ON THE LARGE ERROR INTRODUCED IN THE ESTIMATE OF THE DENSITY OF MEMBRANE PORES FROM PERMEABILITY MEASUREMENTS WHEN DIFFUSION IN "UNSTIRRED LAYER" WITHIN THE CELLS IS DISREGARDED

GILBERT N. LING
Department of Molecular Biology, Pennsylvania Hospital, Eighth and Spruce Streets, Philadelphia, PA 19107.

● *After the development of the "black lipid membrane" techniques, studies of the permeability of labeled water and nonelectrolytes across these artificial membranes have yielded permeability constants comparable in magnitude to those obtained from tracer studies of living cell membranes. This general agreement has affirmed the belief that the living cell membranes are indeed closely similar to these bilayer phospholipid membranes.*

In this report, we draw attention to a hidden assumption behind such comparisons made: the assumption that labeled material passing through the cell membrane barriers instantly reaches diffusion equilibrium inside the cell.

The permeability constants to labeled water (and nonelectrolytes) across lipid layers were obtained using setups in which the lipid membrane was sandwiched between aqueous compartments both of which were vigorously stirred. In studies of permeability of living cell membranes only the outside solution was stirred, the intracellular water remained stationary. Yet the calculations of permeability constants of the cell membrane were made with the tacit assumption, that once the labeled materials pass through the cell membrane, they were instantly mixed with the entire cell contents as if a stirrer operating at infinite speed had been present inside the cells. Ignoring this unstirred condition of the intracellular water, in fact, lumped all the real-life delay due to diffusion in the cytoplasm and added it to the resistance to diffusion of the membrane barrier. The result is an estimated membrane permeability to labeled water (and nonelectrolytes) many times slower than it actually is. The present report begins with a detailed analysis of a specific case: tritiated water diffusion from giant barnacle muscle fibers and two non-living models, one real, one imagined. It shows how the conventional way of determining the cell membrane permeability constant and the percentage areas of aqueous channels on the cell membrane might involve error as high as 100,000 fold.

The overall conclusion is that the living cell membrane is vastly more permeable to water and to nonelectrolytes than the phospholipid bilayer models with or without additives of polyene antibiotics. The real-life permeability rates are in harmony with the theory that large domains of water intensely polarized in multilayers in the cell membrane offer the main pathway for water and solute traffic in and out of living cells.

Accurate studies of the permeability of living cells began with the availability of radioisotopes. The conventional way to determine the permeability constant of the cell membrane involves loading the cells with the radioactively labeled substance under study and washing the loaded cells in a continuous stream or successive portions of a Ringer solution containing no radioactive isotope but otherwise identical in composition to the incubation solution. The logarithm of the remaining radioactivity in the cells at time t is then plotted on the ordinate against t as abscissa according to the equation

$$ln\left(\frac{\overline{C}}{C_i}\right) = -\frac{A}{V}\kappa t, \qquad (1)$$

where C_i and $\overline{C}$ are respectively the concentrations of the labeled substance in the cell initially and at time t, A and V are the surface and volume of the cell and κ is the "permeability constant" in cm/sec. As a rule, when the curve is not entirely rectilinear, the earlier departure is usually ignored (see below). From the rectilinear portion of the curve, the half time of exchange of the labeled substance ($t_{1/2}$) is obtained. From this $t_{1/2}$ value, κ is determined from the relationship:

$$\kappa = \frac{V}{A}\frac{ln\,2}{t\%}. \qquad (2)$$

The standard permeability constant, P, refers to diffusion through a 1 cm^2 of the surface of a 1 cm thick, homogeneous isotropic medium and is in units of cm^2 per second (see Crank, 1956, p. 43); κ is, in fact $\Phi P/d$, where d is the thickness of the cell membrane and Φ, a dimensionless number, is the percentage of the membrane occupied by aqueous "pores". Now

$$P = Dq, \qquad (3)$$

where D is the diffusion coefficient of the labeled substance in a homogeneous membrane and q is the equilibrium distribution coefficient of the labeled substance in the same. Therefore

$$\kappa = \frac{\Phi P}{d} = \frac{\Phi D q}{d}, \qquad (4)$$

where D and q now refer to the diffusion coefficient and equilibrium distribution coefficient of the labeled substance in the water filled membrane pores. Rearranging, we have

$$\Phi = \frac{d}{D}\frac{\kappa}{q}. \qquad (5)$$

Equation 5 provides a way to estimate Φ from κ.

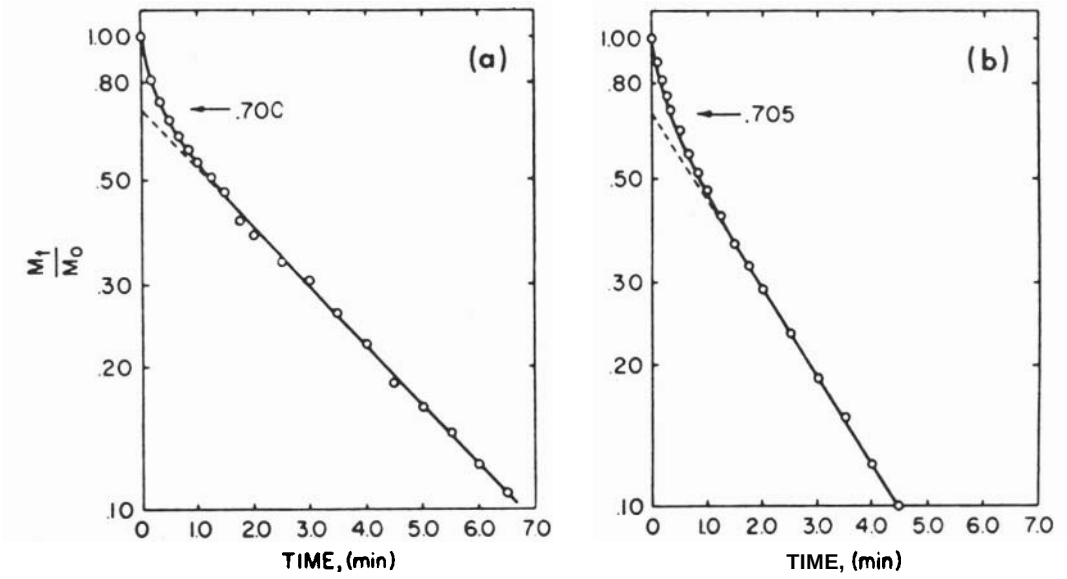

FIGURE 1. Efflux of ^3HHO from an intact isolated muscle fiber. (a): muscle a; (b): muscle d. The fibers were previously equilibrated for at least one hour in barnacle Ringer solution containing 10 μCi/ml of ^3HHO. The graphs are semilogarithmic plottings of the fraction of initial ^3HHO activity as a function of time. The experimental points do not follow a simple straight line. The intercepts of the final straight line are 0.700 and 0.705 respectively. (Reisin and Ling, 1973)

In the following I shall use Equation 5 to calculate Φ from the efflux data of THO labeled water from one living cell and two models of the living cell, one real and another imagined.

1. ***Giant Barnacle Muscle Fiber.*** Figure 1 taken from Reisin and Ling (1973) shows efflux curves of THO from two single giant barnacle muscle fibers. The straight line portions of eight similar curves yields an average $t_{1/2}$ of 89.0 seconds. The average radius of the cylindrical muscle fiber is 0.068 cm, the volume surface ratio is therefore equal to 0.0681 2 = 0.034 cm. From Equation 2, κ = 0.034 X 0.693189 = 2.64 X 10^{-4} cm/sec. This value is very close to that reported by Bunch and Edwards (1969), i.e., 2.6 X 10^{-4} cm/sec. In both, following traditional beliefs, the assumption was made that the cell membrane is the rate-limiting barrier to THO exchange between the muscle cell and its environment. Indeed virtually all available data of cell membrane permeability to labeled water and many other solutes were based on this assumption.

Now the equilibrium distribution coefficient of THO in water within the membrane pore must be close to unity. Assuming that the diffusion coefficient of THO in the membrane pores equals that in free water (2.44 X 10^{-5} cm^2/sec.) (Wang et al., 1953) and that the membrane is 60 Å thick, we have from Equation 5.

$$\Phi = \frac{60 \times 10^{-8}}{2.44 \times 10^{-5}} \cdot \frac{2.64 \times 10^{-4}}{1} = 6.49 \times 10^{-6}.$$

This data shows that out of 1 cm^2 of the barnacle muscle surface membrane only 0.00065% is occupied by aqueous pores! If one assumes that this total diffusing area is divided into n round pores all with a radius of 2.5 Å, then in one μm^2 of the cell surface we have

$$n = \frac{6.49 \times 10^{-6}}{\pi (2.5 \times 10^{-8})^2} \times (10^{-4})^2 = 33.1$$

or 33.1 pores per μm^2 of the cell surface, which is not too far from estimates of the density of Na channels in nerve and muscle cell membranes (Bogart, 1981).

2. ***Cylindrical Filament of Agar Gel.*** A 4% agar gel filament freshly prepared, with dimensions similar to those of the giant barnacle muscle fiber, was loaded with THO and its efflux curve followed. The experimental result, also taken from Reisin and Ling (1973), is reproduced as Figure 2. The radius of the filament studied was 0.052 cm, the $t_{1/2}$ of the straight line portion of the efflux curve is 21 seconds, and the volume/surface = 0.05212 = 0.026 cm, κ = (0.693121) X 0.026 = 8.58 X 10^{-4} cm/sec.

Let us assume arbitrarily that the agar filament like the muscle cell also has a membrane 60 Å thick and that the q value of labeled water in the pore water is unity and that the diffusion of labeled water THO is D = 2.44 X 10^{-5} cm^2/sec. Then

$$\Phi = \frac{60 \times 10^{-8}}{2.44 \times 10^{-5}} \cdot \frac{8.58 \times 10^{-4}}{1} = 2.11 \times 10^{-5}.$$

Thus the hypothetical membrane pores subtend only a volume equal to 0.00211% of the total surface area!

Something is seriously wrong here, because the agar gel filament was prepared in such a way that no drying occurred at its surface and there was no "membrane" of any kind to speak of. All parts of the agar filament should have uniform properties and Φ should be not too far from the water content of the agar gel, i.e., 96%.

3. A ***Pure Water Filament.*** Next, let us imagine a cylindrical filament of pure water which by some magic force is kept from dispersing in the surrounding aqueous solution.

Let this imaginary water filament have a diameter similar to the barnacle muscle fiber or agar gel filament, i.e., r = 0.06 cm and also loaded with THO and washed in a continuous stream of water containing no THO. Then the concentration of THO ($\bar{C}$) in the cylinder at time t, as a fraction of its initial concentration (Ci) is given by (Dunwald and Wagner, 1934):

$$\frac{\bar{C}}{C_i} = \sum_{\nu=1}^{\nu=\infty} \frac{4}{\xi_\nu^2} \exp\left(-\xi_\nu^2 \cdot \frac{D}{r^2}\right) t, \qquad (6)$$

where ξ_ν is the root of the Bessel function of zero order (ξ_ν = 2.405, 5.520 . . .), D is the diffusion coefficient of THO (2.44 X 10^{-5} cm^2/sec.), and r is the radius of the water cylinder. An approximation of Equation 6 is

$$\frac{\bar{C}}{C_i} \simeq \frac{4}{2.405^2} \cdot \exp\left(-\frac{2.405^2\, D}{r^2} t\right), \qquad (7)$$

This equation can also be written in a logarithmic form:

$$\log\left(\frac{\bar{C}}{Ci}\right) \cong -\frac{5.78\, D}{r^2} t - 0.160. \qquad (8)$$

Based on Equations 7 and 8, one finds that this straight-line portion of the semi-logarithmic plot of the diffusion profile yields a $t_{1/2}$ of 17.6 sec., and $\kappa = (0.693/17.6) \times 0.00612 = 1.18 \times 10^{-4}$ cm/sec. Again assuming a hypothetical membrane 60 Å thick covering the water filament we find that

$$\Phi = \frac{60 \times 10^{-8}}{2.44 \times 10^{-5}} \cdot 1.18 \times 10^{-4} = 2.90 \times 10^{-6}$$

Thus again the area subtended by the surface membrane pores through which labeled water travels is only 0.00029% of the total surface of this hypothetical water filament. This is, of course, absurd. Being nothing else than pure water, the entire surface of the imaginary water filament is equally available to THO diffusion; Φ should be 100%.

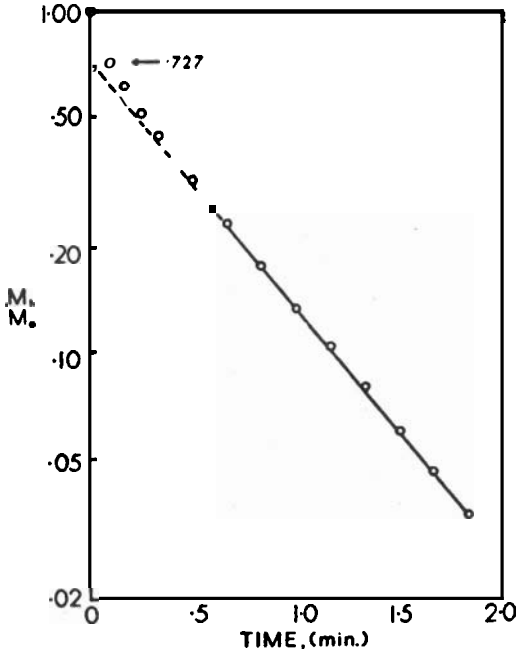

FIGURE 2. Efflux of 'HHO from a filament of agar gel. The filament of agar gel 4% (w/v) is 0.0520 cm in radius. The final straight line obtained by regression has an intercept of ln 0.727. (Reisin and Ling, 1973)

DISCUSSION

Clearly in the case of the imaginary cylinder of water with uniform diffusion coefficient throughout, the diffusion of labeled water is not limited by the surface but is "bulk-phase" limited. (Ling, 1966; Ling et al., 1967). By making the incorrect assumption that the diffusion was surface or membrane limited, we rejected the initial rapid exchange part of the diffusion curve and selected only the slow phase of diffusion for the basis of our calculation. This created one part of the error. The same error was also committed in dealing with the agar gel filament and the

barnacle muscle fiber (see below). Only here an additional error was made because the diffusion coefficient of THO was not 2.44 X 10^{-5} cm^2/sec as in free water but significantly less (1.44 X 10^{-5} cm^2/sec. for agar gel and 1.75 X 10^{-5} cm^2/sec. for barnacle muscle) (see below).

By far the most important error that led to this extremely low percentage of the area subtended by water channels, f, was that in its estimate one assumed that the labeled THO within the membrane was in instant equilibrium with water elsewhere in the cell or its model, as if all the water in the cell or model interior was constantly stirred at infinite speed. In the study of permeability through isolated membrane, like frog skin, or model lipid bilayers, vigorous stirring of solution bathing ***both*** sides of the membrane was, as a rule, both feasible and essential. Failure to stir effectively led to what is called the problem of the "unstirred layer" (see Noyes and Whitney, 1897; Ginzburg and Katchalsky, 1963; Andreoli, et al., 1969). Since in the study of living cells, the inside of the cell is, as a rule, not stirred, the result is that the realistic diffusion through a stationary cell interior is erroneously attributed to that of a thin membrane barrier. As a result Φ is made much smaller than the correct value. This error increases with increasing size of the cell and becomes enormous when dealing with giant barnacle muscle fibers and large frog eggs. The underestimation of permeability produced by the unjustified assumption of infinite speed of diffusion inside the cytoplasm was mentioned by Dick in 1959.

With all these in mind, I would like to discuss two relevant issues relating to water permeability of living cells and model systems.

1. ***The Bulk-Phase-Limited Diffusion of Water in Frog Eggs and Barnacle Muscle and the Cause of the Gross Error Introduced by the Conventional Method of Assessing Water Permeability.*** In 1966 *I* introduced a simple method called the "influx profile analysis" to determine the rate-limiting step in the exchange of a solute between the inside and outside of living cells (Ling, 1966). With this method, Ling, Ochsenfeld, and Karreman (1967) demonstrated that the exchange of THO between frog ovarian eggs is, contrary to conventional belief, bulk-phase limited, i.e., the diffusion coefficient of THO is essentially uniform throughout the entire cell including the cell membrane. Later, using a similar approach, Reisin and Ling (1973) reached a similar conclusion for the exchange of THO of giant barnacle muscle fiber. These data yielded the diffusion coefficient of THO in frog egg cytoplasm (0.72 – 1.47 X 10^{-5} $cm^2/sec.$) and the (radial) diffusion coefficient of THO in giant barnacle muscle cytoplasm (1.35 X 10^{-5} cm^2/sec.). These estimated diffusion coefficients have been repeatedly confirmed from studies where the water motion examined did not involve its passage across the cell surface. (For tabulation of data see Caillé and Hinke, 1974; Ling, 1984, Table 12.3). These quantitative accords affirmed the validity of our conclusion that the cell membrane does not offer resistance to THO movement greater than that offered by the cytoplasm: A bulk-phase limited diffusion of THO in frog ovarian eggs and giant barnacle muscle fiber also implies that the relative area subtended by water at the cell surface is not materially different from that in the cytoplasmic path of an outwardly or inwardly diffusing THO. Quantitatively speaking, this means that the percentage of the surface of the cells covered by water approximates the water content of the cell. For barnacle muscle with a water content of 78.7% ± 3.0%, Φ should be not too far from this figure*. The astronomical discrepancy between 80% and the Φ calculated on the assumption of membrane limited diffusion and instant intracellular mixing (0.00065%) are comparable with similar discrepancies seen in the case of the

two models considered (agar gel, 0.0021 1%; water filament, 0.00029%) for essentially similar reasons.

The assumption of instant intracellular mixing gives rise to large error for both bulk-phase limited and surface limited exchange of labeled substances between the cell and its environment. It is the bulk-phase limited diffusion of THO in these systems that gives us the opportunity to recognize errors of this magnitude. The following analysis offers some additional quantitative insight into this error.

The surface/volume ratio (A/V) of long cylinders is 2/r. Substituting this and Equation 4 into Equation 1, one finds

$$ln\left(\frac{\overline{C}}{C_i}\right) = -\frac{2q\Phi D}{rd}t. \qquad (9)$$

When a bulk-phase limited diffusion is incorrectly regarded as a membrane-limited diffusion, one in fact mistakes Equation 8 for Equation 9, and therefore, has taken $\frac{5.98\ D_{cyt.}}{r^2}$ for $\frac{2q\Phi D_{m.p.}}{rd}$, where $D_{m.p.}$ and $D_{cyt.}$ are respectively the diffusion coefficient in the membrane pore and in the bulk phase cytoplasm.

This mistaken identity then generates a fallacious Φ_f where

$$\Phi_f = \frac{2.89\ d\ D_{cyt.}}{q\ r\ D_{m.p.}} \qquad (10)$$

This Φ_f is obviously not the true area subtended by membrane pores, but an artifact dominated by the ratio of the membrane thickness (d) divided by the radius of the cell, r. With d at 60 Å, and a radius of giant barnacle muscle fiber equal to 0.068 cm or 6.8 X 10^6 Å, this dominating factor is 2.89 X 60/(6.8 X 10^6) = 2.6 X 10^{-5}, which is not far from the Φ we calculated above for the barnacle muscle and its real and imaginary models.

In the AI hypothesis, water in the cytoplasm as well as in the membrane pores (see Ling, 1965) exists in the state of polarized multilayers but the intensity of polarization is higher in the membrane pores than the average intensity of water in the cytoplasm. The study of permeation of labeled water and other solutes through other living cell membranes including those of frog egg and barnacle muscle has revealed that for many other substances their passage through the cell membrane are rate limiting — in agreement with the theory that while water in the state of polarized multilayers offers no greater diffusion barrier than normal liquid water to small and simple molecules (e.g., O_2, N_2, THO), it does offer increasingly higher resistance toward molecules of increasing sizes and complexities ("the size rule") and that this differentiation becomes more pronounced in water more intensely polarized as postulated for water in the cell membrane.

Witness that the data of Figure 1 which demonstrates the bulk-phase limited diffusion with its implication of very large area of membrane subtended by water, also yields a Φ of only 0.00065% when it is arbitrarily assumed that the exchange is rate-limited by its passage through the cell membrane and that

*This percentage estimated is based on the assumption that the solid content of the cell is uniform throughout the cell. If this is not the case, this percentage may be overestimated, though definitely not by orders of magnitude. For example, if the cell solids are arrayed in the form of concentric shells of higher solid density than the average solid density, the diffusion of THO will remain bulk phase limited but the rate of diffusion may be reduced by a factor corresponding to the ratio of the solid density in the concentric shells over the average solid density of the cell. However, the demonstrated generally similar diffusion coefficient of water in the muscle cell (see Ling, 1984, Table 12.3) for water movement in different direction in muscle cytoplasm shows that there is no evidence of concentric rings of significantly lower water contents in the barnacle muscle cell and frog eggs.

diffusion rate of THO within the cell is infinite.

2. ***Does the Water Permeability of Lipid Bilayer Membranes Quantitatively Jibe with the Water Permeability of Natural Cell Membranes?*** In Overton's lipoidal membrane theory (Overton, 1899) cells are covered with a continuous layer of neutral lipids sharing the properties of olive oil, a model made famous by the studies of Collander and his coworkers (Collander, 1959). However, two attributes of neutral lipid membranes are at variance with those of the natural cell membranes: (1) Neutral lipid membranes are "anti-semipermeable", being more permeable to ethanol than to water because the oil/water distribution coefficient for ethanol is some 75 times higher than water (Collander, 1959); (2) A covering of neutral lipids would endow living cells with much higher surface tensions than those measured. To overcome the first difficulty, the mosaic membrane with pores for water and other small molecules was suggested; to overcome the surface tension difficulties lipid bilayers were sandwiched between hydrophilic globular proteins in the Davson-Danielli model (Davson and Danielli, 1943).

The extensive research stimulated by the introduction of "black lipid membranes" technique (Langmuir and Waugh, 1938; Miiller, et al., 1962), often considered as directly in support of the Overton-Collander-Barlund-Davson-Danielli membrane, in fact have departed significantly from it in more than one way.

As mentioned above, in the original Overton lipoidal membrane theory, although lipids and phospholipids have been referred to indiscriminately, there was no doubt that Overton meant neutral lipids. The lipid bilayers studied in the black lipid membrane technique are primarily phospholipids (which are not neutral lipids) and cholesterol (which is not lipid at all, but a sterol) (see Dewey and Barr, 1970); though unquestionably these ***are*** the substance of the real cell membrane lipids.

Phospholipids carry both cationic (e.g., choline in lecithin) (or P sites) and anionic groups (e.g., phosphate in lecithin) (or N sites) and are thus more hydrophilic than neutral lipids. It is not surprising that the interfacial tension between black lipid membranes and water is lower than that at a neutral lipid-water interface, thereby partly obviating the need of covering the lipid layers with continuous layers of globular proteins in order to reduce the interfacial tension in the Pauci-molecular membrane model.

Certain black lipid membranes show high permeability to water (Cass and Finkelstein, 1967; Andreoli et al., 1969). Since the oil/water distribution coefficient for water is too low to account for this high water permeability and since here the existence of permanent aqueous channels is not likely, a logical solution is that there are unstable aqueous channels which have only momentary existence. It is possible that phospholipids with their N and P sites, in conjunction with cholesterol favor this transient aqueous channel formation by polarizing water in multilayers in the memrane, a tendency apparently further enhanced by the presence of polyene antibiotics (see Andreoli et al., 1969).

The question may be raised, "With the "high" water permeability through the lipid bilayers established, would it be possible that living cells covered with a similar lipid bilayer exhibit a bulk-phase limited diffusion of water as we have demonstrated for frog egg and barnacle muscle?"

As mentioned above, the permeability constant, κ, for THO of giant barnacle muscle fiber given by Bunch and Edwards is 2.6×10^{-4} cm/sec. For the much smaller human erythrocytes, κ measured was 5.3×10^{-3} cm/sec. (Paganelli and Solomon, 1957; Villegas et al., 1958). The permeability of the lipid bilayer to THO was given by Andreoli et al. (1969) as $1.08 \times 10^{-3} \pm 0.24 \times 10^{-3}$

cm/sec. in general agreement with the data of Cass and Finkelstein (1967) and others, apparently also in agreement with the living cell data cited above. This agreement would seem to offer an affirmative answer to the question raised. In fact, this agreement is only apparent for two reasons:

First, the agreement of the water permeability data of lipid bilayers is with the data obtained by the a priori and erroneous assumption that water exchange is membrane limited, an assumption made by Bunch and Edwards and by most scientists studying permeabilities of the living cell. As discussed above, this erroneous assumption grossly underestimated the true rate of exchange of water.

Secondly, there is a profound difference in the way the data were obtained in the lipid bilayer membrane and in the living cells. In Andreoli et al's lipid bilayer studies, both sides of the aqueous phases bathing the lipid membrane were vigorously stirred; in the living cell study, only the external side was stirred. As a result, while Andreoli et al's data are probably close to the true permeability of the bilayer membrane; the permeability of the living cell membrane estimated was grossly underestimated for the reasons cited above.

The conclusion is that even though phospholipid-cholesterol bilayer membranes have much higher permeability to water than the oil/water distribution coefficient predicts, its permeability for water is far below that of the living cell membranes. A living cell covered with a lipid bilayer therefore cannot possibly exhibit a bulk phase limited diffusion for water as established for the two cells large enough to be carefully studied: frog ovarian egg and barnacle muscle.

The above considerations once more leads to the conclusion that polarized water rather than lipid bilayers is more likely the major component of the cell membranes of frog egg, and barnacle muscle. A similar conclusion was reached from the indifference of the K^+ permeability to K^+-ionophores of the two kinds of cells (Ling and Ochsenfeld, 1986) along with the plasma membrane of the squid axon (Stillman et al., 1970) and the inner membrane of liver mitochondria (Maloff et al., 1978). These data taken as a whole suggest that, for most living cells, polarized water (dominated by fixed ions) may be the chief component of most living cell membranes.

The foregoing work was supported by NIH Grants 2-ROI-CAI6301 and 2-ROI-GM11422, and by the Office of Naval Research Contract N00014-85-K-0573.

REFERENCES

Andreoli, T. E., Dennis, V. W., and Weigl, A. M. (1969) *J. Gen. Physiol. 53:*133.

Bogart, R. (1981) *Ann. Rev. Physiol. 43:*711.

Bunch, W., and Edwards, C. (1969) *J. Physiol. 202:*683.

Caillé, J. P., and Hinke, J. M. (1974) *Canad. J. Physiol. Pharmacol. 52:*814.

Cass, A., and Finkelstein, A. (1967) *J. Gen. Physiol. 50:*1765.

Collander, R. (1959) in *Plant Physiology* (Vol. 2) (F. C. Steward, ed.), Academic Press, New York.

Crank, J. (1956) *The Mathematics of Diffusion,* Clarendon Press, Oxford.

Davson, H., and Danielli, J. F. (1943) *The Permeability of Natural Membranes,* 2nd ed., Cambridge University Press, London.

Dewey, M. M., and Barr, L. (1970) *Curr. Top. in Memb. and Trans. 1:*1.

Dick, D. A. T. (1959) *Exp. Cell Res. 17:*5.

Dunwald, H., and Wagner, C. (1934) *Z. physik. Chem. B24:*53.

Ginzburg, B. Z., and Katchlsky, A. (1963) *J. Gen. Physiol. 47:*403.

Langmuir, I., and Waugh, D. F. (1938) *J. Gen. Physiol. 21:*745.

Ling, G. N. (1966) *Ann. NY Acad. Sci. 137:*847.

Ling, G. N. (1965) *Fed. Proc. Symp. 24:*S103.

Ling, G. N. (1984) *In Search of the Physical Basis of Life,* Plenum Publ. Corp., New York.

Ling, G. N., and Ochsenfeld, M. M. (1986) *Physiol. Chem. Phys. and Med. NMR* 18: 109.

Ling, G. N., Ochsenfeld, M. M., and Karreman, G. (1967) *J. Gen. Physiol.* 50: 1807.

Maloff, B. L., Scordillis, S. P., Reynolds, C., and Tedeschi, H. (1978) *J. Cell Biol.* 78: 199.

Müller, P., Rudin, D. O., Tien, H. T., and Wescott, W. C. (1962) *Nature 194:*979.

Noyes, A. A., and Whitney, W. R. (1897) *Z. physik. Chem. 23:*689.

Overton, E. (1899) *Vierteljahrsschr. Naturforsch. ges Zurich 44:*88.

Paganelli, C. V., and Solomon, A. K. (1957) *J. Gen. Physiol. 41:*259.

Reisin, I. L., and Ling, G. N. (1973) *Physiol. Chem. Phys.* 5: 183.

Robertson, J. D. (1960) *Prog. Biophys. Biophys. Chem. 10:*343.

Stillman, I. M., Gilbert, D. L., and Robbins, M. (1970) *Bioch. Biophys. Acta 203:*338.

Villegas, R., Barton, T. C., and Solomon, A. K. (1958) *J. Gen. Physiol. 42:*355.

Wang, J. H., Robinson, C. V., and Edelman, I. S. (1953) *J. Amer. Chem. Soc. 74:*466.

Reviewed August 25, 1987