

Coupling of Solute and Solvent Flows in Porous Lipid Bilayer Membranes

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ABSTRACT The present experiments were designed to evaluate coupling of water and nonelectrolyte flows in porous lipid bilayer membranes (i.e., in the presence of amphotericin B) in series with unstirred layers. Alterations in solute flux during osmosis, with respect to the flux in the absence of net water flow, could be related to two factors: first, changes in the diffusional component of solute flux referable to variations in solute concentrations at the membrane interfaces produced by osmotic flow through the unstirred layers; and second, coupling of solute and solvent flows within the membrane phase. Osmotic water flow in the same direction as solute flow increased substantially the net fluxes of glycerol and erythritol through the membranes, while osmotic flow in the opposite direction to glycerol flow reduced the net flux of that solute. The observed effects of osmotic water flow on the fluxes of these solutes were in reasonable agreement with predictions based on a model for coupling of solute and solvent flows within the membrane phase, and considerably in excess of the prediction for a diffusion process alone.

INTRODUCTION

Andersen and Ussing first observed that the net flux of solutes such as thio-urea or acetamide through the isolated frog skin exposed to vasopressin was increased in proportion to the rate of osmotic water flow in the same direction (1). They termed this phenomenon "solvent drag" and attributed it to a coupling of solute flux to solvent flow within aqueous membrane channels. Subsequently, Hays and Leaf observed similar effects of water flow on urea fluxes in the vasopressin-treated urinary bladder of the toad (2), and Kedem and Katchalsky (3) developed quantitative expressions which described such interactions between solute and solvent flows within membranes in the terminology of irreversible thermodynamics. Thus, the demonstration of apparent

solvent drag has been widely regarded as a useful criterion for the presence of aqueous channels, or pores, within membranes (1, 2, 4).

In earlier studies, we suggested that the interactions of amphotericin B with appropriate membrane sterols resulted in the formation of aqueous pores in lipid bilayer membranes (5, 6). It was relevant in this regard to examine the possibility of the coupling of solute and solvent flows in these membranes. However, a number of observations indicate that, in certain instances, phenomena other than solute-solvent interactions in aqueous membrane pores account for apparent coupling of solute and solvent flows. Sidel and Hoffman (7) noted apparent solvent drag for urea in nonporous liquid membranes, mesityl oxide, separating two aqueous phases. Furthermore, apparent electrokinetic phenomena, in plant cells (8, 9), the gallbladder (10), and squid axon (11), may be referable at least in part to changes produced by water flow in the ionic composition of unstirred layers at the membrane interfaces, rather than to coupling of ionic and water flows in the membrane phases.

In the preceding paper we presented evidence for the presence of unstirred layers in series with lipid bilayer membranes (12). Consequently, alterations in the flux of solutes during osmosis across these membranes, in the presence of amphotericin B, could not be regarded a priori as evidence for solvent drag. The present experiments were designed to distinguish between the contributions of unstirred layers and solvent drag to the fluxes of nonelectrolytes across such membranes in series with unstirred layers. The results indicate that coupled flows between solute and solvent may occur in lipid bilayer membranes exposed to amphotericin B.

METHODS

The experimental techniques and the lipid solutions used to form lipid bilayer membranes were identical to those described in the preceding paper (12). The pH of the unbuffered aqueous phase was 5.8-6.0; the temperature was $26.5^{\circ}\text{C} \pm 0.5^{\circ}\text{C}$. The composition of the aqueous phases is given in the text. Unless otherwise indicated, the experiments were carried out when the aqueous phases contained $0.8\text{-}1.0 \times 10^{-6}\text{ M}$ amphotericin B. As in the preceding paper (12), the lipid bilayer membranes exposed to these concentrations of amphotericin B will be termed porous. In particular, it should be noted that the electrical resistances of the porous membranes in the present experiments were similar to those described in the preceding paper (12).

RESULTS

A. Theoretical

Following Kedem and Katchalsky (3), the dissipative transport of the i th nonelectrolyte between two aqueous phases, I and II, separated by a membrane of unit area, may be described completely by the expression:

$$J_i = P_{m_i}(C_i^{m\text{ I}} - C_i^{m\text{ II}}) + J_v(1 - \sigma_{m_i})\bar{C}_i, \quad (1)$$

where J_i is the net flux of solute (moles $\text{sec}^{-1} \text{cm}^{-2}$) across the membrane, and P_{m_i} (cm sec^{-1}) and σ_{m_i} are, respectively, the membrane permeability coefficient for diffusion (12) and the membrane reflection coefficient of the i th solute. For dilute solutions, J_v , the volume flow is $\sim J_w$, the net water flow ($\text{ml sec}^{-1} \text{cm}^{-2}$) across the membrane. The terms C_i^{mI} and C_i^{mII} are, respectively, the aqueous concentrations of the i th solute at the interfaces of the membrane with solutions I and II , and \bar{C}_i is, to a sufficient approximation (3, 13, 14):

$$\bar{C}_i = \frac{C_i^{mI} + C_i^{mII}}{2}. \quad (1a)$$

In the case of porous lipid bilayer membranes in series with unstirred layers, the problem is to evaluate the terms in Equation 1. J_i and J_w may be measured experimentally (5, 12). In the preceding paper (12), we estimated P_{m_i} from the observed solute permeability coefficients (P_{D_i} , cm sec^{-1}) and the sum of the effective thicknesses of the unstirred layers in series with the membranes ($\alpha^i \sim 110 \times 10^{-4} \text{ cm}$). The subsequent sections are concerned with an evaluation of first, C_i^{mI} and C_i^{mII} , and second, σ_{m_i} .

THE CONCENTRATIONS OF SOLUTES AT THE MEMBRANE INTERFACES

The formulation of the problem, following Hertz (15, 16), Manegold and Solf (17), and Dainty and House (18), is illustrated schematically in Fig. 1 for phase I. Both the osmotic water flow, J_w , and the net flux of solute, J_i , are from phase I to phase II. Solution I consists of a bulk phase in series with an unstirred layer, α^i . Since, in our experiments, the aqueous chambers are symmetrical, we assume α^i is one-half α^i , the total thickness of the unstirred layers (12).

As in the previous paper (12), the unstirred layer thickness is an operational term, assuming that D_i^0 , the free diffusion coefficient of the i th solute, is the

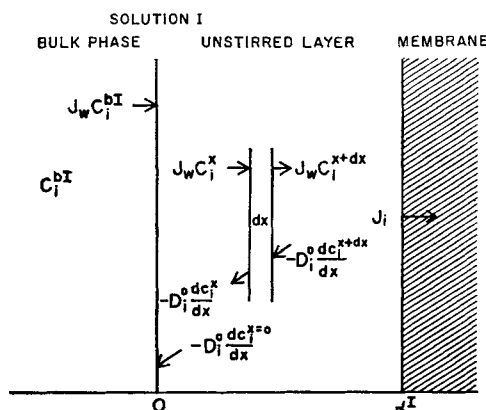


FIGURE 1. Schematic representation of an unstirred layer in series with a membrane having unit area. Details are in the text.

same in bulk solution and unstirred layer, and does not connote the necessary existence of an actual, discrete boundary in a continuous aqueous solution. However, by assigning a finite value for the thickness of the unstirred layer, the latter may be considered, for analytical purposes, as a membrane in which aqueous solutes have a zero reflection coefficient, with respect to the contiguous bulk solution.

Consider an infinitely thin lamella of thickness dx within the unstirred layer. The convective and diffusional flows of the i th solute into and out of dx during osmotic water flow are indicated in Fig. 1, where C_i^x and C_i^{x+dx} refer to the solute concentrations at unstirred layer thicknesses of, respectively, x and $(x + dx)$. In the steady state, the equation of continuity for dx is:

$$J_w C_i^x + D_i^o \frac{d C_i^{x+dx}}{dx} - \left(J_w C_i^{x+dx} + D_i \frac{d C_i^x}{dx} \right) = 0. \quad (2)$$

The Taylor series expansion of Equation 2, neglecting differentials higher than second order, is:

$$D_i^o \frac{d^2 C_i^x}{dx^2} - J_w \frac{d C_i^x}{dx} = 0. \quad (3)$$

Equation 3 may be integrated to:

$$C_i^x = A \exp \frac{J_w^x}{D_i^o} + B, \quad (4)$$

where A and B are the constants of integration. These constants may be evaluated from a consideration of the boundary conditions (Fig. 1).

Following Kirkwood (19) and Katchalsky and Curran (20), the chemical potential is continuous and the gradient of potential discontinuous at a phase boundary. Thus, at $x = 0$, Equation 4 becomes:

$$C_i^{b \ 1} = A + B. \quad (4a)$$

Similarly, solute may diffuse from the unstirred layer into the bulk solution according to:

$$D_i^o \frac{d C_i^{x=0}}{dx},$$

where the gradient of concentration is evaluated at $x = 0$. Furthermore, at $x = 0$, solute enters the unstirred layer with osmotic water flow,

$$J_w C_i^{b \ 1}.$$

At $x = \alpha^1$, J_i is the flux of solute through the membranes. Accordingly, at

the boundaries of the unstirred layers,

$$\frac{d C_i^{x=0}}{dx} = \frac{J_w C_i^{b I} - J_i}{D_i^0}. \quad (5)$$

The first derivative of Equation 4 is:

$$\frac{d C_i^x}{dx} = \frac{J_w}{D_i^0} A \exp \frac{J_w x}{D_i^0}, \quad (6)$$

and, evaluated at $x = 0$,

$$\frac{d C_i^{x=0}}{dx} = \frac{J_w}{D_i^0} A. \quad (6a)$$

Thus, from Equations 5 and 6 a:

$$A = C_i^{b I} - \frac{J_i}{J_w}. \quad (7)$$

Substituting Equations 7 and 6 a into Equation 4, we have, for $x = \alpha^I$:

$$C_i^{m I} = \left(C_i^{b I} - \frac{J_i}{J_w} \right) \exp \frac{J_w \alpha^I}{D_i^0} + \frac{J_i}{J_w}. \quad (8)$$

The corresponding equation for aqueous solution II, in series with the other membrane interface, is:

$$C_i^{m II} = \left(C_i^{b II} - \frac{J_i}{J_w} \right) \exp - \frac{J_w \alpha^{II}}{D_i^0} + \frac{J_i}{J_w}. \quad (8a)$$

It is evident that J_i is not dependent on J_w when $J_w = 0$. Accordingly, by applying L'Hospital's rule for J_w approaching zero, Equations 8 and 8 a become, respectively:

$$\left(C_i^{m I} = C_i^{b I} - \frac{J_i \alpha^I}{D_i^0} \right) \quad (9)$$

and

$$C_i^{m II} = C_i^{b II} + \frac{J_i \alpha^{II}}{D_i^0}. \quad (9a)$$

Thus, when $J_w = 0$, the solute concentration profile changes linearly with the thickness of the unstirred layer (Equations 9 and 9 a). However, Equations 8 and 8 a show that, as J_w increases, $C_i^{m I}$ and $C_i^{m II}$ will, respectively, increase and decrease exponentially. With reference to Equation 1, these obser-

vations indicate clearly that, for a membrane in series with unstirred layers, increments in the flux of a nonelectrolyte during osmosis, with respect to the flux when $J_w = 0$, are referable to two factors: first, a rise in the diffusional component of the flux, determined by the increase in $(C_i^{mI} - C_i^{mII})$; and second, coupling of solute and solvent flows within the membrane phase, described by the term $J_s(1 - \sigma_{m_i})\bar{C}_i$.

The net fluxes of nonelectrolytes through the membranes during osmosis may now be expressed in terms of the bulk phase solute concentrations and parameters which correct for the effects of unstirred layers. Substituting for C_i^{mI} and C_i^{mII} in Equations 1 and 1 *a* from Equations 8 and 8 *a* and rearranging terms, we have:

$$J_i = \frac{C_i^{bI} \left[1 + \frac{J_w(1 - \sigma_{m_i})}{2P_{m_i}} \right] \exp \frac{J_w \alpha^I}{D_i^o} - C_i^{bII} \left[1 - \frac{J_w(1 - \sigma_{m_i})}{2P_{m_i}} \right] \exp - \frac{J_w \alpha^{II}}{D_i^o}}{\frac{1}{P_{m_i}} + \frac{1}{J_w} \left[\exp \frac{J_w \alpha^I}{D_i^o} - \exp - \frac{J_w \alpha^{II}}{D_i^o} \right]} \quad (10)$$

$$+ \frac{(1 - \sigma_{m_i})}{P_{m_i}} \left[\exp \frac{J_w \alpha^I}{D_i^o} + \exp - \frac{J_w \alpha^{II}}{D_i^o} - 2 \right]$$

It is also instructive to evaluate the diffusional component of solute flux, J_{d_i} , during osmosis. From Equation 1:

$$J_{d_i} = P_{m_i}(C_i^{mI} - C_i^{mII}), \quad (11)$$

and, substituting for C_i^{mI} and C_i^{mII} in Equation 11 from Equations 8 and 8 *a*:

$$J_{d_i} = \frac{C_i^{bI} \exp \frac{J_w \alpha^I}{D_i^o} - C_i^{bII} \exp - \frac{J_w \alpha^{II}}{D_i^o}}{\frac{1}{P_{m_i}} + \frac{1}{J_w} \left[\exp \frac{J_w \alpha^I}{D_i^o} - \exp - \frac{J_w \alpha^{II}}{D_i^o} \right]} \quad (12)$$

Equations 10 and 12 may be used to describe the coupling of solute and solvent flows in porous lipid bilayer membranes in series with unstirred layers, provided that σ_{m_i} can be evaluated.

EVALUATION OF σ_{m_i}

Dainty and Ginzburg (21) pointed out that, for a membrane in series with unstirred layers, the observed solute reflection coefficients (σ_{m_i}) may be erroneously low in the case of relatively permeable solutes, since the solute concentration differences at the membrane interfaces may be less than those between

bulk solutions. In these porous lipid bilayer membranes, the observed solute permeability coefficients, (P_{D_i}), were considerably less than the membrane permeability coefficients, (P_{m_i}), for both urea and glycerol (reference 12, Table V). Consequently, it is likely that the observed reflection coefficients for these solutes, reported previously (5), were erroneously low.

Kedem and Katchalsky (22) showed that, for two series membranes, denoted as a and b , the relationship between nonelectrolyte permeability coefficients and reflection coefficients may be expressed as:

$$\sigma_{o_i} = \sigma_{a_i} \frac{P_{D_i}}{P_{a_i}} + \sigma_{b_i} \frac{P_{D_i}}{P_{b_i}}, \quad (13)$$

where σ_{o_i} and P_{D_i} are, respectively, the overall reflection coefficient and permeability coefficient of the i th solute, and the subscripts a and b refer, respectively, to membranes a and b . When the unstirred layers are considered as a membrane in series with a lipid bilayer membrane:

$$\sigma_{o_i} = \sigma_{\alpha_i} \frac{P_{D_i}}{P_{\alpha_i}} + \sigma_{m_i} \frac{P_{D_i}}{P_{m_i}} \quad (13a)$$

where the subscripts α and m refer, respectively, to the unstirred layer and the lipid membrane. Since σ_{α_i} is zero for aqueous solutes (cf. above), we have:

$$\sigma_{m_i} = \sigma_{o_i} \frac{P_{m_i}}{P_{D_i}}. \quad (13b)$$

Table I lists the values for σ_{m_i} in these membranes which were computed according to Equation 13 *b* and the previously reported values of σ_{o_i} , P_{m_i} , and P_{D_i} (5, 12). In agreement with the observations on P_{m_i} in the preceding paper (reference 12, Table V), the observed reflection coefficients for urea and glycerol were substantially different from the true, or membrane, reflection coefficients for these solutes.

B. *Experimental*

THE MODE OF NET SOLUTE FLUX

The values of P_{D_i} listed in Table I (cf. reference 12) were computed from unidirectional tracer fluxes carried out when the two aqueous phases bathing the membranes were identical, except for the concentration of isotope, and the net volume flow was zero. These observations did not exclude the possibility that interactions such as "single-file" flux (23) or exchange diffusion contributed to the net movement of solutes across porous lipid bilayer membranes. In this connection, Pagano and Thompson (24) demonstrated that Cl^- permeation, in unmodified spherical lipid bilayer membranes, was at least par-

tially dependent on an exchange diffusion process. Consequently, it was necessary to examine net solute flux in the absence of net water flow prior to evaluating the effects of osmosis.

For a diffusion process, we have:

$$P_{D_i}^o = \frac{J_i^o}{A_m(C_i^{bI} - C_i^{bII})}, \quad (14)$$

where $P_{D_i}^o$ (cm sec^{-1}) is the apparent solute permeability coefficient computed from the net solute flux (J_i^o , moles sec^{-1}) in the absence of osmotic water flow, and A_m is the membrane area. The relevant experimental observations are in Fig. 2 and Table II. In these experiments, the concentration differences for

TABLE I
CORRECTED SOLUTE REFLECTION
COEFFICIENTS FOR POROUS
LIPID BILAYER MEMBRANES

| Solute | P_{D_i} | P_{m_i} | σ_{o_i} | σ_{m_i} |
|-------------------------|----------------------------------|-----------|----------------|----------------|
| | $\text{cm sec}^{-1} \times 10^4$ | | | |
| Urea | 9.91 | 38.50 | 0.08 | 0.31 |
| Glycerol | 2.57 | 3.88 | 0.43 | 0.65 |
| <i>Meso</i> -erythritol | 0.64 | 0.71 | 0.64 | 0.71 |

P_{D_i} and P_{m_i} are the mean values from Table V (reference 12). The values of σ_{o_i} for urea and glycerol were obtained from earlier studies (5). σ_{o_i} for *meso*-erythritol was measured as described previously (5), when the aqueous phases contained 10^{-6} M amphotericin B; the value shown in the table is the mean of four observations. The values for σ_{m_i} were computed from Equation 13 b and the indicated values of P_{D_i} , P_{m_i} , and σ_{o_i} .

nonelectrolytes between the two aqueous phases were kept sufficiently small to minimize the contributions of osmotic water flow to net solute flux (Equations 1 and 10; cf. also Table III).

Fig. 2 indicates that the net flux of glycerol was linearly related to the solute concentration in phase I, when solution II contained no glycerol. Moreover, $P_{D_{\text{glycerol}}}^o$ computed from these experiments was the same, within experimental error, as $P_{D_{\text{glycerol}}}$ (Table I), which was measured from unidirectional tracer fluxes when net solute flux was zero (5). A comparison of Tables I and II shows that the results were similar for both urea and *meso*-erythritol. These data indicate that the net fluxes of these nonelectrolytes in porous lipid bilayer membranes, in the absence of osmotic water flow, may be described in terms of simple diffusion.

THE EFFECTS OF OSMOTIC WATER FLOW ON NET SOLUTE FLUXES

Table III illustrates the effects of osmotic water flow, in either direction, on the net fluxes of solutes from solution I to solution II across porous lipid bi-

layer membranes. The net water fluxes were produced by adding sucrose, whose reflection coefficient for these membranes is unity (5), to one aqueous phase. Osmotic flow from solution I to solution II; i.e., in the same direction as solute flow, was considered positive. Since the experiments were carried out in open chambers (5, 12), the water fluxes could not be measured simultane-

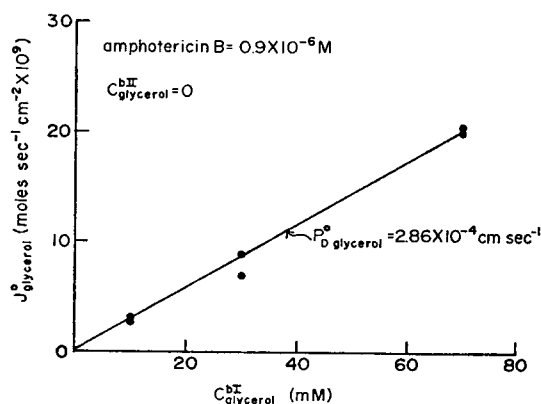


FIGURE 2. The net fluxes of glycerol in porous lipid bilayer membranes. The aqueous phases uniformly contained 0.01 M NaCl, 10^{-6} M amphotericin B. Solution I contained ^{14}C -glycerol and the indicated concentrations of glycerol, and solution II contained no glycerol. The net fluxes of glycerol were measured as described previously (5, 11) and $P_{D_{\text{glycerol}}}^o$ was computed from the data according to Equation 14. In these experiments, R_m ($\text{ohm}\cdot\text{cm}^2 \times 10^{-2}$) was 0.68 ± 0.24 (6).

TABLE II
NET SOLUTE FLUXES IN POROUS
LIPID BILAYER MEMBRANES

| Solute | Solution I | Solution II | J_i^o | $P_{D_i}^o$ | R_m |
|-----------------|---------------------------------|-------------|--|----------------------------------|---|
| | M | M | $\text{moles sec}^{-1} \text{cm}^{-2} \times 10^9$ | $\text{cm sec}^{-1} \times 10^4$ | $\text{ohm}\cdot\text{cm}^2 \times 10^{-2}$ |
| Urea | 0.01 urea, 0.01 NaCl | 0.01 NaCl | 10.30 ± 1.7 (4) | 10.30 | 0.74 ± 0.18 (4) |
| Meso-erythritol | 0.01 meso-erythritol, 0.01 NaCl | 0.01 NaCl | 0.73 (3) | 0.73 | 0.62 ± 0.25 (3) |

The aqueous phases ($26.5^\circ\text{C} \pm 0.5^\circ\text{C}$) contained 10^{-6} M amphotericin B. The appropriate ^{14}C -tagged isotope was added to solution I, and the net solute fluxes were measured as described previously (5, 12). R_m , the dc membrane resistance, was measured (12) concomitantly. The results are expressed as the mean \pm standard deviation for the number of observations in parentheses, and $P_{D_i}^o$ was computed according to Equation 14.

ously with the tracer fluxes. However, in the preceding paper (12), P_f , the osmotic water permeability coefficient, was determined with sucrose for membranes formed from the lipid preparation used in the present experiments. Accordingly, the water fluxes shown in Table III were computed from the relations:

$$J_w = L_p \Delta\pi \quad (15)$$

and

$$L_p = \frac{P_f \bar{V}_w}{RT}, \quad (15a)$$

where L_p ($\text{cm sec}^{-1} \text{atm}^{-1}$) is the coefficient of hydraulic conductivity, $\Delta\pi$ is the difference in osmolality in solutions I and II, \bar{V}_w is the partial molar volume of water, and P_f was $404.7 \times 10^{-4} \text{ cm sec}^{-1}$ (reference 12, Table III).

Table III lists the predicted values which were computed from Equations 10 and 12 for, respectively, the diffusional fluxes and the net fluxes of the different solutes. For these calculations, the values of P_{m_i} and σ_{m_i} were obtained from Table I. In the preceding paper, the total thickness (α^I) of the unstirred layers was estimated to be approximately $110 \times 10^{-4} \text{ cm}$ (12); since the aqueous chambers used in these experiments were symmetrical, α^I and α^{II} in Equations 10 and 12 were each taken to be $55 \times 10^{-4} \text{ cm}$.

A comparison of Table III with Fig. 2 and Table II indicates that osmotic water flow, under these experimental conditions, had no significant effect on the net fluxes of urea, but increased substantially the net fluxes of both glycerol and *meso*-erythritol, when the water flux was in the same direction as solute flow. Moreover, as shown in Table III, the observed net fluxes of the latter two solutes, during osmotic flow in the same direction, were considerably in excess of the flux values predicted from Equation 12, and in reasonable agreement with those predicted from Equation 10. Similarly, osmotic flow in the opposite direction from solute flow reduced the net flux of glycerol to a value somewhat less than that predicted by Equation 12, and in good agreement with the predicted value for coupling of solute and solvent flow in the membrane phase (Equation 10).

DISCUSSION

The experiments described in this paper were intended to evaluate the dissipative transport of nonelectrolytes through porous lipid bilayer membranes in series with unstirred layers. In the absence of osmotic water flow, a simple diffusion mechanism could account for the net flux of solutes (Fig. 2 and Table II). Under these conditions, the ratio $P_{D_i}:P_{m_i}$ was an index of the relative contributions of the membranes and unstirred layers to the frictional resistance to solute diffusion (Table I of this paper; reference 12, Equation 1 and Table V). From Table I, the membranes provided approximately 26, 66, and 90% of the total diffusional resistance for, respectively, urea, glycerol, and *meso*-erythritol, while the remainder was due to the unstirred layers.

The net fluxes of glycerol and *meso*-erythritol were substantially greater during osmosis, with respect to $J_w = 0$, and in reasonable agreement with the values predicted from Equation 10 (Fig. 2, Tables II and III). With respect

TABLE III
THE EFFECTS OF OSMOTIC WATER FLOW
ON NET SOLUTE FLUXES IN POROUS
LIPID BILAYER MEMBRANES

| Solute | Solution I | Solution II | R_m | J_w | Net solute flux | |
|-----------------|------------------------------------|------------------------|----------------------------------|---|---------------------|-----------|
| | | | | | Observed | Predicted |
| | M | M | $\text{ohm-cm}^2 \times 10^{-3}$ | $\frac{\text{ml sec}^{-1} \text{cm}^{-2}}{\times 10}$ | J_i | J_i |
| Urea | 0.01 urea, 0.01 NaCl | 0.5 sucrose, 0.01 NaCl | 0.70 | 3.67 | 10.02 | 11.2 |
| Glycerol | 0.01 glycerol, 0.01 NaCl | 0.6 sucrose, 0.01 NaCl | 0.81 ± 0.30 (12) | 4.40 | 4.93 ± 0.86 (12) | 3.40 |
| Glycerol | 0.01 glycerol, 0.01 NaCl | 0.01 NaCl | 0.82 ± 0.25 (5) | -3.67 | 1.47 ± 0.25 (5) | 2.02 |
| Meso-erythritol | 0.01 meso-erythritol, 0.01 NaCl | 0.5 sucrose, 0.01 NaCl | 0.75 ± 0.28 (4) | 3.67 | 1.61 ± 0.35 (4) | 0.83 |

The aqueous phases ($26.5^\circ\text{C} \pm 0.5^\circ\text{C}$) contained $0.8 \times 10^{-6} M$ amphoterin B. The appropriate ^{14}C -tagged isotope was added to solution I, and the net solute fluxes were measured as described previously (5, 12). R_m , the dc membrane resistance, was measured (12) concomitantly. The results are expressed as the mean \pm standard deviation for the number of observations listed in parentheses. The control fluxes (i.e., when $J_w = 0$) are shown in Table II and Fig. 2 as J_i^0 . The predicted values for the diffusional components of the fluxes (J_{d_i}) and the total fluxes (J_i) were computed from, respectively, Equations 12 and 10 as described in the text.

to the latter, it is noteworthy that the parameter J_w was determined from net water flux experiments (reference 12, Table III), while the set of parameters α^I , α^{II} , P_{m_i} , and σ_{m_i} was derived from the results of zero volume flow experiments at varying aqueous phase viscosities (Table I of this paper; reference 12, Tables IV and V). Consequently, the similarities in Table III between the observed and predicted net solute fluxes during osmosis (it is noteworthy that there is similar agreement between the observed and predicted fluxes at $J_w = 0$; Fig. 3) are based on comparisons among three different groups of experiments. These observations, taken together, imply that the fluxes of glycerol and *meso*-erythritol, at a minimum, were coupled in the membrane phase to water flow. According to this view, it is likely that Equation 10 describes, to a reasonable approximation, the fluxes of nonelectrolytes through these membranes during osmosis.

Fig. 3 compares the effects of osmotic water flow on the net solute fluxes and the diffusional components of the fluxes. The curves were drawn from

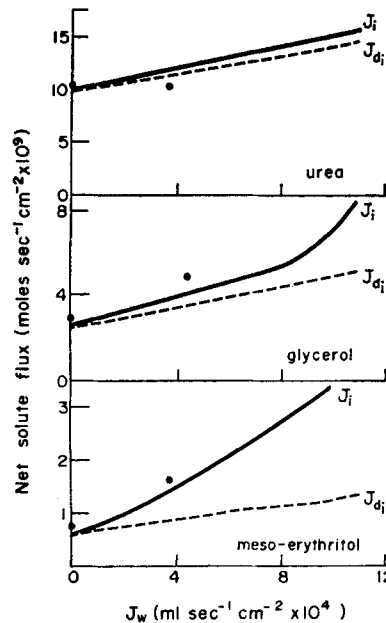


FIGURE 3. The effect of osmotic water flow on net solute flux. The curves for the net flux (J_i) and the diffusional component of the flux (J_{d_i}) for each solute were drawn from Equations 10 and 12, respectively for the experimental conditions in Fig. 2 and Tables II and III; the parameters P_{m_i} and σ_{m_i} were obtained from Table I and α^I and α^{II} were each taken to be 55×10^{-4} cm (12). J_w was computed from Equations 15 and 15 *a*, using $P_f = 404.7 \times 10^4$ cm sec⁻¹ (reference 12, Table III), when $\Delta\pi$ was in the approximate range from 0 to 40 atm. The points represent the observed fluxes, and were obtained from Fig. 2 and Table II, for $J_w = 0$, and from Table III, during osmotic water flow.

Equation 10, for J_i , and from Equation 12, for J_{d_i} , for these experimental conditions; the points represent the experimental values at $J_w = 0$ (Fig. 2 and Table II) and during osmosis (Table III). It is evident that the component of solute flux referable to coupling between solute and water flow in the membrane phase, i.e. the difference between J_i and J_{d_i} , becomes increasingly significant with respect to total solute flux as solute permeability diminishes. Table I illustrates that, for the sequence urea, glycerol, and meso-erythritol, the relative increase in σ_{m_i} was considerably less than the comparable reduction in P_{m_i} . In terms of Equations 1 and 10, these observations indicate that the relative magnitude of $(1 - \sigma_{m_i})$, with respect to P_{m_i} , increased for decreasing solute permeability. The curves in Fig. 3 also show the increments which may be expected in the diffusional fluxes (J_{d_i}) of more permeable solutes, for values of J_w less than 8×10^{-4} ml sec⁻¹ cm⁻² with respect to $J_w = 0$, because of increases in the solute concentration differences at the interfaces between membrane and unstirred layers referable to water flow through the latter (Equations 8 and 8 a).

It is of interest to consider prior instances of apparent solvent drag on nonelectrolytes in the context of the present observations. Sidel and Hoffman noted that, in mesityl oxide membranes, osmotic water flow resulted in urea flux ratios ranging from 1.15 to 1.49 when the aqueous solutions contained equal urea concentrations (7). Since the liquid, nonaqueous membranes were presumably not porous, it is possible that the deviations from a urea flux ratio of unity in their experiments were dependent on phenomena related to unstirred layers. Similarly, recent studies have indicated that unstirred layers may contribute significantly to the total resistance to water diffusion in the presence of vasopressin both in the isolated frog skin (18, 25) and in the urinary bladder of the toad (26). Consequently, the extent to which solvent drag contributes to the acceleration of nonelectrolyte flux during osmosis in such tissues (1, 2) requires further consideration. It should be noted in this connection that, in the presence of vasopressin, the reflection coefficient of urea in the toad urinary bladder is 0.70 (27) while those of thiourea and acetamide in frog skin have been estimated to be, respectively, 0.89 and 0.98 (28). Accordingly, the increments in the flux of such solutes across epithelial tissues during osmosis (1, 2), in contrast to the results with urea in the present experiments (Fig. 3), may depend on the fact that $\sigma_{m_{urea}}$ was only 0.31 in these lipid bilayer membranes (Table I).

The experiments in this paper and in the preceding paper (12) provide additional support for the hypothesis that interactions such as hydrogen bonding between electronegative moieties on amphotericin B and equatorial protons on 3-OH groups of appropriate sterols result in pore formation in lipid bilayer membranes (5, 6). However, it should be stressed that quasilaminar osmotic water flow (5, 12), restricted diffusion of solutes (Tables I and

references 5, 6, 12), and the solvent drag phenomenon (Fig. 3) constitute, in our view, necessary, but not sufficient, criteria for the presence of membrane pores. In the case of these membranes, more detailed information concerning the chemical interactions between cholesterol and amphotericin B and their stoichiometry may increase our understanding of the nature of such pores.

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