A Selective Membrane for Transporting Sodium Ion against Its Concentration Gradient

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Abstract: A carrier-containing membrane has been developed which can selectively move sodium ion from a region of low concentration to a region of high concentration. The energy for this movement comes from the simultaneous countertransport of protons. The transport is unaffected by electrostatic and osmotic effects but is dominated by the chemical characteristics of the mobile carrier. Since the membrane is chemically well defined, its operation is understood on a molecular level and its behavior can be successfully predicted from a simple theory.

We have developed a membrane which will selectively move sodium ion against its concentration gradient. In this paper we exploit the fact that the membrane is defined chemically to investigate the origins of the selectivity and to verify the behavior predicted theoretically.

The specific membrane system developed here[1] contains a mobile carrier, the macrocyclic antibiotic monensin[2,3] which reacts selectively with sodium and transports it across the membrane. Previous systems of this sort have shown less selectivity and have not focussed on the chemical details within the membrane.[4] The carrier simultaneously transports protons in the opposite direction; indeed, it is this proton flux which supplies the energy for the movement of sodium. Thus the membrane studied here provides a chemically exact analog to the phenomena of "coupled facilitated diffusion" and "counterflow" occurring in biological membranes.[5]

These effects will be clearer if we first give a qualitative description of the membrane's operation. A schematic drawing of the diffusion cell used is shown in Figure 1. The top compartment contains a 0.1 *N* sodium hydroxide solution and the bottom compartment contains 0.1 *N* sodium chloride and 0.1 *N* hydrochloric acid. The membrane contains the mobile carrier monensin in octanol solution.

During the course of the experiment, the sodium ion concentration difference across the membrane rises from zero, its initial value, to 0.16 *M* (curve 1, Figure 1). Simultaneously, the acid concentration differences drop to zero (curve 2, Figure 1). As this limit is reached, the sodium ion concentration difference reaches a maximum. These effects are much smaller when monensin is absent (curve 3, Figure 1).

We believe that the results in Figure 1 are best explained by the mechanism shown schematically in Figure 2. The carrier, which is a carboxylic acid, is much less soluble in the surrounding solutions than in the membrane, shown schematically by the two vertical lines. After the rapid reaction shown in step 1, the complex diffuses slowly to the right down its concentration gradient (step 2). After a second rapid reaction in which sodium is replaced by a proton (step 3), the monensin diffuses down its concentration gradient back across the membrane (step 4). The net result is that sodium ion is moved from left to right against its concentration difference across the membrane. The ways in which this effect can be generalized to provide industrially promising alternatives to ion exchange will be described in subsequent papers.

Theory

Exact Chemical Description. The physical behavior of the membrane depicted in Figures 1 and 2 can be better explained by considering the chemistry involved in greater detail. First, solutes in the adjacent solutions may dissolve in the membrane

$$Na^+ + OH^- \overset{k_{NaOH}}{\rightleftharpoons} \mathbf{NaOH} \quad (1)$$

$$H^+ + Cl^- \overset{k_{HCl}}{\rightleftharpoons} \mathbf{HCl} \quad (2)$$

$$H_2O \overset{k_{H_2O}}{\rightleftharpoons} \mathbf{H_2O} \quad (3)$$

where the boldface indicates solutes within the membrane and the k_i are partition coefficients given by, for example

$$c_{NaOH} = k_{NaOH}(C_{Na} + C_{OH^-}) \quad (4)$$

Because the membrane solvent has such a low dielectric constant, the vast majority of ions form ion pairs, rather than existing as free ions.

After these solutes are dissolved in the membrane, they undergo a variety of further reactions

$$\mathbf{NaOH} + \mathbf{HCl} \overset{k_{NaCl}}{\rightleftharpoons} \mathbf{NaCl} + \mathbf{H_2O} \quad (5)$$

$$\mathbf{NaCl} + \mathbf{HOR} \overset{k_{NaOR}}{\rightleftharpoons} \mathbf{NaOR} + \mathbf{HCl} \quad (6)$$

$$\mathbf{NaOR} + \mathbf{HM} \overset{k_{NaM}}{\rightleftharpoons} \mathbf{NaM} + \mathbf{HOR} \quad (7)$$

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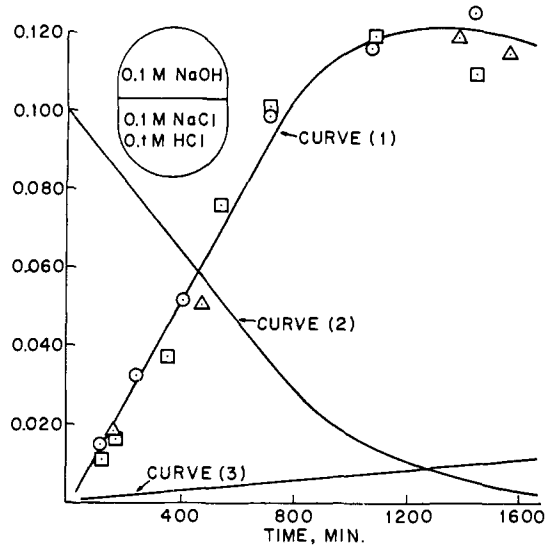

Figure 1. Sodium transport with monensin. Curve 1 and the open circles represents the sodium transported with monensin; curve 2 is the acid transported with monensin; and curve 3 represents the sodium transported without monensin. The squares illustrate sodium transport with the membrane clamp, and the triangles represent sodium moved with added sucrose.

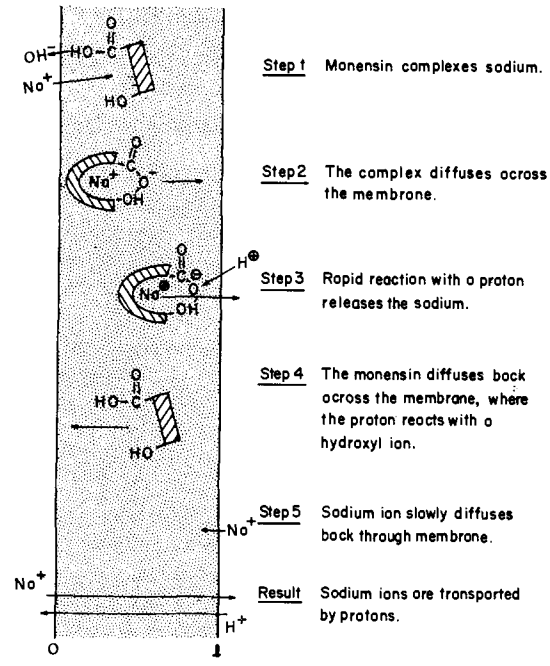

Figure 2. Mechanism for sodium ion transport.

where the symbols OR and M are for the octanate and monensin anions, respectively, and the K are association constants, for example

$$c_{NaCl}c_{H_2O} = K_{NaCl}c_{NaOH}c_{HCl} \tag{8}$$

The first of these reactions represents the obvious acid–base interaction; the sum of the second and third represent the reactions responsible for steps 1 and 3 in Figure 2. In fact, the second represents the membrane solvent octanol acting as a mobile carrier as well.

These reactions are by no means the only ones occurring in the membrane. For example, we have neglected the ionization of the sodium chloride ion pairs. We have chosen these six reactions because they provide a simple mathematical formulation of the most important reactions occurring in the membrane. Other equivalent sets could have been chosen.[6] If there are additional important reactions, the results of this derivation will not agree with the experimental results.

The chief assumption of this theory is that all of these reactions occur much faster than the diffusion steps 2 and 4 in Figure 2. More exact criteria for these relative speeds have been presented in terms of the second Damköhler numbers by several other investigators.[7,8] We know of no direct measurements of the reaction kinetics of the carriers used here. Kinetic studies of monactin[9] and the macrocyclic polyethers,[10] which are similar to our carriers, show that the reaction is much faster than diffusion when the membrane is thicker than 1000 Å. Ours are. Moreover, since our carriers are soluble only in the membrane, reactions outside of the membrane are negligible.[11]

Flux Equations. The second major assumption of this development is that the diffusion coefficients of all species within the membrane are equal; thus the flux equation is

$$j_i = -D(\partial c_i/\partial x) \tag{9}$$

where j_i is the flux and c_i is the concentration, both within the membrane, and the diffusion coefficients D are assumed the same for all species. In this assumption we are neglecting cross-diffusion effects, osmotic effects, and electrostatic effects. This assumption can be largely avoided,[12] but the resulting equations become extremely elaborate and cumbersome, and the physical consequences of the reactions given above are obscured. That these approximations are justified is shown by the experiments reported later.

With these assumptions, one may show that the average total concentration of each carrier, including both complex and uncomplexed forms, is independent of position within the membrane, *i.e.*,

$$c_{NaOR} + c_{HOR} = \bar{c}_{OR} \tag{10}$$

$$c_{NaM} + c_{HM} = \bar{c}_{M} \tag{11}$$

where $\bar{c}_{OR}$ and $\bar{c}_{M}$ are the average concentrations of octanol and monensin, respectively. Details of this proof are given elsewhere.[13] The continuity equation for each of the species within the membrane is

$$0 = -\frac{\partial}{\partial x}j_i + \sum_j r_{ij} \tag{12}$$

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where the r_{ij} are the reactions of solute i with solute j. For example, the equation for sodium chloride includes two reactions given by eq 5 and 6.

The total sodium flux across the membrane is the sum of fluxes of all sodium-containing species within the membrane.

$$J_{Na^+} = j_{NaOH} + j_{NaCl} + j_{NaOR} + j_{NaM}$$

Because sodium ion is not irreversibly altered by passing across the membrane, the sum over all the reactions is zero, and the total sodium flux is

$$J_{Na^+} = -\frac{D}{l}[\Delta c_{NaOH} + \Delta c_{NaCl} + \Delta c_{NaOR} + \Delta c_{NaM}] \quad (13)$$

Using the various reactions outlined in eq 1 through 8 and the mass balances in eq 10 and 11, we may now rewrite these concentration differences within the membrane in terms of the concentrations in the solutions adjacent to the membrane

$$J_{Na^+} = -\frac{D}{l}\left[\Delta\{k_{NaOH}c_{Na^+}c_{OH^-}\} + \Delta\left\{\frac{k_{NaOH}k_{HCl}K_{NaCl}c_{Na^+}c_{OH^-}c_{H^+}c_{Cl^-}}{k_{H_2O}c_{H_2O}}\right\} + \Delta\left\{\bar{c}_{OR}\Big/\left(1 + \frac{k_{H_2O}c_{H_2O}}{k_{NaOH}K_{NaCl}K_{NaOR}c_{Na^+}c_{OH^-}}\right)\right\} + \Delta\left\{\bar{c}_{M}\Big/\left(1 + \frac{k_{H_2O}c_{H_2O}}{k_{NaOH}K_{NaCl}K_{NaOR}K_{NaM}c_{Na^+}c_{OH^-}}\right)\right\}\right] \quad (14)$$

Thus this result shows that the flux varies with a difference of products in NaCl concentrations and partition coefficients, both of which can differ across the membrane. A similar equation developed for the total flux of protons is given elsewhere.[13]

The physical significance of eq 14 can be clarified by comparing it to eq 13. In both equations, the first and second terms within the square brackets represent the flux of ion pairs of NaOH and NaCl, respectively. Both these terms are negligible here because these solutes have such a low solubility within the membrane; *i.e.*, k_{NaOH} and $(k_{NaOH}k_{HCl}K_{NaCl})$ are both small numbers.[14] The third term in the square brackets represents the sodium flux assisted by the octanol acting as a nonselective mobile carrier. This is the term responsible for curve 3 in Figure 1.

The major effect, responsible for curve 1 in Figure 1 and outlined schematically in Figure 2, arises from the fourth term in the square brackets in eq 13 and 14. This is the term responsible for moving sodium ion selectively against its concentration gradient. Because this term is dominant, we can simplify eq 14 using the fact that c_{OH^-} is very small on the acidic side of the membrane

$$J_{Na^+} \approx -\frac{D\bar{c}_M}{l}\Big/\left\{1 + \frac{k_{H_2O}c_{H_2O}}{k_{NaOH}K_{NaCl}K_{NaOR}K_{NaM}c_{Na^+}c_{OH^-}}\right\}_0$$

where the subscript zero refers to the basic side of the membrane. Concentrations on the acidic side of the membrane do not appear in this equation because c_{OH^-} on the acidic side is much less than c_{OH^-} on the basic side except at the very end of the experiment. Thus J_{Na^+} should be linear in the total carrier concentration $\bar{c}_M$, and $1/J_{Na^+}$ should be linear with $1/(c_{Na^+}c_{OH^-})$. We have already verified this former prediction;[13] the latter one is discussed later in this paper.

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Experimental Section

Materials. Sodium monensin (Eli Lilly and Co.) was recrystallized from toluene and dried in a vacuum oven at 100°. It had a melting point of 273–274° as compared to 267–269° given in the literature.[3] The solubility of sodium monensin in water-saturated octanol is 14 wt %, a 0.21 *m* solution. 5β-Cholanic acid (Steraloids Inc.) was purified by first dissolving it in chloroform and then adding wet methanol. It had a melting point of 165–167° as compared to the literature value of 167–169°.[15] The solubility of cholanic acid in water saturated with octanol is 11 wt % or a 0.31 *m* solution. Sodium cholanate was prepared by dissolving an excess of sodium hydroxide in methanol and adding to it a solution of cholanic acid in chloroform.[16] The resulting precipitate of sodium cholanate was washed with distilled water, methanol, and chloroform and dried in a vacuum oven. Double distilled water was used in all experiments. All other salts and solvents were reagent grade and were used as received.

Apparatus. The diffusion apparatus used here was a modified diaphragm cell with a removable diaphragm,[17,18] which was the liquid membrane. The cell consisted of two 15-cm³ compartments constructed from O-ring joints of 2.5 cm i.d. The two compartments, which support the membrane between them, were clamped together with standard triangular pipe clamps and Buna-N O-rings. Each cell compartment contained a Teflon screen midway up the compartment on which rested a Teflon coated magnetic stirrer. Thus, both compartments could be stirred by rotating two large horseshoe magnets outside of the cell.[18] On both ends of the cell, 14/35 ground glass joints were closed with solid Teflon stoppers pierced by 19 gauge syringe needles. The needles in turn were sealed with Luerlok syringe valves.

The chief experimental problem in using this cell is to obtain a liquid membrane that remains intact. We achieved this by using a composite membrane consisting of a layer of dialysis paper cut from tubing, a piece of glass fiber paper (Reeve Co.), and then a second layer of dialysis paper. The glass filter paper and the dialysis paper were 0.05 and 0.02 cm thick, respectively; the membrane had a cross section area of 5.0 cm². We found the glass paper superior to ordinary filter paper, with which we were unable to maintain an effective barrier for small electrolytes. Earlier experiments using conventional filter paper gave a sodium concentration difference only a third of that shown in Figure 1. Using this composite membrane, we did not find it necessary to increase the pressure on the membrane liquid.[19]

There were two problems with the use of this composite membrane. First, it did give an apparent induction time for diffusion, of about an hour, giving the data a slight sigmoidal shape. This induction time, caused by the initial condition in the membrane,[20] does not significantly alter the effects observed here. Second, the membrane involves a series of diffusional resistances. The first resistance arises from the decreased stirring in our cells compared to those originally developed by Stokes.[18] We showed this was negligible by obtaining cell calibration constants within 5% for KCl, sucrose, and urea. The second resistance comes from the dialysis paper. We showed that this resistance had only a negligible effect on membrane transport in the absence of carriers but could contribute as much at 15% of the diffusional resistance of a carrier-containing membrane. Since dialysis paper was necessary for stability of the membrane, we did not remove it.

Procedure. The cell was assembled in the following series of steps. A piece of dialysis paper was conditioned by soaking it in the acid solution and a second piece by soaking it in the basic solution. A piece of glass fiber paper was impregnated with water-

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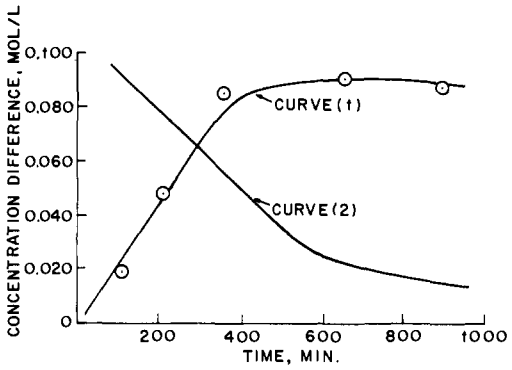

Figure 3. Sodium transport with cholanic acid. Curve 1 and curve 2 represent the sodium ion transported and the protons transported, respectively.

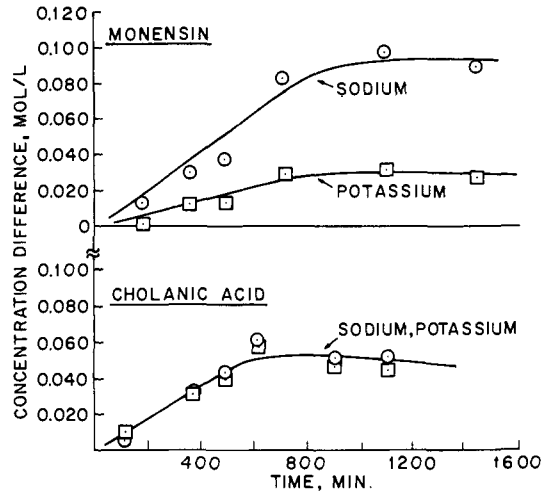

Figure 4. The relative selectivity of two mobile carriers. In both parts of the figure, the circles and squares represent the sodium and potassium ions transported, respectively.

saturated octanol-carrier solution. The composite membrane was then assembled. The bottom half of the diffusion cell with its O-ring in place was filled to the maximum extent possible with the acid solution saturated with octanol. The membrane assembly was then carefully slid across the top of the O-ring in order to exclude air bubbles from the bottom compartment. The O-ring and top compartment were then put in position. The compartments were clamped together and the top compartment was rinsed and filled with the basic solution. The assembled cell was enclosed in a polyethylene bag to prevent contamination or dilution of the membrane constituents through the edge of the membrane. The entire cell was then placed in a 25 ± 0.003° temperature bath for the duration of the experiment. At the end of a run, the cell solutions were titrated for acid, base, and chloride concentrations. The alkali metal concentrations were determined by atomic adsorption. Some experiments were made using voltage clamps[21,22] in order to maintain a 0 emf across the membrane. Diffusion coefficients in the membrane solution were measured using a conventional Stokes diaphragm cell filled with this solution.

Results and Discussion

In this section, we first illustrate the effect of adding mobile carriers to our liquid membranes; second, we examine selectivity and electrostatic and osmotic effects; finally, we test the theory developed above. The first part thus discusses the chemical bases and limitations of the membrane systems studied here. The second part involves phenomena important in many biological membranes. The final part again uses the complete chemical definition of these membranes to verify *a priori* predictions of observed behavior. In all these parts, the fact that all components of the membrane are known permits a much more complete understanding.

Effects of Mobile Carriers. As outlined in the introductory section, the sodium ion can be moved against its concentration gradient by use of a liquid membrane containing a mobile carrier. The results using monensin as the carrier have already been given in Figure 1; those using cholanic acid are given in Figure 3. For both carriers, the amount of sodium ion moved against its gradient is increased in order of magnitude by the addition of the mobile carrier. Other experiments[14] show that the permeability of the liquid membrane to the sodium ion has been increased at least two orders of magnitude by this addition.

Nevertheless, after one understands this effect in terms of the mechanism given in Figure 2, one wonders why it is not bigger. After all, the transport of each sodium ion in Figure 2 is achieved at the cost of only one proton. Thus the transport of 0.1 *M* H^+ should produce the transport of 0.1 *M* Na^+ and hence a concentration difference 0.2 *M* Na^+. In addition, a significant sodium ion concentration difference develops *without* mobile carrier.

The reason for this behavior can be seen from a more careful examination of the role of the membrane solvent, octanol. We found that both the acids and the sodium ion can, under some conditions, move reasonably rapidly across an intact membrane of octanol alone. The acids do this because of their solubility in octanol; *i.e.*, k_{HCl} in eq 2 is relatively large. Experimentally, the fluxes of HCl and $HClO_4$ are about five times less through octanol in the absence of added carrier than in its presence. This acid leak is significant because it limits the amount of sodium ion which can be moved.

The second problem with octanol results because this solvent itself acts as a mobile carrier. On the basic side of the membrane, the octanol reacts with sodium hydroxide to form sodium octanate and water; on the acidic side, the sodium octanate and proton react to produce sodium ion and octanol. However, when the relative concentrations of octanol and monensin are taken into account, the alcohol is seen (Figure 1, curve 3) to be a poor carrier by comparison. We found that we could eliminate this unwanted carrier effect by using membrane solvents like dichlorobenzene.

Nevertheless, we decided octanol was the best solvent for use with these specific carriers for several reasons. The carriers are very soluble in octanol, both as salt and as acid. Moreover, these membrane solutions have relatively high viscosities and low solubilities, which make it considerably easier to obtain a stable, reproducible liquid membrane. As a result, we continued using octanol in spite of its disadvantages and included its effect in our theory (eq 6).

Membrane Selectivity. The ability of these carrier-containing membranes to discriminate between alkali metal ions is illustrated by Figure 4 and Table I. The experiments shown in the figure initially involve a solution of 0.1 *M* NaCl, 0.1 *M* KCl, and 0.1 *M* HCl separated by the membrane from a solution containing 0.1 *M*

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Table I. Relative Selectivity of Mobile Carriers

	Na:Li	Na:K	Na:Cs
Monensin	8:1	3:1	4:1
Cholanic acid	1:1	1:1	1:1
Octanol	1:1	1:1	1:1

NaOH and 0.1 *M* KCl. In other words, the experiments differ from those reported in Figures 1 and 3 only in the addition of 0.1 *M* KCl to both cell compartments.

When monensin is the mobile carrier, the maximum concentration difference generated for sodium ion is three times larger than that generated for potassium ion. However, when cholanic acid is used, the concentration differences generated for the two ions are about equal. The same is true for octanol, as shown in Table I.

The reason for this selectivity is most easily understood in terms of the structures shown in Figure 5. Monensin with its six ether groups resembles a macrocyclic polyether, whose cation complexing properties are well known.[23,24] As in the polyethers, the existence of these groups enables monensin to wrap around metal cations through ion–dipole interactions between the cation and the ether oxygens, resulting in a hydrophilic hole which complexes ions only over a narrow size range. Moreover, the structure of monensin is such that configurational changes are permitted upon deprotonation. Cholanic acid, on the other hand, lacks such a complexing mechanism; its basic configuration is that of a steroid. Except for the butanoic acid group, the structure is rigid with little configurational changes possible, and the interaction of anion and cation occurs only through coulombic forces. As a result, it does not complex selectively with cations but only forms simple ion pairs.

Electrostatic and Osmotic Effects. Electrostatic and osmotic effects have frequently been found to be important in studies of membrane transport systems.[5] The electrostatic effects originate because an emf exists across the membranes. In our case, this force is initially 60 mV and drops to a value of 40 mV when the maximum sodium ion concentration difference occurs. It acts in the same direction as the sodium ion flux and hence could be responsible for part of this flux.

We removed this electrostatic potential by maintaining the emf across the membrane at 0 mV with a voltage clamp. This made no difference in our results; the data with the voltage clamp (the triangles in Figure 1) fall on the same curve as the results without the voltage clamp (the circles in Figure 1). This is in sharp contrast to the behavior expected from biological studies. In our case, the lack of an electrostatic effect is a consequence of the exchange of a sodium ion for a proton (*cf.* Figure 2) and of diffusion of uncharged species through the membrane.

Osmotic effects across these membranes may occur because the ionic strength on the basic side is less than that on the acid side. The difference might be expected to decrease the sodium ion concentration difference developed by accelerating the movement of HCl and

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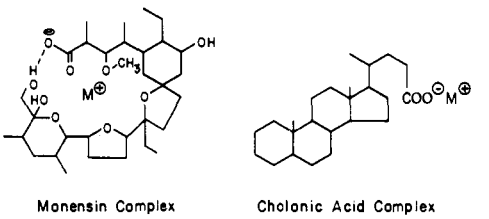

Figure 5. Chemical structures of monensin (a) and cholanic acid (b).

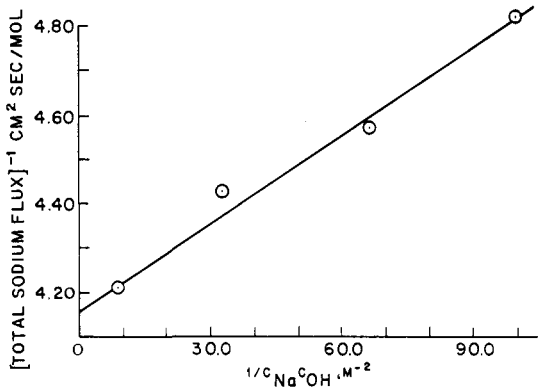

Figure 6. Test of eq 15 with the mobile carrier cholanic acid.

NaCl across the membrane to the basic side. Equalization of the osmotic effects by the addition of sucrose on the basic side resulted in no effect. The results with added sucrose (squares in Figure 1) fall on the same curve as those without sucrose.

Verification of Theory. The theoretical development earlier in this paper predicted that the reciprocal of the total sodium ion flux $1/J_{Na^+}$ should be linear in the reciprocal of the product of sodium ion and hydroxide ion concentrations $c_{Na^+}c_{OH^-}$ on the basic side of the membrane. This type of plot represents a linearization similar to plots used in enzyme kinetics, although the substrate dependence here is more complex. The theory also predicts that the sodium flux should be independent of both sodium and hydroxide concentrations on the acidic side. The key assumptions in deriving this relation are that all reactions are fast relative to diffusion, that all diffusion coefficients are equal, and that the added mobile carrier dominates the sodium ion flux.

The results in Table II and Figure 6 verify this theory.

Table II. The Initial Concentration of Sodium Ion and Hydroxide Ion Used in the Determination of $1/J_{Na}$

C_{Na^+}, *M* (basic)	C_{OH^-}, *M* (basic)	C_{Na^+}, *M* (acidic)	C_{OH^-}, *M* (acidic)	$1/J_{Na} \times 10^{-5}$, (cm^2 sec)/mol
Cholanic Acid (0.25 *M*)				
1.100	0.100	1.100	10^{-13}	1.01
0.300	0.100	0.300	10^{-13}	1.11
0.150	0.100	0.150	10^{-13}	1.15
0.100	0.100	0.100	10^{-13}	1.23
Sodium Monensin (0.17 *M*)				
1.100	0.100	1.100	10^{-13}	4.22
0.300	0.100	0.300	10^{-13}	4.43
0.150	0.100	0.150	10^{-13}	4.57
0.100	0.100	0.100	10^{-13}	4.79

These experiments, using either cholanic acid or monensin as carriers, were most conveniently made at relatively high sodium and hydroxide concentrations. This ensures that the bulk of the ionic solutes in the membrane are present as ion pairs, consistent with eq 1-8; however, it also means that the total flux of sodium ion does not change radically with altered c_{Na^+} and c_{OH^+}. Thus, Table II and Figure 6 represent not only a test of eq 15 but also additional evidence of our experimental reproducibility.

To check this equation more completely, we also measured the specific constants involved for the cholanic acid system. For this system, the intercept in Figure 6 has the value 1.0×10^5 cm^2 sec/mol. According to eq 15, this intercept equals $[l/(D\bar{c}_M)]$. We determined the diffusion coefficients D of sodium cholanate and cholanic acid to be 0.84×10^{-5} and 0.63×10^{-5} cm^2/sec, respectively. This gives an average value of 0.74×10^{-5} cm^2/sec. We used an average carrier concentration $\bar{c}_M$ of 0.25 mol/l. in all runs. From the intercept and $[l/(D\bar{c}_M)]$, an effective membrane thickness l of 0.2 cm was calculated, which is about three times greater than the actual membrane thickness. Since the effective membrane thickness l differs from the actual thickness by a tortuosity factor on the order of three, we feel that this is a good agreement.

For the cholanic acid system, the slope in Figure 6 is found experimentally to be 2.2×10^{-4} (mol sec)/cm^4. According to eq 15, this slope is

$$\frac{l}{D\bar{c}_M}\left(\frac{k_{H_2O}c_{H_2O}}{k_{NaOH}K_{NaCl}K_{NaOR}K_{NaM}}\right)$$

We took $(l/D\bar{c}_M)$ to be the experimental value of 1.0×10^5 (cm^2 sec)/mol. The various equilibrium constants are as follows: $k_{H_2O}c_{H_2O} - 0.83$ mol/l.;[25] $k_{NaOH} = 2 \times 10^{-2}$ l./mol;[14] $K_{NaCl}K_{NaOR} = 0.6$;[26] and $K_{NaM} = 5.2 \times 10^4$.[14] The value of the slope calculated from these constants is 1.9×10^{-4} (mol sec)/cm^4, again in good agreement with the experimental value.

The correlation of the flux and the product of c_{Na^+}-c_{OH^-} shown in Table II and Figure 6 is frankly better than we expected. Indeed, Figures 1 and 3 show a concentration difference varying linearly with time for surprisingly long times. Why this is so can be seen by reexamining the cause of the major effect, *i.e.*, the difference between the basic and acidic sides of the product ($c_{Na^+}c_{OH^-}$) (*cf.* eq 14). At the start of the experiment (*cf.* Figure 1), the product is 10^{-2} mol^2/l.^{2} on the basic side, and 10^{-14} mol^2/l.^{2} on the acidic side. As a result, the concentrations on the basic side are not important, as indicated by eq 15. However, even after 12 hr, the product is about 0.16×10^{-2} mol^2/l.^{2} on the basic side and is about 4×10^{-14} on the acidic side. Thus the acidic side concentrations are still unimportant and eq 15 is valid beyond the range originally expected.

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