An Analysis of Unstirred Layers in Series with "Tight" and "Porous" Lipid Bilayer Membranes

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ABSTRACT The present experiments were designed to evaluate the effective thickness of the unstirred layers in series with native and porous (i.e., in the presence of amphotericin B) lipid bilayer membranes and, concomitantly, the respective contributions of membranes and unstirred layers to the observed resistances to the diffusion of water and nonelectrolytes between aqueous phases. The method depended on measuring the tracer permeability coefficients for the diffusion of water and nonelectrolytes ($P_{D}$, cm sec$^{-1}$) when the aqueous phase viscosity ($\eta$) was increased with solutes having a unity reflection coefficient, such as sucrose or dextran. The effective thickness of the unstirred layers ($\alpha'$, cm) and the true, or membrane, permeability coefficients for diffusion of water and nonelectrolytes ($P_{m}$, cm sec$^{-1}$) were computed from, respectively, the slope and intercept of the linear regression of $1/P_{D}$ on $\eta$. In both the native and porous membranes, $\alpha'$ was approximately $110 \times 10^{-4}$ cm. The ratio of $P_{T}$, the osmotic water permeability coefficient (cm sec$^{-1}$) to $P_{m}$ was 1.22 in the native membranes and 3.75 in the porous membranes. For the latter, the effective pore radius, computed from Poiseuille's law, was approximately 5.6 Å. A comparison of $P_{m}$ and $P_{D}$ indicated that the porous membranes accounted for 16, 25, and 66% of the total resistance to the diffusion of, respectively, H$_2$O, urea, and glycerol, while the remainder was referable to the unstirred layers.

INTRODUCTION

In 1904, Nernst formulated the general hypothesis that stationary, or diffusion-limited, liquid layers at the boundaries between liquid and other phases might affect reactions within heterogeneous systems (1). Subsequently, Osterhout (2), Jacobs (3), and Teorell (4) called attention to the potential relevance of unstirred layers in regulating the transport of materials across
biological interfaces. More recently, this question has been considered with regard to the differences between coefficients for water flow through membranes measured either during isotopic diffusion ($P_{D_{\text{H}2O}}$, cm sec$^{-1}$) or osmotic water flow ($P_f$, cm sec$^{-1}$) experiments (5). In particular, Dainty has suggested (5) that $P_f/P_{D_{\text{H}2O}}$ ratios in excess of unity could depend on unstirred layers rather than on quasilaminar osmotic water flow through aqueous membrane channels (6–8), since the unstirred layers may impede isotopic diffusion to a greater degree than net water flux.

In this connection, Dainty and House observed in frog skin that the values of $P_{D_{\text{H}2O}}$ were affected to a considerably greater extent than those of $P_f$ by the magnitude of the unstirred layers (9). Ginzburg and Katchalsky noted that the values of $P_{D_{\text{H}2O}}$ in artificial cellulose membranes, were directly related to the rate of stirring in the aqueous phases (10). Similarly, Hays demonstrated that the vasopressin-dependent increment in $P_{D_{\text{H}2O}}$, for the toad urinary bladder, could be raised from less than twofold to approximately fivefold, when the aqueous phases were stirred vigorously (11). In the case of unmodified, or native, lipid bilayer membranes, there is reasonable experimental evidence from a number of laboratories (12–14) which supports the view that the primary mode of water transport through the membranes during osmosis is by diffusion, and that differences between $P_f$ and $P_{D_{\text{H}2O}}$ depend on the thickness of the unstirred layers.

Earlier communications from this laboratory suggested that the amphotericin B-dependent increments in the water, electrolyte, and nonelectrolyte permeability of lipid bilayer membranes containing appropriate sterols could be rationalized in terms of the formation of aqueous pores (14, 15). Accordingly, it was relevant to assess explicitly both the effective thickness of the unstirred layers in series with lipid bilayer membranes in the absence and presence of amphotericin B, and concomitantly, the contributions of the membranes to the observed resistances to the diffusion of water and solutes between aqueous phases.

This paper presents the results of such an analysis based on the differences in the observed permeability coefficients for diffusion of water and nonelectrolytes at varying aqueous phase viscosities. The results provide, first, an estimate, independent of $P_f$ measurements, for the effective thickness of the unstirred layers in series with such membranes, and second, additional evidence for quasilaminar water flow during osmosis in membranes exposed to amphotericin B. In a companion manuscript (16), we have evaluated the effects of osmotic water flow on the flux of solute across membranes exposed to amphotericin B, with particular regard to the question of coupling of solvent and solute flows under these conditions.

A preliminary report of these observations has appeared elsewhere (17).
METHODS

The experimental procedures for the formation and study of lipid bilayer membranes separating two aqueous phases have been presented in detail in earlier publications (14, 15). Except for the modifications described below, these techniques were employed without change in the present studies.

The lipid solutions used to form membranes contained equimolar amounts of high-potassium (HK) sheep red cell phospholipids (18) and cholesterol dissolved in decane; the total lipid concentration was in the range from 25 to 30 mg/ml. In an attempt to minimize experimental variations due to differences in lipids, a number of HK sheep red blood cell lipid preparations were pooled into a single preparation which was used in these and subsequent (16) studies.

The experiments were carried out in water-jacketed chambers identical to those described previously (15). In particular, the polyethylene diaphragms on which the membranes were formed were approximately $1.25 \times 10^{-2}$ cm thick (14); the diameter of the membrane apertures varied from 0.15 to 0.25 cm. The composition of the aqueous phases is indicated in the text. The pH of the unbuffered aqueous phases was 5.8-6.0 and the aqueous phase temperature was $26.5^\circ C \pm 0.5^\circ C$.

$P_f$ (cm sec$^{-1}$), the osmotic water coefficient, was estimated from net water fluxes as described previously (14, 15). Sucrose, in the concentration range from 0.01 to 0.6 M, was used to vary the osmolality of the aqueous phases, since, for these membranes, the reflection coefficient of sucrose is approximately one in the absence and presence of amphotericin B (14). $P_{Di}$ (cm sec$^{-1}$), the permeability coefficient for diffusion of water or the $i$th solute, was measured from unidirectional tracer fluxes at zero volume flow (14). In these and subsequent (16) experiments, the radioactive tracer was added to one aqueous phase approximately 3 min before the flux periods were begun.

In some instances, the DC resistance was measured as described previously (18). In the majority of the experiments, however, it was preferable to measure resistance with a four-electrode voltage clamp apparatus. Fig. 1 is a simplified block diagram of the system, which was constructed by Mr. John Adams, Physiology Instrument Shop, Duke University, Durham, N. C. The closed triangles indicate operational amplifiers (Analog Devices, Inc., Cambridge, Mass.), $\epsilon_1$ and $\epsilon_2$ represent 2% agar saturated KCl bridges to calomel-KCl electrodes in chamber I and $\epsilon_3$ and $\epsilon_4$ denote identical electrodes in chamber II. $R_m$ and $C_m$ indicate, respectively, membrane resistance and capacitance. The magnitude of the DC pulses was regulated by adjusting potentiometer $P_1$ in the feedback loop of amplifier $A$ (Model 105 C). The membranes were placed in the feedback loop for amplifier $B$ (Model 142 C) when the one-turn potentiometers, $P_2$ and $P_3$, which were joined together, were in the positions shown in Fig. 1. The membrane potential ($V_m$) was multiplied 10-fold in a differential electrometer amplifier (Keithley Instrument Co., Cleveland, Ohio, Model 604) and returned to the input of amplifier $B$. $V_m$ was monitored on channel one of a dual channel recorder (Model G-2000, Varian Instrument Co., Walnut Creek, Calif.) by reducing the voltage output of the differential electrometer 10-fold with amplifier $E$ (Model 105 C).
Membrane currents ($I_m$) were measured from the voltage output (monitored on channel two of the recorder) of amplifier $D$ (Model 142 C) in series with amplifier $C$ (Model 310 J) and the membrane. The switches selecting the feedback resistors in amplifiers $C$ and $D$ were joined together, permitting the seven indicated feedback configurations. Under these conditions, a 1 v output from amplifier $D$ corresponded to membrane currents ranging, in 10-fold increments, from $10^{-9}$ (position 7) to $10^{-3}$.

Figure 1. A block diagram of the electrical apparatus. Details are given in Methods.

The aqueous phase viscosities were measured at $26.5 \pm 0.5^\circ C$ with a modified Ostwald viscometer having an efflux volume of 1.4 ml, in the manner described by Schultz and Solomon (19).

Dextran (type 60 C; average mol wt = 77,500) was purchased from Sigma Chemical Co., St. Louis, Mo. Amphotericin B was kindly provided by Miss Barbara Stearns, Squibb Institute for Medical Research, New Brunswick, N.J.
RESULTS

A. Theoretical

Following Dainty (5), the relationship between $P_{D_i}$ and the thickness of the unstirred layers may be described by the series expression:

$$\frac{1}{P_{D_i}} = \frac{\alpha^t}{D^t} + \frac{1}{P_{m_i}},$$

(1)

where $\alpha^t$ is the sum of the effective thicknesses of the unstirred layers in both aqueous phases adjacent to a membrane, $D^t$ is the free diffusion coefficient of water or the $i$th solute, and $P_{m_i}$ is the true, or membrane, permeability coefficient for diffusion of water or the $i$th solute. Equation 1 defines $\alpha^t$ as the unstirred layer thickness when it is assumed that the frictional constraints to diffusion in the unstirred layer are the same as in bulk solution. In the present studies, $\alpha^t$ will be used as an operational quantity, without necessarily connoting the state of water in the unstirred phases (cf. Discussion). The free diffusion coefficient may be expressed in terms of the Stokes-Einstein relationship:

$$D^t = \frac{RT}{6\pi Na_i \eta},$$

(2)

where $a_i$ is the hydrodynamic radius of water or the $i$th solute and $\eta$ is the viscosity of the solvent. Since $D^t$ and $\eta$ are inversely related, it seemed reasonable to assess the unstirred layer thickness by varying aqueous phase viscosity.

One potential difficulty in such an approach depends on the fact that the Stokes coefficient, $6\pi$, is probably incorrect for small molecules such as water or urea (20, 21). However, the product $D^t \eta$, which will be termed $\beta_i$, is relatively constant in aqueous solutions over a wide range of temperatures (21–23). Accordingly,

$$D^t \eta \simeq \beta_i,$$

(3)

and by substitution:

$$\frac{1}{P_{D_i}} = \frac{\alpha^t}{\beta_i \eta} + \frac{1}{P_{m_i}}.$$  

(4)

Equation 4 provides the basis for the experiments. Thus, if $\alpha^t$ and $P_{m_i}$ remain constant, their values may be computed from, respectively, the slope and intercept of the relationship between $1/P_{D_i}$ and $\eta$. In this regard, it should be noted that $P_{m_i}$ may be estimated from Equation 4 independently of $P_i$. Accordingly, a comparison of $P_i$ and $P_{m_i}$ should provide information concerning the mode of water transport in the membrane phase.
B. Experimental

THE RELATIONSHIP OF PD$_{4}$ TO MEMBRANE CONDUCTANCE

In our earlier studies (14, 15, 18), measurements of dc resistance and the permeability coefficients for diffusion of water and nonelectrolytes, in the presence of polyene antibiotics, were often carried out on different membranes. Recently, Holz and Finkelstein have indicated that the amphotericin B–dependent values of PD$_{4}$, when corrected for unstirred layer effects, are linearly related to membrane conductance (24). These workers have reported their PD$_{4}$ values, in the presence of either nystatin or amphotericin B, by normalizing their data for a membrane resistance of 10$^{2}$ Ω cm$^{2}$ in 0.1 M NaCl.

Fig. 2 illustrates the relationship between the amphotericin B–dependent dc membrane conductance in 0.01 M NaCl and the PD$_{4}$ values for meso-erythritol; i.e., a solute for which the unstirred layer correction is relatively small (Table V). Although many of the experimental points agreed reasonably well with a line having a unity slope and zero intercept, the correlation was not uniform (Fig. 2). Moreover, in agreement with Holz and Finkelstein (24), the observed, rather than corrected, values of PD$_{4}$ for more permeable molecules such as H$_{2}$O or urea, in which the unstirred layer effect is large (Tables III and V), were not linearly related to membrane conductance.

![Figure 2. The relationship between PD$_{4}$ and dc membrane conductance (Gm).](https://example.com/f2.png)

The aqueous phases contained 0.01 M NaCl, 0.01 M meso-erythritol, and 0.8–1.0 × 10$^{-4}$ amphotericin B (pH $\approx$ 5.8, 26.5°C ± 0.5°C). Electrical measurements were carried out with the voltage clamp apparatus. The arbitrary line was drawn for a slope of unity and a zero intercept. Experimental details are given in Methods.
Accordingly, the amphotericin B-dependent tracer flux experiments in these and subsequent (16) studies were carried out on membranes having similar DC resistances, and the data were tabulated directly.

**THE EFFECT OF AQUEOUS PHASE VISCOSITY ON \( P_{D_{\text{H}20}} \)**

Tables I and II indicate the effects of varying aqueous phase viscosity on \( P_{D_{\text{H}20}} \) in the absence and presence of amphotericin B. For convenience sake, these membranes, in the presence of amphotericin B, will be referred to as porous. In order to increase the resistance of the aqueous, but not the membrane phases to tracer diffusion, the relatively impermeable solutes sucrose (14) and dextran were used to alter viscosity.

**TABLE I**

**THE EFFECT OF AQUEOUS PHASE VISCOSITY ON THE PERMEABILITY COEFFICIENT FOR WATER DIFFUSION IN NATIVE LIPID BILAYER MEMBRANES**

| Aqueous phase          | \( \eta \) | \( P_{D_{\text{H}20}} \)     |
|------------------------|------------|-------------------------------|
| 0.01 m NaCl, 0.01 m sucrose | 9.06 | 8.20±0.55 (8)                 |
| 0.01 m NaCl, 1.7% dextran      | 13.30 | 6.45±0.68 (5)                 |
| 0.01 m NaCl, 0.75 m sucrose    | 18.90 | 5.48±0.83 (9)                  |
| 0.01 m NaCl, 3% dextran      | 19.50 | 5.06±0.52 (5)                  |
| 0.01 m NaCl, 0.95 m sucrose    | 24.20 | 4.60±0.45 (7)                  |

The aqueous phase temperature was 26.5°C ± 0.5°C, and the average mol wt of the dextran was 77,500. The values of \( P_{D_{\text{H}20}} \) are expressed as the mean ± standard deviation for the number of observations indicated in parentheses. Each set of experiments was carried out on a minimum of three membranes. The DC membrane resistance was \( \geq 10^8 \) ohm-cm² in all cases. Experimental details are given in Methods.

In agreement with previous studies (14, 15), the values of \( P_{D_{\text{H}20}} \) were approximately twofold greater in the porous than in the native membranes (Tables I and II, 9.06 \( \times \) 10⁻³ poise). Furthermore, in both instances, when the aqueous phase viscosity was increased, \( P_{D_{\text{H}20}} \) was reduced proportionately. It is noteworthy in this connection that the dextran concentrations used in these experiments did not exceed approximately 0.5 \( \times \) 10⁻⁴ M. Consequently, the reductions in \( P_{D_{\text{H}20}} \) were neither uniquely dependent on the solute concentration in the aqueous phases nor related to possible changes in membrane structure attributable to the relatively high osmolality of the sucrose solutions (25). Moreover, since the electrical membrane resistances at the different viscosities were quite similar, it is unlikely that the higher concentrations of
sucrose or dextran used in these and subsequent experiments (16) modified either the interactions of amphotericin B with the membranes, or, presumably, the fractional pore area (14).

Figs. 3 and 4 illustrate, respectively, the data in Tables I and II plotted according to Equation 4. In each instance, the linear regression of $1/P_{DH0}$ on $\eta$ implies that, for these experimental conditions, $\alpha^t$ and $P_{m\eta0}$ remained relatively constant. Thus, these observations permitted an estimate of both the unstirred layer thickness and the contributions of the membranes to the total resistance to water diffusion. The values of $\alpha^t$ were in the same range,

| Aqueous phase | $\eta$ (poise X 10$^3$) | $P_{DH0}$ (cm sec$^{-1}$ X 10$^4$) | $R_m$ (ohm cm X 10$^{-2}$) |
|---------------|-------------------------|----------------------------------|--------------------------|
| 0.01 M NaCl, 0.01 M sucrose | 9.06 | 17.10±2.20 (8) | 0.78±0.35 (8) |
| 0.01 M NaCl, 0.25 M sucrose | 11.10 | 13.99±1.8 (4) | — |
| 0.01 M NaCl, 0.4 M sucrose | 13.32 | 12.14±0.66 (5) | 0.62±0.27 (5) |
| 0.01 M NaCl, 0.6 M sucrose | 16.50 | 10.47 (2) | 0.80 |
| 0.01 M NaCl, 3% dextran | 19.60 | 9.5±1.81 (6) | 0.70±0.30 (6) |
| 0.01 M NaCl, 0.9 M sucrose | 23.30 | 7.22±0.56 (7) | 0.58±0.22 (7) |
| 0.01 M NaCl, 4.25% dextran | 25.08 | 6.72±0.9 (4) | 0.64±0.29 (4) |

The aqueous phases, 26.5°C ± 0.5°C, contained 0.8-1.0 X 10$^{-4}$ M amphotericin B. The DC membrane resistances ($R_m$) and the values of $P_{DH0}$ are expressed as the mean ± standard deviation for the number of observations indicated in parentheses. Experimental details are given in Methods.

approximately 110 X 10$^{-4}$ cm, for both the native (Fig. 3) and the porous (Fig. 4) membranes. However, $P_{m\eta0}$, with respect to $P_{DH0}$ at 9.06 X 10$^{-3}$ poise, was less than twice as great in the native membranes (Table I and Fig. 3) but approximately six times greater in the porous membranes (Table II and Fig. 4).

Table III summarizes the relationship of the diffusional to osmotic water permeability coefficients in these membranes. The values of $P_{DH0}$ (at 9.06 X 10$^{-3}$ poise) and $P_{m\eta0}$ were obtained from Tables I and II and Figs. 1 and 2. The indicated values of $P_f$ were obtained on the same lipid preparation and are similar to previously reported values (14, 15). In particular, the value of $P_f$ was increased 20- to 25-fold in the presence of amphotericin B. Assuming
that $P_{\text{mH}_2\text{O}}$ represents the membrane permeability coefficient for water diffusion, the ratio $P_f: P_{\text{mH}_2\text{O}}$ may be used to evaluate water transport through these membranes during osmosis.

In the native membranes, the $P_f: P_{\text{mH}_2\text{O}}$ ratio was 1.22. Using an alternative approach, Everitt et al. (13) computed a $P_f: P_{\text{mH}_2\text{O}}$ ratio of approximately one, and an unstirred layer thickness of $70 \times 10^{-4}$ cm for unmodified lipid bilayer membranes. Thus, these observations are consistent with the view (12-14) that water traverses such membranes primarily by diffusion.
In contrast, the $P_f : P_{m,0}$ ratio in the presence of amphotericin B was 3.75 (Table III). During osmosis through aqueous channels the relationship among laminar, or Poiseuille, water flux, diffusional water flux, and the equivalent pore radius may be expressed as (7, 8, 14):

$$J_p = \frac{P_f}{P_{m,0}} - 1 = \frac{r^2RT}{8\eta D_{H_2O} V_w},$$

where $J_p$ and $J_d$ represent, respectively, laminar and diffusional water fluxes, $r$ is the equivalent pore radius, and $V_w$ is the partial molar volume of water. For a $P_f : P_{m,0}$ ratio of 3.75, the effective pore radius was approximately 5.6 Å, and laminar water flow accounted for nearly three-fourths of the net water flux through the membranes during osmosis.

### Table III

| Amphotericin B | $P_{D_{H_2O}}$ | $P_{m,0}$ | $P_f$ | $P_f : P_{m,0}$ |
|----------------|---------------|-----------|-------|-----------------|
| $M$            | cm sec$^{-1} \times 10^4$ |           |       |                 |
| 0              | 8.20±0.55 (8) | 13.8      | 16.8±2.8 (6) | 1.22            |
| 0.8-1.0×10$^{-4}$ | 17.10±2.20 (8) | 107.5     | 404.7±59.5 (5) | 3.75            |

The values for $P_{D_{H_2O}}$ at 9.06×10$^{-4}$ poise are from Tables I and II. The values of $P_{m,0}$ are from Figs. 1 and 2. The values of $P_f$, observed with the same lipid preparation at 26.5 °C ± 0.5°C, are expressed as the mean ± standard deviation for the number of observations listed in parentheses. Experimental details are given in Methods.

### The Effect of Aqueous Phase Viscosity on $P_{D_{H_2O}}$

Table IV illustrates the effect of varying aqueous phase viscosity on $P_{D_{H_2O}}$ for urea and glycerol, in porous membranes. In accord with the observations on $P_{D_{H_2O}}$ (Tables I and II), both $P_{D_{urea}}$ and $P_{D_{glycerol}}$ were proportionately reduced when $\eta$ was increased. Moreover, in each instance, the value of $\alpha'$ required to rationalize the differences between the $P_{D_{H_2O}}$ values in terms of Equation 4, assuming that $P_{m,1}$ was constant, was in close agreement with the values for $\alpha'$ computed from the $P_{D_{H_2O}}$ experiments (Figs. 3 and 4). Taken together, these data indicate that 110×10$^{-4}$ cm is a reasonable approximation for the effective thickness of the unstirred layers in series with native or porous lipid bilayer membranes.

Assuming this value, Table V lists the values of $P_{m,1}$, computed from $P_{D_{H_2O}}$ and Equation 4, for three solutes having varying degrees of permeability in the porous membranes. It is evident that there is a considerable discrepancy between the values of $P_{D_{H_2O}}$ and $P_{m,1}$ for the more permeable solutes urea and glycerol, but not for the relatively impermeable solute meso-erythritol. Stated
in another way, these data indicate that, for both urea and glycerol, a substantial fraction of the total resistance to diffusion was referable to the unstirred layers, rather than to the membranes. Specifically, from the ratio, $P_{Di}:P_{m}$ (Tables III and V), the porous membranes accounted for 16, 25,
the contributions of the membranes to the observed resistances to the diffusion of water and solutes between aqueous phases. First, in order to assess the mode of water transport through the membranes during osmosis, both in the absence and presence of amphotericin B, $P_f$ and $P_{m_{n0}}$ were evaluated independently. Second, it is possible that the properties of the unstirred layers were determined, at least in part, by the effects of the surface monolayers of the lipid membranes on the state of water in vicinal aqueous lamellae (cf. below). Accordingly, $\alpha'$ was evaluated when the aqueous phases were in series with these membranes, rather than other materials (13).

The results indicate that increments in aqueous viscosity, with either sucrose or dextran, affected primarily the resistance of the unstirred layers, rather than the membranes, to the diffusion of water (Equation 4, Figs. 3 and 4) and, by inference, nonelectrolytes (Table IV). In the present experiments, $\alpha'$ was approximately $110 \times 10^{-4}$ cm, both in the absence (Table I and Fig. 3) and presence (Tables II, IV and Fig. 4) of amphotericin B. Moreover, a comparison of $P_f$ and $P_{m_{n0}}$ implies that quasilaminar osmotic water flow occurred in membranes exposed to appropriate concentrations of amphotericin B (Table III). These observations are consistent with the view that the interactions of amphotericin B with such membranes result in pore formation (14). However, the effective pore radius computed in the present experiments, approximately 5.6 Å (Table III), is smaller than the values derived previously, approximately 7–10.5 Å, from an analysis of both $P_D$ and reflection coefficient measurements (14). In this regard, Tables III and V indicate the considerable disparities which may exist between $P_{m'}$ and $P_{D'}$ values for permeable substances as a result of unstirred layers. Similar considerations suggest that unstirred layers may also result in erroneously low reflection coefficients for more permeable solutes (16, 24, 28, 29).

The variation of $P_D$ with aqueous phase viscosity, described previously (17) and in the present studies, has also been utilized by Holz and Finkelstein (24), who computed a value of $170 \times 10^{-4}$ cm for the thickness of unstirred layers in series with similar porous membranes and corrected their observed $P_{D'}$ values in terms of Equation 1. Accordingly, it is relevant to compare the results of our studies with those obtained by Holz and Finkelstein.

The present experiments were carried out on porous membranes having electrical resistances in the range of 0.58 to $0.85 \times 10^2$ ohm-cm², when the aqueous phases contained 0.01 M NaCl (Fig. 2 and Tables II, IV, and V). However, the DC resistance of such membranes in the presence of polyene antibiotics is a linear function, with a slope of unity, of the aqueous salt concentration (18). Accordingly, since the results of Holz and Finkelstein were normalized for membrane resistances of $10^2$ ohm-cm² in 0.1 M NaCl (24), their data may be multiplied 15-fold (i.e., normalized to approximately $0.66 \times 10^4$ in 0.01 M NaCl) for comparison with the present experiments. In this context, the $P_{m_{n0}}$ value of $6.0 \times 10^{-4}$ cm sec⁻¹ (at $10^2$ ohm-cm² in
0.1 M NaCl) obtained by these workers for amphotericin B–treated membranes becomes \( \approx 90 \times 10^{-4} \) cm sec\(^{-1}\) (normalized to 0.66 \( \times 10^{-3} \) ohm-cm\(^2\) in 0.01 M NaCl); i.e., in reasonable accord with the results of the present experiments (Tables II, III and Fig. 2). However, the \( P_f: P_{m_o} \) ratio of 3 reported by these workers is slightly less than the value of 3.75 obtained in the present experiments (Table III). Similarly, the \( P_{m_i}: P_{m_o} \) ratios of Holz and Finkelstein (24), for both urea and glycerol, were lower than those obtained by us (Tables III and IV). It is noteworthy in this respect that the amphotericin B–dependent pore radius computed by us was approximately 5.6 Å (Equation 5) while that indicated by Holz and Finkelstein was 4.0 Å. Thus, it is possible that the effective pore radius in lipid bilayer membranes exposed to amphotericin B may vary inversely with the electrical membrane resistance. Hopefully, studies currently in progress in the laboratory may provide additional information concerning this issue.

Considerable experimental evidence has now been accumulated which indicates that unstirred layers may modify, at a minimum, diffusion processes in a number of systems other than lipid bilayer membranes (12–14), including cellulose membranes (10), plant cells (5, 25, 29, 30), and epithelial tissues (9, 11, 31). Similarly, it has been suggested that cytoplasmic diffusion may retard significantly the dissipative movement of water and solutes into certain cells (32, 33). Furthermore, Colton has suggested that the major fraction of the total resistance to diffusion of solutes from blood to bathing medium, during hemodialysis, may be referable to unstirred layers in the blood phase (34). Thus, phenomena relating to unstirred layers have clinical, as well as conceptual, significance. Accordingly, it is relevant to consider certain physical factors which might be responsible for unstirred layers.

In a real sense, it is improbable that a continuous aqueous phase could include a well-stirred aqueous solution demarcated by a discrete boundary from an adjacent, entirely stationary layer. Thus, a number of reports have shown that the magnitude of the unstirred layers in series with membranes is inversely related to the rate of stirring in bulk phases (9–12). In this connection, Kaufmann and Leonard (35) indicated, in terms of fluid mechanics, that the interfacial resistance to diffusion in the aqueous phase adjacent to a membrane includes a region in which convective flow may occur but diminishes progressively, approaching zero at the membrane interface. Expressed in this context, \( \alpha^i \) in Equation 4 is an operational term for lamellae which may be partially as well as completely diffusion–limited. In the case of lipid bilayer membranes, Cass and Finkelstein suggested that convective stirring of aqueous phases in the regions near the membranes may be limited by the thickness of the diaphragm on which the membranes are formed (12). It is noteworthy, in the present experiments, that the value of \( \alpha^i \approx 110 \times 10^{-4} \) cm, approximated the thickness of the polyethylene diaphragms, 125 \( \times 10^{-4} \) cm.

Finally, it is evident that a complete understanding of phenomena related
to unstirred layers depends on detailed information concerning the state of water in such phases. The fact that the frictional resistances for diffusion between water and water (Tables I and II and Figs. 3 and 4) or water and solute (Table IV) in the unstirred layer were altered in direct relationship to bulk aqueous viscosity does not indicate that the magnitude of these resistances in the bulk and unstirred phases was the same. Thus, these observations do not exclude the possibility that hydrophobic interactions (36–38) between the membrane interfaces and adjacent layers of water reduced considerably the values of $D_t$ in these regions. In that case, the actual values of $\alpha^t$ would be proportionately less than those computed in the present experiments (cf. Equation 4). Clearly, an answer to this question requires additional studies.

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