeability to the great majority of substances. This specialization of the membrane is responsible for the phenomenon of what has come to be known as catalyzed permeability or facilitated transfer (Davson and Reiner,42 Stein and Danielli48) and represents an adaptation of the cell membrane to permit certain physiologically important molecules and ions. This is especially manifest in the ability of certain erythrocytes to permit sugars (LeFevre and LeFevre,44 Willbrandt15) to pass through rapidly. Such an adaptation might well be confined to a limited area of the membrane and, at any rate, might well be a modification of the protein constituent of the membrane since, so far as sodium permeability in the cat erythrocyte is concerned, the specialized region behaves in a way reminiscent of an enzyme; moreover, the permeases of bacteria, about which we shall hear more, are also analogous with enzymes.

This consideration of membrane specialization to meet certain specific requirements of the cell makes several other phenomena intelligible: for example, Ballentine and Parpart46 observed that treatment of the erythrocytes of some species with lipase, which apparently split off a fatty acid residue from the lecithin molecule, actually decreased the permeability of the rabbit’s erythrocyte to glycerol, but had little effect on the permeability to nonpolyhydroxy compounds, and had no effect on ox cells’ permeability. Now the ox cells do not have this membrane specialization in respect to polyhydroxy compounds, so that the lipase action seems to be directed at this special mechanism, but this does not mean that the lipids are necessarily concerned structurally in this membrane specialization, because the lipase may simply have split off a long-chain fatty acid, which itself becomes adsorbed in a quite unspecified manner onto the specialized region and reduces its permeability.*

Narcotic Action

We have seen how fat-soluble substances may attack the cell membrane, causing increased cell permeability and rupture of the membrane when in high enough concentration; many of these substances are typical narcotics, such as ether, chloroform, urethane, alcohol, and so on. So far as permeability in general was concerned it was found that this was increased, e.g., the permeability to water of the ox erythrocyte;47 and the permeability of the large unicellular alga Chara ceratophylla to various nonelectrolytes (Bärlund48). Anselmino and Hoenig49 showed that the permeability of human erythrocytes to glycerol, erythritol, etc., was strikingly reduced by narcotics, and an extension of their work to different species shows that it is only the erythrocytes of those species, such as the rabbit, man, and monkey, which have this unusually higher permeability to glycerol, that were sensitive to the narcotics. In other species, where glycerol permeability was slow and more consistent with respect to other molecules of similar partition coefficient, e.g., in the ox and sheep, the narcotics only accelerated permeability. Where the passive permeability of ions is concerned, we also see this phenomenon of increased permeability to one type of ions but not to another; for example, in the cat erythrocyte, passive permeability to sodium is completely inhibited by narcotics, while that to potassium is only accelerated. In this case the permeability to sodium is apparently facilitated by an enzymelike system,42 while that to potassium is not. Again, the transport of anions, such as chloride and bicarbonate, through the erythrocyte membrane is extremely rapid and so presumably

*Ballentine and Parpart stated that, although the lipase split a fatty acid residue from the membrane, this did not appear in the suspension medium, so that this interpretation of its action is highly probable, especially since this narcotic action is by no means confined to the compounds commonly described as narcotics; the soaps of long-chain fatty acids are highly effective.27
depends on some facilitating mechanism. The permeability to anions is, consistent with the other facts, inhibited by narcotics.50

Thus the influences of fat-soluble and surface-active substances on permeability now fall into an intelligible pattern. The substances presumably penetrate into the lipid membrane and tend to reduce its stability, and this leads to increased permeability. These substances also block enzymatic groupings in a nonspecific fashion and thus attack the facilitated transfer mechanisms. Consequently they slow the rates of penetration of molecules and ions that depend for their speedy transfer across the membrane on this mechanism.

To summarize, then, we may say that our gropings into the fine structure of the cell membrane had brought us, in the late thirties of this century, to the realization that what we may call the blanket permeability properties of the cell membrane might be accounted for by a thin layer of lipid and that this layer was probably present, presumably stabilized by protein, with which it could form a mixed film. Certain features of cell permeability revealed, however, that the membrane could not be homogeneous, so that we have to invoke specialized regions, which impart to the membrane some of the characteristics of an enzyme, notably in accelerating transport of specific molecules, in the dependence of this transport on pH and temperature such that optima corresponding to physiological optima are present, and in sensitivity to narcotics and to heavy metals such as copper.

In later papers we shall see that the phenomena of active transport, involving the supply of energy to the process of moving molecules and ions into or out of the cell, demands the presence of catalytic systems in the membrane, i.e., not only must we postulate special regions for facilitating transfer but also regions where metabolic reactions may take place to provide energy for transport. Since the period covered by this historical sketch, a great deal of evidence indicating the presence of catalytically active enzymes in the cell membrane has appeared.

but it is worth harking back to a phenomenon that bore strongly on this point before the later evidence accrued. Fluoride inhibits glycolysis, the metabolic process that provides the energy of the mammalian erythrocyte; in attempting to reverse the effect while studying its influence on erythrocyte permeability, calcium was added to the medium in order to precipitate the fluoride. It was found31 that the added calcium caused hemolysis; presumably the fluoride was tightly bound to the cell membrane, and the attempt to remove it by making it insoluble with calcium caused the partial destruction of the membrane. The effect must almost certainly have been a surface effect, if only because calcium penetrates the cell so slowly, so that we can conclude that fluoride attaches itself to the surface of the cell. Thus glycolysis is apparently inhibited by this ion which attaches itself to the surface, so that it seems very likely, on this ground, that the enzyme concerned with glycolysis, and poisoned by fluoride, is actually in the cell membrane.

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HUGH DAVSON

_Circulation_. 1962;26:1022-1037
doi: 10.1161/01.CIR.26.5.1022

_Circulation_ is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7322. Online ISSN: 1524-4539

The online version of this article, along with updated information and services, is located on the World Wide Web at:
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