MEMBRANE LIPID, NOT POLARIZED WATER, IS RESPONSIBLE FOR THE SEMIPERMEABLE PROPERTIES OF LIVING CELLS

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ABSTRACT It has recently been proposed that the semipermeable surface barrier of living cells is in fact provided by water polarized in multilayers by the cellular protein, and not by the lipids of the cell membrane as had been widely supposed. This review paper summarizes a large and diverse body of recent experimental work, concerned with the structure and function of biological membranes in general and with the passive permeability properties of cell surface membranes in particular, which does not support the polarized water hypothesis. Indeed, there exists a great deal of experimental evidence which supports the concept that the lipid bilayer is a central structural feature of cell surface membranes and that one of the major functions of this lipid bilayer is to provide the selective, semipermeable barrier found at the surface of living cells.

INTRODUCTION

In a paper appearing in this journal, Dr. G. N. Ling proposed that water polarized in multilayers by the cellular proteins, and not membrane lipid, provides individual living cells with their semipermeable surface barrier (1). In support of this proposal Dr. Ling presented experimental data demonstrating a close correlation between the permeability of reversed frog skin and synthetic cellulose-acetate sheets to water and nine other hydroxylic nonelectrolytes. He also contended that the lipid membrane is not an adequate model for biological membranes because it is not a bona fide semipermeable membrane and cited several older papers in the membrane literature in support of his suggestion that lipids are not important structural or functional components of cellular membranes. In this brief review paper I will show that Dr. Ling's frog skin permeability data is probably not relevant to the question of the permeation of nonelectrolytes across the membranes of individual living cells and thus cannot be used as evidence against the involvement of lipids in the maintenance of the cellular permeability barrier. I will also show that *lipid bilayer* membranes do in fact behave as bona fide semipermeable membranes and do provide good models for the selective surface barrier of individual living cells. Finally, I will briefly review a large body of sound experimental evidence not cited in Dr. Ling's paper which provides strong support for the concept

that a lipid bilayer is a central structural feature of all biological membranes and that this lipid bilayer functions as the major permeability barrier in living cells.

INTERPRETATION OF EPITHELIAL MEMBRANE PERMEABILITY COEFFICIENTS

Dr. Ling extrapolates without qualification his data on the permeability of reversed frog skin to the semipermeable properties of individual living cells. There exists good theoretical and experimental evidence that such an extrapolation is completely unjustified (2–12). The skin of the frog is of course a complex, macroscopic structure composed of a very large number of individual cells of many different histological types, arranged in two major layers separated by a basement membrane. The dermal and epidermal layers themselves consist of several different sublayers, each of which may be several cells thick. Many investigators have demonstrated that nonelectrolytes and ions can pass through complex, multicellular structures such as frog skin by several different mechanisms. A particular permeant may traverse this structure primarily by the sequential passage through a series of individual component cells (the intracellular pathway). In this case the rate-limiting step in the overall permeation process *may* be the passive diffusion across the surface membranes of one or more individual cells. However, diffusion through the cellular cytoplasm or within membrane-bounded intracellular structures could also be rate-limiting even for a transcellular permeation route. Alternatively, a particular permeant may traverse the skin of the frog primarily by diffusion within the intercellular spaces and/or through specialized junctional regions which link certain component cells (the extracellular pathways). In this latter case the major portion of the permeant molecules may pass through a multicellular structure without diffusing across the surface membranes of individual cells. Of course many permeants utilize a combination of intra- and extra-cellular pathways. In either case the effect of unstirred water layers on apparent permeability coefficients must be considered. Unstirred water layers, which are certainly present within the frog skin structure, are likely to significantly retard the free diffusion of nonelectrolytes. Lateral diffusion within the intercellular spaces can also affect the apparent permeability coefficients of a multilayered structure such as frog skin. Good evidence exists that in frog skin, and even in many structurally simpler epithelial membranes, extracellular pathways of permeation can be significant or predominant and that the effects of unstirred water and diffusion within the lateral intercellular spaces on the apparent permeabilities of these structures must be considered (2–12). The permeability data presented by Ling is essentially uninterpretable on a molecular basis since both the major diffusional pathway for the various nonelectrolytes tested and the rate-limiting step or steps in what could be a series of rather complex overall permeation processes are not known in his reversed frog skin preparation. The implicit assumption made by Ling, that nonelectrolyte diffusion through a complex, multicellular structure such as frog skin, which is hundreds of microns in thickness, is essentially equivalent to nonelectrolyte diffusion across the plasma membrane of a single living cell, which has a thickness of about 0.01

of a micron, is not valid. The good correlation noted by Ling between the nonelectrolyte permeability coefficients of frog skin and cellulose-acetate sheets is probably due to the fact that the unstirred water layers of the cellulose-acetate sheet provide a good model for similarly immobilized water in the intra- and/or extra-cellular spaces of reversed frog skin. Certainly this correlation does not constitute strong evidence against a role for lipids in maintaining the selective surface barrier of individual living cells.

EXISTENCE OF LIPID BILAYERS IN BIOLOGICAL MEMBRANES

In his introduction Dr. Ling cites two papers, both published in 1967, which showed that the appearance of the trilaminar "unit membrane" structure observed with the electron microscope was not materially altered by the removal of 95% of the membrane lipid (13, 14). From this simple observation the conclusion was drawn that biological membranes do not contain a central lipid bilayer structure and that indeed the unit membrane structure is in fact entirely proteinaceous. Even if one accepts the questionable supposition that the electron micrographic techniques available at that time produced images which could be interpreted at the molecular level, other interpretations of this observation are possible. Since it has been demonstrated that the stains utilized in these studies interact primarily with the membrane protein even in native membranes, several workers have interpreted these results to mean simply that the original distribution of the protein layers on each side of the lipid bilayer was not destroyed by extraction of the membrane lipid under certain carefully controlled conditions (15, 16). Support for this latter view has come from experiments which demonstrated that the unit membrane appearance is preserved only after glutaraldehyde prefixation and the use of specific staining procedures (13) and then only with certain membrane systems (14). Freeze-fracture electron microscopy, which does not involve potentially disruptive chemical fixation and staining, has in fact provided strong evidence for the existence of lipid bilayers in cell membranes (17–21; for reviews see 22–24). Dr. Ling also ignores the large body of experimental work published in the last six years which directly demonstrates both the presence of extensive areas of lipid bilayer in biomembranes and the excellent correspondence between many of the properties of biological and artificial lipid bilayer membranes. Space limitations do not permit even a cursory review of this extensive literature here, but the reader may consult any of a number of review articles for a summary of this information (16, 25–32). It is of interest to note that, to the best of my knowledge, every author of a major review article on membrane structure and function since 1970 has found the evidence for the existence of lipid bilayers in cellular membranes to be compelling.

SEMIPERMEABLE PROPERTIES OF LIPID BILAYER MEMBRANES

Another argument advanced by Dr. Ling against a role for lipids in maintaining the cellular permeability barrier is the fact that the "lipid" membrane model of Overton and Collander is not a bona fide semipermeable membrane, since water and certain

other small polar nonelectrolytes permeate cell membranes many times faster than would be predicted simply by their oil/water partition coefficients. However, the widely acknowledged inadequacy of a simplified lipoid membrane model some three-quarters of a century old is hardly a creditable argument against a functional role for lipids in the membranes of living cells. The rate of permeation of nonelectrolytes through a lipid bilayer membrane, which is of course the currently relevant model system, depends on the size, shape, and chemical structure of the permeant molecule, as well as on its oil/water partition coefficient, just as is the case for biological membranes (for reviews, see 33 and 34). Indeed, the close correspondence which exists between the passive permeability characteristics of bimolecular lipid membranes and cellular membranes is good evidence for a functional role for a lipid bilayer in maintaining the permeability barrier in living cells (34). Lipid bilayer membranes *are* of course semipermeable, showing a higher permeability to water than to other nonelectrolytes (33, 34).

MEMBRANE LIPIDS AND THE SEMIPERMEABLE PROPERTIES OF LIVING CELLS

Dr. Ling is apparently unaware of a group of papers published in the last five years which directly address the question of whether or not the lipids of the plasma membrane function as the major nonelectrolyte permeability barrier in intact cells (35–40). In these studies the fatty acid composition and cholesterol content of the membrane lipids of the simple procaryote *Acholeplasma laidlawii* B were systematically altered and the rates at which a number of nonelectrolytes passively diffuse into intact cells and into liposomes (closed, spherical lipid bilayer membranes) prepared from the total membrane lipid were measured at a variety of temperatures. Since alterations in fatty acid composition and cholesterol content do not significantly alter the qualitative or quantitative distribution of the membrane proteins (41) or polar lipid headgroups (39), it is possible to selectively study the effect of variations in the nature of the hydrophobic core of the plasma membrane of this organism on its passive permeability properties. The permeabilities of intact cells and derived liposomes are markedly dependent on the chemical structure and chain length of the membrane lipid fatty acids. The incorporation of branched-chain or unsaturated fatty acids, or fatty acids of reduced chain length, increases nonelectrolyte permeability to a similar extent in both cells and liposomes (35, 37–40). The nonelectrolyte permeability of both the plasma and liposomal membranes is reduced by the incorporation of cholesterol (35, 38–40). The mean activation energy values calculated for the permeation of several nonelectrolytes into intact cells and into liposomes are the same, within experimental error, and suggest that these nonelectrolytes permeate both biological and artificial membranes as single, fully dehydrated molecules (35, 36, 39). In contrast to permeation rates, which are dependent on both permeant structure and membrane lipid composition, apparent activation energy values for the overall permeation process are dependent only on permeant structure and are not significantly affected by variations in the fatty acid

composition or cholesterol content of the artificial or biological membrane systems (35, 36, 39). The permeability of intact cells and derived liposomes is a function of the fluidity of the membrane lipids as measured by their thermotrophic gel to liquid-crystalline membrane lipid phase transition temperatures (39, 40). *A. laidlawii* B is equipped with a mechanism to control the fatty acid composition of its membrane lipids, and thus the fluidity and permeability of its plasma membrane, within certain limits (37). Many of the experiments described above were also performed on animal erythrocytes, with similar results (36, 42).

It has also been established that small changes in the chemical structure of the cholesterol molecules present in the membrane of *A. laidlawii* or animal erythrocytes can significantly alter the passive permeability of intact cells by altering the physical state of the membrane lipids (38, 43). The presence of cholesterol itself decreases the fluidity of the hydrocarbon chains of the membrane lipids, increases the tightness of molecular packing in the lipid bilayer and reduces the nonelectrolyte permeability of both intact cells and liposomes. The incorporation of epicholesterol, the 3α-hydroxy isomer of cholesterol, has no detectable effect on membrane lipid fluidity or packing and also does not alter the nonelectrolyte permeability of the artificial or biological membrane systems. On the other hand, the presence of cholest-3-one, a keto analogue of cholesterol which actually increases the fluidity of the membrane lipids and results in a more loosely packed bilayer structure, increases the nonelectrolyte permeability of both intact cells and liposomes (38, 42, 43). The studies discussed above clearly demonstrate that the physical state of the membrane lipids, as determined by the fatty acid composition and sterol content, determines the nature of the nonelectrolyte permeability barrier of living cells. It is difficult to see how alterations in the hydrophobic core of a lipid bilayer could produce marked and characteristic changes in the nature of polarized water multilayers at the cell surface, as proposed by Ling.

A great deal of other evidence also supports the concept that the membrane lipids, and not polarized water multilayers, function as the major permeability barrier in living cells. The extensive treatment of intact cells with proteases which remove most of the protein molecules exposed on the outer surface of the cell membrane does not destroy or markedly alter the cellular permeability barrier, as would be expected if the speculations of Ling were correct (for reviews, see 27, 30, 44). On the other hand, the treatment of susceptible cells with purified phospholipase A_2, which cleaves fatty acyl groups from membrane phospholipids, destroys the semipermeable properties of living cells and leads to cell lysis, as would be expected if the hydrophobic core of the lipid bilayer functions as the major cellular permeability barrier (for review, see 30). Lipid-soluble polyene antibiotics have been shown to destroy the permeability barrier of intact cells by forming pores in the plasma membrane. These antibiotics function in an entirely analogous fashion with artificial lipid bilayer membranes which contain no protein. Interestingly, these antibiotics are completely inactive toward both cells and liposomes which do not contain cholesterol, since the pores formed in the plasma membrane are composed of antibiotic-cholesterol complexes (45–47). Cultivation of *Lactobacillus plantarum* under conditions of impaired fatty acid biosynthesis results in the

production of cells with lipid-deficient, hyperpermeable membranes (48, 49). Restoration of normal cellular permeability can be accomplished by the addition of appropriate lipid supplements even under conditions which preclude additional protein synthesis (48, 49). Again these results can be easily explained with the bimolecular lipid membrane model but are difficult to reconcile with the hypothesis proposed by Ling. Many other observations could be cited which are incompatible with the polarized water hypothesis.

CONCLUSION

The experiments briefly summarized above, and the results of many other studies which cannot be discussed in detail here, clearly establish that membrane lipid, not polarized water, is responsible for the semipermeable properties of living cells. The limited and largely uninterpretable permeability data and the unconvincing and irrelevant theoretical arguments presented by Ling are not a creditable challenge to our current ideas concerning the structural and functional importance of lipid bilayers in biological membranes.

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