Chloride Transport in Porous Lipid Bilayer Membranes

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ABSTRACT This paper describes dissipative Cl⁻ transport in "porous" lipid bilayer membranes, i.e., cholesterol-containing membranes exposed to 1-3 × 10⁻⁷ M amphotericin B. The diffusional permeability coefficient for Cl⁻, estimated from unidirectional ⁸⁶Cl⁻ fluxes at zero volume flow, varied linearly with the membrane conductance (Gm, Ω⁻¹·cm⁻²) when the contributions of unstirred layers to the resistance to tracer diffusion were relatively small with respect to the membranes; in 0.05 M NaCl, P_DCI was 1.36 × 10⁻⁴ cm·s⁻¹ when Gm was 0.02 Ω⁻¹·cm⁻². Net chloride fluxes were measured either in the presence of imposed concentration gradients or electrical potential differences. Under both sets of conditions: the values of P_DCI computed from zero volume flow experiments described net chloride fluxes; the net chloride fluxes accounted for ~90-95% of the membrane current density; and, the chloride flux ratio conformed to the Ussing independence relationship. Thus, it is likely that Cl⁻ traversed aqueous pores in these anion-permselective membranes via a simple diffusion process. The zero current membrane potentials measured when the aqueous phases contained asymmetrical NaCl solutions could be expressed in terms of the Goldman-Hodgkin-Katz constant field equation, assuming that the P_DNa/P_DCl ratio was 0.05. In symmetrical salt solutions, the current-voltage properties of these membranes were linear; in asymmetrical NaCl solutions, the membranes exhibited electrical rectification consistent with constant-field theory. It seems likely that the space charge density in these porous membranes is sufficiently low that the potential gradient within the membranes is approximately linear; and, that the pores are not electrically neutral, presumably because the Debye length within the membrane phase approximates the membrane thickness.

INTRODUCTION

Lipid bilayer membranes formed from a variety of lipid preparations, including sheep red blood cell lipids dissolved in decane (1), have electrical resistances in the vicinity of 10⁸ Ω·cm². Presumably, these high membrane resistances are referable to the electrical properties of the surface monolayers, which are largely independent of the thickness of the lipid medium which separates them (2).
Pagano and Thompson have compared isotopic and electrical measurements of ion permeability in spherical lipid bilayer membranes formed in NaCl solutions (3). These workers noted that there was reasonable agreement between the observed unidirectional fluxes of $^{22}\text{Na}^+$ at zero volume flow and the fluxes predicted for simple ionic diffusion from electrical measurements, including membrane conductances and ionic transference numbers. In contrast, comparable unidirectional $^{36}\text{Cl}^-$ fluxes exhibited saturation kinetics and were several orders of magnitude greater than the values expected from electrical data, assuming simple ionic diffusion. Accordingly, Pagano and Thompson suggested that Cl$^-$ transport across those membranes was mediated by an exchange diffusion process (3). Discrepancies between tracer flux experiments and electrical measurements in bilayer membranes have also been noted in other instances. In lipid bilayer membranes exposed to valinomycin, the transference number for K$^+$ ($t_K$), estimated from zero current potential measurements, was unity (4). However, voltage-dependent $^{42}\text{K}^+$ fluxes on identical membranes exposed to O$_2$ and equal concentrations of KCl indicated that $t_K$ was approximately 0.4 (5). On the basis of such observations, it is evident that detailed descriptions of ionic transport processes in lipid bilayer membranes require both electrical and isotopic flux measurements.

There is reasonable experimental evidence from this (6) and other laboratories (7) which supports the hypothesis that the interaction of polyene antibiotics such as amphotericin B with lipid bilayer membranes containing appropriate sterols (8, 9) results in the formation of aqueous pores. The electrical conductances of such porous membranes are considerably greater than those of unmodified bilayer membranes (8). Furthermore, ionic transference numbers estimated from electrical measurements have indicated that the porous bilayer membranes are approximately 10 times more permeable to anions (e.g. Cl$^-$) than to cations (e.g. Na$^+$, K$^+$) (8).

The purpose of the present experiments was to evaluate the relationship between $^{36}\text{Cl}^-$ fluxes, membrane currents, and membrane conductances in porous bilayer membranes. The results are consistent with the view that Cl$^-$ transport through pores in these membranes is a simple diffusion process which conforms to the Ussing flux ratio relationship (10). Moreover, the current-voltage properties of these membranes may be described by the Goldman constant-field equation (11, 12), if it is assumed that the membrane thickness approximates the effective Debye length within the membrane, so that microscopic electroneutrality does not obtain.

**METHODS**

The experimental procedures have been presented in detail previously (6, 13). Lipid bilayer membranes separating two aqueous phases were formed on a 2.0 mm diameter
aperture in a polyethylene diaphragm separating two aqueous chambers, front and rear (6). The lipid solutions used to form membranes contained equimolar amounts of high-potassium sheep red cell phospholipids and cholesterol dissolved in decane to a total lipid concentration in the range 25–30 mg/ml (8, 9). The pH of the unbuffered aqueous phases was 5.8–6.0 and the aqueous temperature was 26.5 ± 0.5°C. Unless otherwise indicated, the aqueous phases contained amphotericin B, 1–3 × 10⁻⁷ M. In this paper, lipid bilayer membranes exposed to these amphotericin B concentrations will be termed porous.

The electrical circuit used to measure membrane currents and potentials was identical to one described previously (13). The differential circuit was arranged to monitor the voltage in the rear chamber with respect to that in the front chamber. 

³⁶Cl⁻ fluxes were carried out for 15- to 30-min periods using techniques identical to those described previously for water and nonelectrolyte tracer fluxes (6, 13). The ³⁶Cl⁻ concentration in the hot chamber was, at a minimum, 10⁶ times greater than in the cold chamber throughout all flux periods. The ³⁶Cl⁻ concentration in the cold chamber was zero at the start of a flux period and negligible, during any flux period, with respect to the tracer concentration in the hot chamber, which was very nearly constant. The ³⁶Cl⁻ fluxes, in a given membrane, were proportional to the tracer concentration in the hot chamber, and linearly related to time. Accordingly, \( P_{D_{Cl}} \) (cm²s⁻¹), the diffusion permeability coefficient for Cl⁻ at zero volume flow (Figs. 1–3), was computed from (6):

\[
P_{D_{Cl}} = \frac{[^{36}\text{Cl}^-]_c V_c}{[^{36}\text{Cl}^-]_h Am \Delta t}
\]

where \([^{36}\text{Cl}^-]_c\) and \([^{36}\text{Cl}^-]_h\) are, respectively, the ³⁶Cl⁻ concentrations (cpm·ml⁻¹) in the cold and hot chambers, \(V_c\) is the volume of the cold chamber (cm³), \(A_m\) is the membrane area (cm²), and \(\Delta t\) is the duration of the flux period (seconds). Similarly, unidirectional chloride fluxes \(J_{Cl}|_{eq} \cdot s^{-1} \cdot cm^{-2}\) in the presence of imposed NaCl concentration gradients (Figs. 7 and 8) or electrical gradients (Figs. 9 and 10) were computed from the expression:

\[
J_{Cl} = \frac{[^{36}\text{Cl}^-]_c V_c}{X_{[^{36}\text{Cl}^-]} Am \Delta t},
\]

where \(J_{Cl}\) is the chloride flux from the hot to the cold chamber, \([^{36}\text{Cl}^-]_c\) and \(V_c\) are, respectively, the ³⁶Cl⁻ concentration and volume in the cold chamber and \(X_{[^{36}\text{Cl}^-]}\) is the specific activity (cpm·eq⁻¹) in the hot chamber.

Amphotericin B was kindly provided by the Squibb Institute for Medical Research (New Brunswick, N. J.). ³⁶Cl⁻ was obtained from New England Nuclear Corp. (Boston, Mass.).

RESULTS

Unidirectional ³⁶Cl⁻ Fluxes at Zero Volume Flow

In unmodified spherical lipid bilayer membranes, Pagano and Thompson noted that unidirectional ³⁶Cl⁻ fluxes at zero volume flow exhibited satura-
tion kinetics; the maximum $^{36}$Cl$^{-}$ flux occurred when both aqueous solutions contained 0.1 M NaCl (3). In porous lipid bilayer membranes, the diffusional permeability coefficients for water and nonelectrolytes were related linearly to the DC membrane conductances when the contributions of aqueous unstirred layers to the total resistance to tracer diffusion were relatively small (7, 13). The present $^{36}$Cl$^{-}$ fluxes at zero volume flow were carried out at different NaCl concentrations over a relatively wide range of membrane conductances ($G_m$, Ω$^{-1}$·cm$^{-2}$). This range was obtained by varying the aqueous amphotericin B concentration between 1.2 and 2.8 × 10$^{-7}$ M (Figs. 1 and 2).

Figs. 1 and 2 illustrate the relationship between $P_{DCl}$ and $G_m$ for porous bilayer membranes exposed to 0.05 M NaCl (Fig. 1) and 0.15 M NaCl (Fig. 2). In this regard, it should be noted that the electrical conductances of identical membranes in the absence of amphotericin B are in the vicinity of 10$^{-8}$ Ω$^{-1}$·cm$^{-2}$ (1, 8). Furthermore, the apparent $P_{DCl}$ values computed from exchange diffusion $^{36}$Cl$^{-}$ fluxes by Pagano and Thompson, at $G_m$ values of approximately 1.5 × 10$^{-8}$ Ω$^{-1}$·cm$^{-2}$, varied from 10$^{-10}$ to 10$^{-7}$ cm·s$^{-1}$ (3). In contrast, the respective values of $P_{DCl}$ and $G_m$ in Figs. 1 and 2 exceeded, at a minimum, 10$^{-4}$ cm·s$^{-1}$ and 10$^{-4}$ Ω$^{-1}$·cm$^{-2}$. Thus, since the magnitudes of the electrical conductances and the Cl$^{-}$ fluxes in unmodified bilayer membranes are negligible with respect to comparable observations in the present experiments, the origins of Figs. 1 and 2 were taken to be zero.

$P_{DCl}$ increased linearly with membrane conductance when $G_m$ was less than 0.04 Ω$^{-1}$·cm$^{-2}$ (0.05 M NaCl, Fig. 1) or 0.2 Ω$^{-1}$·cm$^{-2}$ (0.15 M NaCl, Fig. 2); at higher membrane conductances, the relationship between $P_{DCl}$ and $G_m$ became nonlinear. If it is assumed that the electrical conductances provided an
index to the number of membrane pore sites, several points are noteworthy. First, the DC conductance of such a porous membrane, at a given concentration of polyene antibiotic, is a linear function of the aqueous phase salt activity (8); moreover, the slope of this relationship is unity (8). The molar activities of 0.05 M NaCl and 0.15 M NaCl are, respectively, 0.041 and 0.113 (14). Accordingly, for a simple diffusion process, the slope of \( P_{DC1} \) with \( G_m \) should be 2.8 times greater in 0.05 M NaCl than in 0.15 M NaCl. In accord with this calculation, when \( G_m \) was 0.02 \( \Omega^{-1} \cdot \text{cm}^{-2} \) the values of \( P_{DC1} \) were \( 1.36 \times 10^{-4} \text{ cm} \cdot \text{s}^{-1} \) (0.05 M NaCl, Fig. 1) and \( 0.45 \times 10^{-4} \text{ cm} \cdot \text{s}^{-1} \) (0.15 M NaCl, Fig. 2). Stated in another way, the slopes of the linear portions of Figs. 1 and 2 could be made nearly identical by dividing the \( G_m \) values in Fig. 2 by 2.8, i.e., by normalizing these conductances for an aqueous phase of 0.05 M NaCl.

Second, it seemed likely that, at relatively high membrane conductances, the constraints imposed by unstirred layers on tracer diffusion became increasingly significant (7, 13). To test this possibility, the data in Figs. 1 and 2 were plotted in reciprocal fashion, shown in Fig. 3 (the conductance values from Fig. 2 were normalized for an aqueous phase of 0.05 M NaCl as indicated above). According to this view the resistance to Cl\(^-\) diffusion at infinitely high membrane conductances (i.e. the zero intercept in Fig. 3) should be determined by the aqueous unstirred layers, and may be expressed as:

\[
R_{DC1} = \frac{\alpha}{D_{Cl}^*},
\]

where \( R_{DC1} \) (s\( \cdot \)cm\(^{-1} \)) = \( 1/P_{DC1} \), \( \alpha \) is the unstirred layer thickness (cm) and \( D_{Cl}^* \) is the free diffusion coefficient for Cl\(^-\). In earlier experiments under identical conditions, the unstirred layer thickness in series with these membranes...
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In 0.05 M NaCl

0.15 M NaCl

\[ R_{m, 0.05 \text{ M NaCl}} = 680 \, \text{s cm}^{-1} \]

**FIGURE 3.** The relationship between \( 1/P_{\text{Cl}^-} \) (\( R_{\text{Cl}^-} \, \text{s cm}^{-1} \)) and \( 1/G_{\text{m}} \) (\( R_{m, \Omega \cdot \text{cm}^2} \)). The values in 0.05 M NaCl were obtained directly from Fig. 1. The values in 0.15 M NaCl, obtained from Fig. 2, were plotted by normalizing the electrical resistances for an aqueous phase of 0.05 M NaCl as indicated in the text. The line was determined from a least-squares regression of the data (correlation coefficient = 0.92). From an analysis of variance, the standard deviations of the slope and intercept were, respectively, 148.0 ± 5.5 \( \Omega^{-1} \cdot \text{cm}^{-2} \) and 680 ± 490 \( \text{s cm}^{-1} \).

was approximately \( 110 \times 10^{-4} \text{ cm} \) (13). Thus, for \( D_{\text{Cl}^-} \approx 1.5 \times 10^{-4} \text{ cm}^2 \cdot \text{s}^{-1} \) (15), the diffusional resistance of the unstirred layers should be \( \approx 730 \, \text{s cm}^{-1} \), in reasonable agreement with 680 ± 490 (SD) \( \text{s cm}^{-1} \), the zero intercept observed in Fig. 3. These observations are consistent with the view that, for the linear ranges of \( P_{\text{Cl}^-} \) with \( G_{\text{m}} \) in Figs. 1 and 2, the contributions of unstirred layers to the resistance to tracer diffusion, with respect to the membranes, were relatively small. Accordingly, the net \( \text{Cl}^- \) flux experiments (Figs. 4–10) were carried out at membrane conductances which, when normalized (as described above) for aqueous phases of 0.05 M NaCl or 0.15 M NaCl, were within the linear ranges of Figs. 1 and 2.

Finally, it is relevant to estimate from these experiments the contribution of \( G_{\text{Cl}^-} \), the \( \text{Cl}^- \) conductance, to \( G_{\text{m}} \), the total membrane conductance. Following Hodgkin (16), the relationship between \( G_{i} \) (\( \Omega^{-1} \cdot \text{cm}^{-1} \)), the conductance of the \( i \)th ion, and \( J_{i} \) (eq \cdot s\(^{-1} \cdot \text{cm}^{-2} \)), the unidirectional flux at zero volume flow of the \( i \)th ion, an independently diffusing species, is:

\[
G_{i} = \frac{F}{RT} Z_{i}^{2} J_{i}, \quad (3)
\]

where \( Z_{i} = \) valence, \( F = \) Faraday's number, \( R = \) gas constant, and \( T = \text{°K} \).

The experiments in Figs. 1 and 2 were carried out at zero volume flow. Assuming that \( \text{Cl}^- \) traversed these membranes as an independently diffusing
species, \( J_{Cl} \) may be predicted from:

\[
J_{Cl} = P_{DCl} f_{Cl} [Cl^-],
\]

where \([Cl^-]\) is the aqueous phase concentration and \(f_{Cl}\) the activity coefficient. Thus:

\[
G_{Cl} = \frac{F^2}{RT} \xi_1 P_{DCl} f_{Cl} [Cl^-],
\]

where \(G_{Cl}\) is the chloride conductance. Table I lists the observed values of \(P_{DCl}\) at \(Gm = 0.02 \Omega^{-1} \cdot \text{cm}^{-2}\) obtained from the linear portions of Figs. 1 and 2, and the values of \(G_{Cl}\) computed from Eq. 5. These observations indicate clearly that the computed Cl\(^-\) conductance accounted for, at a minimum, 95\% of the membrane conductance under these conditions. Furthermore, the data show that, at zero volume flow, Cl\(^-\) traversed pores in these membranes as an independently diffusing species, rather than by an exchange diffusion process.

**Asymmetrical NaCl Solutions: Current and Potential Measurements**

In earlier experiments, zero current electrical potentials measured in the presence of asymmetrical salt solutions indicated that these porous membranes were approximately 10 times more permeable to Cl\(^-\) than to Na\(^+\) or K\(^+\) (8, 9). Accordingly, we evaluated the relationships among membrane conductance, \(P_{DCl}\), zero current membrane potentials, and zero voltage currents (i.e., short-circuit currents) when the membranes were exposed to asymmetrical NaCl solutions. The results are shown in Figs. 4–6.

Each set of experiments was carried out in the following manner. The membrane conductance was first measured in 0.01 M NaCl. Subsequently, the NaCl solutions indicated in Figs. 4–6 were introduced into the front and rear chambers. The zero current membrane potentials (\(V_m\), Figs. 4–6) and zero voltage currents (\(J_{ion}\), Figs. 4–6) were measured under these conditions. In

| NaCl | \(P_{DCl}\) | \(Gm\) | \(G_{Cl}\) |
|------|-------------|--------|--------|
| 0.05 | 1.36 \(\times 10^4\) | 0.02 | 0.021 |
| 0.15 | 0.45 | 0.02 | 0.019 |

The values of \(P_{DCl}\) and \(Gm\), at the indicated NaCl concentrations, were obtained from the linear slopes of Figs. 1 and 2. \(G_{Cl}\) was computed according to Eq. 5 from the values of \(P_{DCl}\).
The relationship between membrane conductance ($G_m$, measured when the front and rear chambers contained 0.01 M NaCl) and the zero current potentials ($V_m$) and zero voltage currents ($J_{ele}$) measured when the front and rear solutions were those indicated in the figure. The solid line relating $J_{ele}$ and $G_m$ was drawn from a regression analysis of the data (correlation coefficient = 0.97). The dotted line was drawn from Eq. 6 as indicated in the text. The solid line in the upper panel is the mean value of $V_m$. The aqueous phases contained $1-3 \times 10^{-7}$ amphotericin B. Experimental details are given in the text.

The experiments shown in Fig. 6, 0.25 M sucrose, whose reflection coefficient, like that of NaCl, is unity in these membranes (6), was added to the front chamber to minimize osmotic water flow. Finally, 0.01 M NaCl was reintroduced into the front and rear chambers, and the membrane conductance was measured again. The values of $G_m$ shown in Figs. 4–6 are the means of $G_m$ measurements in 0.01 M NaCl before and after the corresponding measure-
The relationship between $G_m$, $J_{\text{elec}}$, and $V_m$ was plotted as in Fig. 4. $G_m$ was measured in 0.01 M NaCl and $J_{\text{elec}}$ and $V_m$ when rear and front chambers contained the indicated NaCl solutions. Correlation coefficient for the line relating $G_m$ and $J_{\text{elec}}$ was 0.93. Amphotericin B = 1.0–2.8 × 10⁻⁷ M.

The solid lines shown in Figs. 4–6 were derived from regression analyses of the data. It is evident that, within experimental error, $J_{\text{elec}}$ varied linearly with $G_m$ for all three concentration ratios of NaCl. The corresponding values of $V_m$ were relatively constant over the conductance range shown in Figs. 4–6. In agreement with earlier observations (8, 9), the sign of $V_m$ (the more concentrated NaCl solutions were positive) indicated Cl⁻ permselectivity, and the ratio of $V_m$ to $E_{\text{Cl}}$, the Cl⁻ equilibrium potential, varied with the activity ratio of NaCl in the two aqueous phases (cf. Figs. 11 and 12).

For a simple diffusion process limited primarily by the membranes rather than unstirred layers, the net flux of chloride from rear to front chamber ($J_{\text{Cl}}$, eq·s⁻¹·cm⁻²) at zero membrane potential may be predicted from the Fick equation:

$$J_{\text{Cl}} = -P_{\text{Cl}}(a_{\text{NaCl}} - a'_{\text{NaCl}}).$$

where $a_{\text{NaCl}}$ and $a'_{\text{NaCl}}$ are the NaCl activities in the front and rear chamber.

In these porous membranes, $G_m$ is a linear function with unity slope of the aqueous NaCl concentration (8), and the ratio of the slope of $P_{\text{Cl}}$ with $G_m$ in 0.05 M NaCl (Fig. 1) and 0.15 M NaCl (Fig. 2) was the same, within experimental error, as the ratio of the molar NaCl activities (cf. above). Accordingly, it seemed reasonable to assume that the values of $P_{\text{Cl}}$ in Fig. 1 could be normalized in the same manner for membrane conductances in 0.01 M NaCl,
i.e., by dividing the $G_m$ values in Fig. 1 by 4.55, the activity ratio for 0.05 M NaCl/0.01 M NaCl (14). The dotted lines in Figs. 4–6 were drawn from Eq. 6 and such normalized values of $P_{DCI}$, predicted from the linear slopes of Fig. 1 for the conductances shown in Figs. 4–6. In this connection, it should be noted that the membrane conductances shown in Figs. 4–6, when normalized for NaCl concentrations of 0.05 or 0.15 M, were within the linear range of Figs. 1 and 2. It is clear that the net Cl$^-$ fluxes predicted from Eq. 6 were nearly identical to the observed zero voltage currents. These observations indicate that simple Cl$^-$ diffusion could account, within experimental error, for the observed values of $J_{elec}$.

**Relationship Between Net Cl$^-$ Flux and Electrical Flux**

In order to evaluate directly the contribution of net Cl$^-$ flux to current density, two sets of experiments were performed. First, unidirectional tagged Cl$^-$ fluxes and zero voltage currents were measured simultaneously when the aqueous phases contained unequal NaCl concentrations (Figs. 7 and 8). The experimental conditions for the Cl$^-$ fluxes shown in Figs. 7 and 8, including, specifically, the range of values for $J_{elec}$, were identical, respectively, to those in Figs. 5 and 6. Second, unidirectional tagged Cl$^-$ fluxes and membrane currents were measured simultaneously when the NaCl concentrations in the front and rear chambers were identical and an external electrical potential was imposed (Figs. 9 and 10).

**Figure 7.** The relationship between Cl$^-$ fluxes ($J_{Cl}$) and zero voltage current ($J_{elec}$). The experimental conditions were identical to those in Fig. 5. $J_{Cl}$ was computed from Eq. 2 (Methods). Net $J_{Cl}$, rear to front, (dotted line) was the difference between the two unidirectional Cl$^-$ fluxes. $J_{elec}$ was monitored for the duration of the flux period; the data shown represent mean values. Only those fluxes in which $J_{elec}$ varied by less than 30% during the flux period were reported.
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Figure 8. The relationship between Cl⁻ fluxes (J_{CI}) and zero voltage current (J_{elec}).

The conditions were identical to those in Fig. 6. J_{CI} and J_{elec} were measured as in Fig. 7. The results were expressed as in Fig. 7. The solid line for rear to front unidirectional Cl⁻ flux was computed from a regression analysis of the data (correlation coefficient = 0.91).

The results of previous experiments (8, 9) and the present experiments (Table I; Figs. 4–6) indicate that these porous membranes are significantly, but not exclusively, anion permselective. In agreement with these observations, the net Cl⁻ fluxes in the presence of imposed NaCl concentration gradients exceeded slightly the zero voltage currents (Figs. 7 and 8), presumably because a portion of the Cl⁻ flux through the membranes was accompanied by Na⁺ flux. Thus, for the two different NaCl concentration ratios, the slope of the relationship between net Cl⁻ flux (Figs. 7 and 8; dotted lines, the difference between rear to front and front to rear Cl⁻ fluxes) and zero voltage current (J_{elec}) was 1.07 (Fig. 7) and 1.1 (Fig. 8). In other words, the data indicate that, for these two NaCl concentration ratios, net Cl⁻ flux accounted for approximately 92% of the zero voltage current. Furthermore, a comparison of the net Cl⁻ fluxes shown in Figs. 7 and 8 with, respectively, the Cl⁻ fluxes in Figs. 5 and 6 predicted from Eq. 6 indicates that the values of P_{ncl} measured at zero volume flow (Fig. 1) described the zero voltage net Cl⁻ flux in the presence of imposed concentration gradients.

The Cl⁻ fluxes measured when the aqueous phases contained 0.15 M NaCl and the magnitude of the imposed voltage was either 25 or 86 mV are shown in, respectively, Figs. 9 and 10. The membrane conductances for these experiments, computed from Ohm's law, the imposed voltages, and the observed values for J_{elec} were within the linear range of Fig. 2. In both instances, the slope of the relation between net Cl⁻ flux (Figs. 9 and 10; dotted lines) and J_{elec} was approximately 0.92, i.e., Cl⁻ accounted for ~93% of the membrane current density.
Figure 9. Voltage-dependent Cl⁻ fluxes. The aqueous solutions contained 0.15 M NaCl and 1–2.8 × 10⁻⁷ M amphotericin B. Unidirectional Cl⁻ fluxes from rear to front chamber (JC₉) were measured according to Eq. 2 when potentials of either +25 or −25 mV were applied to the rear chamber. The solid lines were drawn from regression analyses of the data (correlation coefficients = 0.91 and 0.93). Net Cl⁻ flux was computed from the difference between unidirectional fluxes. Jₑlec was monitored for the duration of the flux period. Only those fluxes in which Jₑlec varied by less than 25% during the flux period were reported.

Figure 10. Voltage-dependent Cl⁻ fluxes. The conditions were identical to Fig. 9, except that +86 or −86 mV was the potential applied to the rear chamber. The solid line for −86 mV was computed from regression analysis of the data (correlation coefficient = 0.92). Net Cl⁻ flux was computed from the difference between unidirectional fluxes.
Values of \( P_{\text{DCl}} \) from these experiments may be computed from constant-field theory (11, 12):

\[
P_{\text{DCl}} = \text{net } J_{\text{Cl}} \frac{RT}{Z_{\text{Cl}} V_m P} \left[ 1 - \exp \left( \frac{Z_{\text{Cl}} F V_m}{RT} \right) \right]^\frac{-1}{2} \left( \frac{Z_{\text{Cl}} F V_m}{RT} \right) \]

(7)

In Fig. 9, \( G_m \) and net \( J_{\text{Cl}} \) were, respectively, 0.0154 \( \Omega^{-1} \cdot \text{cm}^{-2} \) and \( 3.8 \times 10^{-9} \) eq \( \cdot \text{s}^{-1} \cdot \text{cm}^{-2} \) when \( J_{\text{ele}} \) was \( 4 \times 10^{-6} \) eq \( \cdot \text{s}^{-1} \cdot \text{cm}^{-2} \). \( P_{\text{DCl}} \) computed at this conductance from Eq. 7, was \( 0.34 \times 10^{-4} \) cm \( \cdot \text{s}^{-1} \); the experimental value of \( P_{\text{DCl}} \) from the linear slope of Fig. 2 was \( 0.35 \times 10^{-4} \) cm \( \cdot \text{s}^{-1} \) at 0.0154 \( \Omega^{-1} \cdot \text{cm}^{-2} \). Similarly, in Fig. 10, \( G_m \) and net \( J_{\text{Cl}} \) were, respectively, 0.0112 \( \Omega^{-1} \cdot \text{cm}^{-2} \) and \( 9.3 \times 10^{-9} \) eq \( \cdot \text{s}^{-1} \cdot \text{cm}^{-2} \), when \( J_{\text{ele}} \) was \( 10^{-8} \) eq \( \cdot \text{s}^{-1} \cdot \text{cm}^{-2} \). The value of \( P_{\text{DCl}} \) at this conductance from Eq. 7 was \( 0.26 \times 10^{-4} \) cm \( \cdot \text{s}^{-1} \); the experimental value of \( P_{\text{DCl}} \), from the slope of the linear part of Fig. 2, was \( 0.25 \times 10^{-4} \) cm \( \cdot \text{s}^{-1} \) at 0.0112 \( \Omega^{-1} \cdot \text{cm}^{-2} \). Thus, it is evident that \( P_{\text{DCl}} \) measured at zero volume flow described net Cl\(^{-}\) flux for symmetrical salt solutions in the presence of electrical potential gradients.

The nature of net Cl\(^{-}\) flux through the membranes may also be evaluated from the data in Figs. 7–10. Thus, for an independently diffusing species in the absence of significant water flow, the flux ratio may be expressed by the Ussing independence relationship (10):

\[
\frac{J_{\text{Cl}}^\text{fr}}{J_{\text{Cl}}^\text{fr}} = \frac{\delta_{\text{NaCl}}}{\delta_{\text{NaCl}}} \exp \left( \frac{Z_{\text{Cl}} F V_m}{RT} \right)
\]

(8)

where \( J_{\text{Cl}}^\text{fr} \) and \( J_{\text{Cl}}^\text{fr} \) are, respectively, the Cl\(^{-}\) fluxes from rear to front and front to rear chambers. Table II lists the values for the observed flux ratios (Figs.

| TABLE II | THE Cl\(^{-}\) FLUX RATIO IN POROUS LIPID BILAYER MEMBRANES |
|----------|----------------------------------------------------------|
| Front    | Rear          | \( \delta_{\text{Cl}} \) | \( J_{\text{Cl}}^\text{fr}/J_{\text{Cl}}^\text{fr} \) | Observed | Predicted |
| M        | \( \text{mV} \) | \( \delta_{\text{NaCl}} \) |                                                              |          |           |
| 0.1      | 0.015         | 0                        | 5.62                                                          | 5.85     |
| 0.15     | 0.004 M NaCl  | 0                        | 26.0                                                          | 30.3     |
| 0.25 M sucrose |          | 25                       | 2.5                                                           | 2.63     |
| 0.15     | 0.15          | 86                       | 27.3                                                          | 28.0     |

The observed flux ratios for the indicated conditions were obtained from the ratios of the slopes of the unidirectional Cl\(^{-}\) fluxes shown in Figs. 7–10. The predicted flux ratios were computed from Eq. 8.
7–10) and those predicted from Eq. 8. The observed flux ratios from the data in Figs. 7, 9, and 10 were in close agreement with the predicted values, and the flux ratio from the data in Fig. 8 was approximately 12% less than predicted. The nature of the latter discrepancy is indeterminate and may reflect experimental error. However, taken together, these data indicate that, to a reasonable approximation, Cl⁻ traversed these membranes as an independently diffusing species in the presence of either imposed concentration (Figs. 7 and 8) or electrical potential (Figs. 9 and 10) gradients.

Asymmetrical NaCl Solutions: Zero Current Potentials

Two different expressions may describe the zero current potentials observed when these membranes are exposed to asymmetrical NaCl solutions. Thus, assuming that Na⁺ and Cl⁻ are the sole current-carrying species, a modified form (17) of the Nernst equation is:

\[ V_m = t_{Na} E_{Na} + t_{Cl} E_{Cl} \]  

where \( E_n \), the equilibrium potential is:

\[ E_{Na} = -E_{Cl} = \frac{RT}{F} 2.3 \log \frac{d_{NaCl}}{d_{Na}}, \]  

and the ionic transference numbers (i.e. the ratio of an ionic conductance to membrane conductance) are related by:

\[ 1 = t_{Na} + t_{Cl}. \]  

By rearranging Eqs 8–10, we have:

\[ V_m = (2 t_{Cl} - 1) E_{Cl}. \]  

Alternatively, \( V_m \) may be expressed in terms of the constant-field equation (11, 12):

\[ V_m = \frac{RT}{F} 2.3 \log \frac{(P_{Na}/P_{Cl}) d_{Na} + a_{Cl}}{(P_{Na}/P_{Cl}) d_{Na} + a_{Cl}} \]  

In unmodified bilayer membranes formed from sheep red cell lipids, the DC resistance was approximately \( 10^4 \Omega \cdot \text{cm}^2 \), the ratio \( t_{Na}/t_{Cl} \) was 4–5, and \( V_m \) was linearly related to the logarithm of aqueous phase salt activity ratio for 10- to 100-fold activity ratios (1). Similarly, in identical membranes exposed to \( 10^{-5} \text{M} \) valinomycin, the DC resistance was approximately \( 10^9 \Omega \cdot \text{cm}^2 \) (in 0.1 M KCl) and \( V_m \) approximated \( E_K \) for 10- to 100-fold KCl concentration ratios (4). Thus, Eq. 12 adequately described the zero current \( V_m \) in these two instances.
In contrast, the relationship between $V_m$ and the logarithm of the NaCl activity ratio in these porous membranes became curved when the activity ratio exceeded 10, in the concentration range 0.0005-0.1 M; moreover, the deviation from linearity persisted when the solutions were made isotonic by adding sucrose to the dilute NaCl solutions (8). The phenomenon is illustrated clearly in Fig. 11. The dotted line was drawn from Eq. 12, assuming $t_{cl} = 0.95$. The experimental conditions included dilute solutions containing either 0.01 M NaCl (●—●, Fig. 11) or 0.001 M NaCl (■—■, Fig. 11), and, the data from Figs. 4-6 (▲—▲, Fig. 11). In Fig. 12, the data from Fig. 11

![Figure 11](image1.png)

**Figure 11.** The zero current membrane potential plotted according to Eq. 12. The dotted line was drawn for $t_{cl} = 0.95$. The triangles (▲—▲) are the data from Figs. 4-6. In the experiments indicated by circles (●—●), the front chamber contained 0.01 M NaCl; the NaCl concentration in the rear chamber was in the range 0.05-0.2 M. In the experiments indicated by squares (■—■), the front chamber contained 0.001 M NaCl; the NaCl concentration in the rear chamber was in the range 0.005-0.05 M. The results are expressed as the mean ± standard deviation. Amphotericin B = 1-3 × 10⁻⁷ M.

![Figure 12](image2.png)

**Figure 12.** The zero current membrane potential plotted according to Eq. 13. The dotted line was drawn for $P_{Na} / P_{Cl} = 0.05$. The data are the mean values of $V_m$ from Fig. 11 and the symbols have the same meaning as in Fig. 11.
were plotted according to the constant-field equation and the dotted line was
drawn assuming that \( P_{DNa}/P_{DCl} \) in Eq. 13 was 0.05. It is evident that, within
experimental error, there was close agreement between the experimental
points and the computed line.

**Membrane Rectification**

The current-voltage relationship for identical unmodified or porous mem-
branes in the presence of symmetrical salt solutions is linear in the range ±
100 mV ([1, 4, 8]; Figs. 9 and 10). The results illustrated in Figs. 11 and 12
indicate that, in the presence of asymmetrical NaCl solutions, the porous
membranes should exhibit rectifying properties predictable from constant-
field theory. Thus, if Na\(^+\) and Cl\(^-\) are the sole current-carrying ionic species,
the membrane current \( I_m \) may be expressed as (11, 12):

\[
I_m = \frac{P_{DCl} V_m A_m F^2}{RT} \left[ \frac{(P_{DNa}/P_{DCl}) \sigma_{Na}^0 + \sigma_{Cl}^0) - (P_{DNa}/P_{DCl}) \sigma_{Na}^0 + \sigma_{Cl}^0 \exp \left( \frac{-FV_m}{RT} \right)}{1 - \exp \left( \frac{-FV_m}{RT} \right)} \right],
\]

where \( A_m \) = membrane area (cm\(^2\)), and the limiting conductance ratio \( G^{+\infty}/
G^{-\infty} \), for \( V_m = \pm \infty \), is (18, 19):

\[
\frac{G^{+\infty}}{G^{-\infty}} = \frac{(P_{DNa}/P_{DCl}) \sigma_{Na}^0 + \sigma_{Cl}^0}{(P_{DNa}/P_{DCl}) \sigma_{Na}^0 + \sigma_{Cl}^0}
\]

Two experiments designed to evaluate this relationship are illustrated in Fig.
13 and Table III.

The experimental protocol was similar to that in Figs. 4–6. The membrane
conductance was measured when both aqueous phases contained 0.01 M
NaCl. Subsequently, either 0.05 M NaCl (Fig. 13, exp A, ○ --- ○) or 0.2
M NaCl (Fig. 13, exp B, ■ --- ■) was introduced into the rear chamber and
the current-voltage characteristics were measured under these conditions. Fi-
nally, 0.01 M NaCl was reintroduced into the rear chamber and \( G_m \) was
again recorded.

Fig. 13 illustrates that, in asymmetrical NaCl solutions, these membranes
exhibited significant rectifying properties. Similar observations have been
made previously in comparable bilayer membranes by Cass et al. (20) and in
thicker inert membranes by others (11, 21, 22). The curves in Fig. 13 were
drawn from Eq. 14 for the parameters \( P_{DCl} \) and \( P_{DNa} \) which yielded the least-
squares fit to the data. Table III indicates clearly that the values of \( P_{DCl} \) re-
quired to rationalize the data in Fig. 13 according to Eq. 14 were consistent
Figure 13. Rectification in asymmetrical NaCl solutions. In exp A ( ), the rear and front chambers contained, respectively, 0.05 M NaCl and 0.01 M NaCl. In experiment B ( ), on a second membrane, the rear and front chambers contained, respectively, 0.2 M NaCl and 0.01 M NaCl. The steady-state currents \( I_m \) were recorded when the membranes were voltage-clamped at the indicated potentials. The curves were drawn according to Eq. 14 as indicated in the text.

Table III

| Experiment | NaCl activity ratio | Gm (0.01 M NaCl) | \( P_{DCl} \) | \( P_{DNa}/P_{DCl} \) |
|------------|---------------------|-----------------|-------------|------------------|
|            |                     |                 | Fig. 1 | Fig. 13 | Fig. 15 |
| A          | 4.55                | 0.22            | 0.07     | 0.084            | 0.032 |
| B          | 16.2                | 0.17            | 0.053    | 0.056            | 0.032 |

Experiments A and B are those indicated in Fig. 13; the NaCl activity ratios are for the rear chamber with respect to the front chamber. The values of Gm are the means of measurements in symmetrical 0.01 M NaCl solutions carried out before and after the current-voltage plots for asymmetrical NaCl solutions. The values of \( P_{DCl} \) from Fig. 1 were obtained by normalizing the conductances observed in experiments A and B with symmetrical 0.01 M NaCl solutions for symmetrical 0.05 M NaCl solutions. The values of \( P_{DCl} \) and \( P_{DNa}/P_{DCl} \) listed for Fig. 13 are the parameters which gave the least-squares fit for Eq. 14 to the data in Fig. 13.
with the $P_{Df}$ values predicted from the observed membrane conductances in symmetrical 0.01 M NaCl solutions and Fig. 1. Likewise, the $P_{DNa}/P_{DCl}$ ratio (0.032; Table III) required for the curves in Fig. 13 was similar to that in Fig. 12 ($P_{DNa}/P_{DCl} = 0.05$). Finally, Fig. 13 indicates that the experimental ratios of the limiting slope conductances were only slightly greater than those expected from Eq. 14.

**DISCUSSION**

The purpose of these studies was to evaluate Cl$^-$ transport in porous lipid bilayer membranes. In this regard, we restate certain assumptions common to all of the experiments. First, the magnitudes of the electrical conductances and coincident values of $P_{DCl}$ (Figs. 1 and 2) were, at a minimum, $10^2$ times greater than comparable observations in similar (3) or identical (1, 8) unmodified bilayer membranes. Thus, for operational purposes, we consider that ions traverse these membranes primarily through aqueous pores; presumably, the dielectric constant of the pore sites approximates that of water rather than the hydrophobic membrane interior. In earlier experiments, the effective radii of the amphotericin B and cholesterol-dependent (8, 9) pores, estimated from water and nonelectrolyte flux data, were approximately 5 Å (13). In the present experiments, the membrane conductances were taken to be an index to the number of pores for a given membrane. Second, in these porous membranes, the relationship between membrane conductance and aqueous phase NaCl concentration, in the range 0.001-0.2 M, is approximately the same as that in bulk solution (8). Similarly, the linear slopes of Figs. 1 and 2 were identical, within experimental error, when the membrane conductances were normalized for aqueous NaCl activity. We have assumed that similar corrections of conductances in 0.01 M NaCl (Figs. 4-8, 13) for 0.05 M NaCl permitted an estimate of $P_{DCl}$ for a given membrane from the data in Fig. 1. Third, the results in Fig. 3 imply that, for the linear ranges of Figs. 1 and 2, Cl$^-$ flux was limited primarily by the membranes rather than unstirred layers.

In symmetrical NaCl solutions, the chloride conductance computed from $P_{DCl}$ was approximately 95% of the membrane conductance (Table I, Figs. 9 and 10). The values of $P_{DCl}$ derived from zero volume flow experiments (Eq. 5; Figs. 1 and 2) described net chloride fluxes in the presence of imposed concentration (Eq. 6; Figs. 7 and 8) or voltage (Eq. 7; Figs. 9 and 10) gradients. Under these conditions, net chloride flux accounted for 90-95% of the membrane current density (Figs. 7-10) and the observed chloride flux ratios were in reasonable agreement with those predicted from the independence relationship (Table II). Accordingly, we conclude that, for the varying experimental conditions described by Eqs. 5-7, $P_{DCl}$ was constant, i.e., the predominant mode of chloride transport through the membrane pores was by a simple diffusion process.
It should be noted parenthetically that the integration of these results with a plausible physical model remains a vexing problem. Making an elementary calculation, the "concentration" of a single chloride ion in a uniform right circular cylinder 5 Å in radius and 100 Å in length would be approximately 0.22 M. Thus, at relatively high bulk phase NaCl concentrations, e.g., ≥ 0.15 M (Figs. 11–13), it may be expected from simple probability considerations that a finite, but indeterminate, number of channels will contain more than one chloride ion during net chloride flux. The present experiments, including both tracer fluxes and electrical measurements, imply that $P_{\text{Cl}}$ is relatively independent of voltage or concentration and that Cl$^-$ is an independently diffusing species. Yet, it is difficult to envision, for example, the simultaneous flux of multiple chloride ions, each having a hydrated radius of approximately 2.1 Å (23), through a 5 Å radius channel without significant cooperative phenomena such as the "single-file" effect (24). Clearly, an analysis of such issues requires explicit information on the molecular structure of these pores.

The data in Figs. 12 and 13 indicate that the zero current membrane potentials and the current-voltage curves in asymmetrical salt solutions may be expressed in terms of traditional constant-field theory (Eq. 13 and 14). The validity of such a description depends, at least in part, on the applicability of the constant-field assumption for these membranes. The latter requires that

\[ \frac{d\psi_m}{dx^2} \sim 0, \quad (15a) \]

and

\[ \frac{d\psi_m}{dx} \sim \frac{V_m}{\Delta x}, \quad (15b) \]

where $\psi_m$ is the electrical potential within the membrane phase, $V_m$ is the externally measured (or applied) electrical potential, and $\Delta x$ is the membrane thickness. Eq. 15a and 15b are valid approximations when the space charge density $\rho_m$ within the membrane phase approaches zero.

In unmodified lipid bilayer membranes exposed to aqueous solutions containing hydrophilic salts, the membrane conductances are in the range of $10^{-8}$ Ω$^{-1}$·cm$^{-2}$ (1). Under such conditions, the membrane concentration of charged species, and hence $\rho_m$, is relatively small (25). (This may not be the case when the aqueous phases contain lipid soluble ions such as tetraphenylborate [26–28].) In regard to the present experiments, there is reasonable evidence which supports the view that the space charge density in porous membranes may also be negligibly small, i.e., that the pores lack significant ion-exchange sites. The permselective properties of porous bilayer membranes are in large part pH independent, in the range 2.4–10.5 (9, 29). Furthermore, N-acetylation...
and/or methyl esterification of, respectively, the amino and carboxyl moieties on amphotericin B does not modify appreciably the effects of that polyene antibiotic on the ionic, water, and nonelectrolyte permeability of native bilayer membranes (9). On the basis of such observations, we (9) and others (29) have suggested that hydroxy or carbonyl moieties on amphotericin B might be responsible for the anion permselectivity of these porous membranes. Finally, the conductance of porous bilayer membranes increase linearly (with a unity slope) with aqueous salt activity in symmetrical NaCl or KCl solutions (8); Barry and Diamond (30) have pointed out that the conductance-concentration relationship should be linear in neutral membranes which have a low space charge density and are sufficiently thin to violate microscopic electroneutrality (cf. below). Thus, it seems reasonable to conclude that $\rho_m$ in porous bilayer membranes is sufficiently small that the electric field is approximately constant.

Nonlinear current-voltage properties may be rationalized, assuming electroneutrality in terms of expressions derived for charged membranes containing either fixed (18, 31) or mobile (32) sites. However, it is unlikely that these porous membranes contain significant ion-exchange sites (cf. above). Alternatively, it is probable that the rectification observed in asymmetrical NaCl solutions (Fig. 13) occurred because lipid bilayer membranes are sufficiently thin, with regard to the Debye length in the membrane, that microscopic electroneutrality need not obtain (20, 30, 33). Thus, it may be shown (30, 34) that a solution of the Nernst-Planck flux equations for a thin membrane, subject to the boundary conditions of low membrane space charge density and non-electroneutrality, results in expressions similar in form to the traditional Goldman-Hodgkin-Katz equations.

In this connection, the Debye length within the channels of a porous bilayer membrane exposed to a symmetrical NaCl solution is:

$$\frac{1}{\delta} = \left( \frac{f^R(a^N_m + a^Cl_m)}{RTD_m e_\infty} \right),$$

where $\delta$ is the Debye length (cm), $\epsilon_\infty$ is the permittivity of free space, $D_m$ is the dielectric constant within the membrane pores, and $a^N_m$ and $a^Cl_m$ are the activities within the pores. $\delta$ may not be evaluated explicitly at present, since $a^N_m$, $a^Cl_m$, and $D_m$ are not known. However, it is possible to indicate by a crude calculation that it is plausible that $\delta \approx 70$ Å, i.e., approximately the thickness of a bilayer membrane. Since these membranes are predominantly anion-selective, it is likely that $a^Cl_m \gg a^N_m$; if it is assumed that the pores are aqueous, $D_m \approx 80$. Thus, for a bulk phase of 0.01 M NaCl at 25°C, a Debye length of 70 Å requires that the time-average value of $a^Cl_m$ be only as low as 0.0036 M; for lower chloride "concentrations" within the pores, $\delta$ would exceed the membrane thickness. It seems reasonable to assume that steric factors, at a mini-
mum, could result in such relatively small constraints to the partition of Cl− between bulk solution and pore phases.

Finally, it is instructive to compare the values of $P_{DCl}$ obtained in the present experiments with similar observations in living systems. Thus, the values of $P_{DCl}$ in a variety of biological membranes, e.g., frog skeletal muscle (17, 35), bullfrog gastric mucosa (36), and the toad urinary bladder (37), are in the range $1 \times 10^{-6} \text{ cm}\cdot\text{s}^{-1}$. The predicted value of $P_{DCl}$ in the present experiments was $2.25 \times 10^{-5} \text{ cm}\cdot\text{s}^{-1}$ for a membrane conductance of $10^{-9} \Omega^{-1}\cdot\text{cm}^{-2}$ in 0.15 M NaCl (Fig. 2). Stated alternatively, for comparable conductances in similar salt solutions, $P_{DCl}$ in these membranes was in the same range as the value observed in various biological membranes. Accordingly, it is possible that the present results in these synthetic membranes may be relevant to the evaluation of dissipative Cl− flux in natural membranes.

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