Membranes Which Pump

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Carrier-containing membranes which can pump a specific ion from a region of low concentration into a region of high concentration have been constructed. This pumping is a large effect, proportional to the amount of carrier present. The main requirement for this type of transport is that the carrier react competitively with two simultaneously diffusing solutes. One solute is pumped; the second supplies the energy for the pumping. A theory based on the assumption that the two carrier reactions are fast predicts that the amount pumped is proportional to the carrier concentration, that the pumping stops when the concentration gradient of the energy supplying solute reaches zero, and that the effect goes through a maximum when the solubility in the membrane of the solute being pumped decreases. These predictions are in agreement with experimental results for sodium transport across membranes containing a variety of carriers, including stearic acid, lecithin, and monensin.

Membranes which can pump a specific solute against its own concentration gradient have been constructed in this laboratory. Such a membrane can move a single solute from a region of low solute concentration into a region of high solute concentration. For example, in one experiment the membrane was placed between two solutions, each of which contained initially 0.10*N* sodium ion. After the experiment, one solution contained 0.04*N* sodium ion and the other contained 0.16*N* sodium ion. Even more importantly, this transport can be specific: a membrane which will pump sodium, but will not pump potassium, has been developed (*1*). These membranes are very important for two reasons: they allow simple in vitro models for some of the membrane transport systems found in living systems; and they have major potential in separating and concentrating specific solutes on an industrial scale.

Living systems contain a large number of membrane transport systems, including processes for moving sodium, calcium, glucose, and phosphate. In several of these systems the flux of a solute is regulated by the concentration gradient of a second solute in a manner similar to that operating here. Industrial separations based on these membranes would probably find greatest application in individual ion recovery from brines and metallurgical wastes. Since the technique can be made very specific, silver ions can be easily separated from copper and bromide ions removed and concentrated from sea water. A long range industrial objective of this work is the development of a membrane to separate racemic lysine to give large inexpensive quantities of L-lysine for use as a food supplement.

These membranes depend for their operation on a carrier, which is retained within the membrane. The carrier reacts competitively with two solutes which are being transported across the membrane by means of the mechanism shown in Figure 1. In this figure two well stirred solutions containing solutes at the concentrations shown are separated by the carrier-containing membrane denoted by the vertical region. One solute, denoted by 1 in the figure, is that being transported against its own concentration difference across the membrane. The second solute, denoted by 2, is transported by the carrier but in the same direction as its concentration difference. However, as is clear from the figure, the solute-carrier complex of either 1 or 2 diffuses in the same direction as its own concentration gradient. The overall result is that the transport of solute 1 can be governed not by its own concentration difference across the membrane, but by the concentration difference of another solute.

The membrane effects described in this paper are similar to the phenomena of coupled facilitated diffusion, countertransport, and counterflow previously reported in the literature (*2* to *6*). They are especially comparable with those observed in the oxygen-carbon dioxide-hemoglobin system (*2, 6*). However, they are emphatically not active transport, which is conventionally restricted to pumping where the energy source is a chemical reaction.

The purpose of this paper is to develop a theory describing membranes containing a carrier which reacts competitively with two solutes, and to compare qualitatively the theoretical predictions with some experimental results. More complete experiments will be published later. The

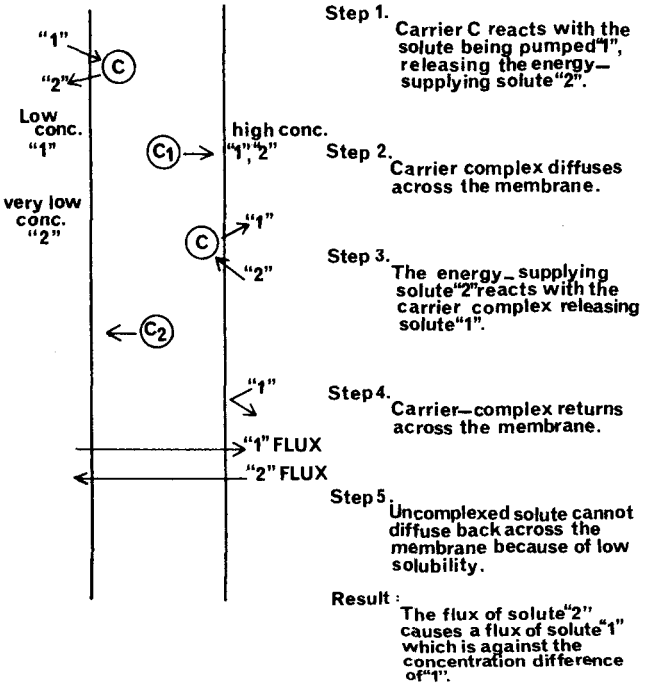

Fig. 1. Method of carrier operation.

contribution of this work is not in the mathematics, which is perfectly straightforward; it is in the application of these calculations to the investigation of novel phenomena of major biological and industrial importance. However, while membrane pumps may eventually be developed for a wide variety of solutes, those in existence at present pump only specific cations.

THEORY

In this section we calculate the steady state flux of two solutes simultaneously diffusing across a carrier containing membrane which separates two well-stirred solutions. The two solutions contain only the two diffusing solutes. The membrane contains a total of five solutes: diffusing solutes 1 and 2, carrier and two carrier solute complexes 13 and 23. The two complexes are formed by very rapid competitive reaction between the solutes and the carrier, so that their concentrations are subject to the equilibrium

$$c_{i3} = K_i c_i c_3 \quad i = 1, 2 \tag{1}$$

Since the carriers studied here have reaction half lives smaller than 10^{-6} sec., the assumption of equilibrium is not critical except near the membrane solution interface. This region is discussed in detail below.

The flux equations for all species within the membrane are assumed to be

$$-\mathbf{j}_i = D\nabla c_i \quad i = 1 \ldots 3, 1s, 2s \tag{2}$$

If the diffusing solutes are ionic, Equation (2) should have the form

$$-\mathbf{j}_i = D_i(\nabla c_i + z_i c_i F \nabla \varphi)$$

But if all D_i are equal, then $\nabla\varphi$ is zero because of the conditions of zero current and of electroneutrality. While the assumption of constant D and the implicit assumption of uncoupled flows [that is, $\mathbf{j}_i \neq f(\nabla c_{j\neq i})$] can be removed (7), the resulting equations show such algebraic complexity that their physical significance is obscure. If these two assumptions are made, attention is clearly focused on the two competing chemical reactions with the carrier.

The actual calculation of the total steady state flux across the carrier-containing membrane begins with the continuity equations for steady state diffusion of a solute, a solute-carrier complex, and the carrier:

$$\frac{\partial j_i}{\partial x} = -r_i \quad i = 1 \ldots 2 \tag{3}$$

$$\frac{\partial j_{is}}{\partial x} = r_i \quad i = 1 \ldots 2 \tag{4}$$

$$\frac{\partial j_3}{\partial x} = -r_1 - r_2 \tag{5}$$

where r_i is rate of formation of complex 3 from solute i and carrier 3. Because the solution volumes are large, the boundary conditions on the ionic species are

$$x = 0 \quad c_i = k_i C_{iB} \quad i = 1, 2 \tag{6}$$

$$x = l \quad c_i = k_i C_{iA} \quad i = 1, 2 \tag{7}$$

The partition coefficient k_i has the same value at $x = 0$ and $x = l$. The carrier is subject to the restraint

$$\frac{1}{l}\int_0^l (c_3 + c_{1s} + c_{2s})\, dx = \bar{c} \tag{8}$$

In addition, the carrier must obey the boundary condition (8):

$$x = 0, l \quad j_3 + j_{1s} + j_{2s} = 0 \tag{9}$$

This condition that the carrier flux is discontinuous at the membrane interface is an approximation implied by the statement that the carrier-solute reaction is very fast, so that Equation (1) holds everywhere. The error introduced by this approximation is much less than 1% for the systems studied here (5).

To determine the concentration profiles of all species, we must first find the free carrier concentration. We sum Equation (4) over both solutes, add the result to Equation (5), and use Equations (8) and (9) to obtain

$$c_3 = \frac{\bar{c}}{1 + K_1 c_1 + K_2 c_2} \tag{10}$$

This relation gives the concentration of unreacted carrier as a function of the cations in the system. The total flux of solute 1 can now be found by combining Equations (2) to (4), integrating, and using Equations (1), (6), (7), and (10) to obtain

$$\begin{aligned} j_1 + j_{1s} = &\left[\frac{Dk_1}{l}\right](C_{1B} - C_{1A}) \\ &+ \left[\frac{Dk_1}{l}(R\,1 + k_2K_2\bar{C}_2)\right](C_{1B} - C_{1A}) \\ &- \left[\frac{Dk_1}{l} Rk_2K_2\bar{C}_1\right](C_{2B} - C_{2A}) \end{aligned} \tag{11}$$

where

$$\bar{C}_i = (C_{iA} + C_{iB})/2$$

$$R = \frac{K_1\bar{c}}{(1 + k_1K_1C_{1A} + k_2K_2C_{2A})(1 + k_1K_1C_{1B} + k_2K_2C_{2B})}$$

The first term on the right-hand side of Equation (11) represents the flux due to ordinary diffusion; the second, which gives flux due to facilitated diffusion, has been derived previously. The third term on the right-hand side gives the pumping, that is, the effect of the two competitive reactions with the carrier. The terms in square brackets in Equation (11) are closely related to the coefficients of solute permeability multiplied by RT, as these coefficients are defined in the biophysics literature (9).

Three special cases of Equation (11) are of interest. In the first case the carrier reacts with only one of the diffusing solutes (for example, $K_2 = 0$). For this solute, Equation (21) reduces to the usual equation for facilitated diffusion:

$$j_1 + j_{1s} = \frac{Dk_1}{l}\left[1 + \frac{K_1\bar{c}}{(1 + k_1K_1C_{1A})(1 + k_1K_1C_{1B})}\right](C_{1B} - C_{1A}) \tag{12}$$

Here, the flux of cation 1 is zero if its own concentration difference across the membrane is zero (that is, $j_1 + j_{1s} = 0$ if $C_{1B} = C_{1A}$).

The second special case is in sharp contrast, because both competitive cation-carrier reactions are involved. In this case, species 1 is present at uniform concentration ($C_{1B} = C_{1A} = \bar{C}_1$) so that Equation (11) reduces to

$$j_1 + j_{1s} = -\frac{Dk_1}{l}$$

$$\left[\frac{K_1k_2K_2\bar{c}\bar{C}_1}{(1+k_1K_1C_{1B}+k_2K_2C_{1B})(1+k_1K_1C_{1A}+k_2K_2C_{2A})}\right] \quad (13)$$
$$(C_{2B}-C_{2A}).$$

The flux of species 1 is caused by the concentration difference of species 2. In other words, species 1 is pumped across the membrane by species 2. Since the quantity in square brackets can be much greater than unity (for example, if $K_1 = K_2 >> 1$ and $k_1 = k_2 < 1$), this pumping can be much greater than ordinary diffusion. This is the effect which can move or pump cation 1 against its own concentration gradient. It is characteristic of a membrane in which a carrier reacts competitively with two cations.

The third and final special case of Equation (11) calculates the maximum concentration difference which can be maintained by this pumping. To simplify the result, we assume that the energy-supplying solute is not present on one side of the membrane ($C_{2A} = 0$). Since $j_1 + j_{1s} = 0$, Equation (11) becomes

$$C_{1B} - C_{1A} = \frac{1}{k_1}\left[\frac{\bar{c}}{1+\dfrac{k_1K_1C_{1B}}{k_2K_2C_{2B}}}\right] \quad (14)$$

This concentration difference can be very large (for example, if $k_1 << 1$ and if $k_1K_1/k_2K_2 \leq 1$). This difference is much larger than that observed in multicomponent diffusion. Again, we see that an important and novel effect occurs in membranes containing competitive reactions with a carrier solute.

EXPERIMENTAL

Reagent grade materials were used as received for all experiments. The sodium salt of monensin used as a carrier in many experiments was kindly supplied by Eli Lilly Company. All diffusion runs were made in diaphragm cells very similar to those described previously (*10*). These cells are similar to those of Stokes (*11*), but are made from a glass pipe joint so that a variety of membrane materials can be clamped across the cell. The majority of the membranes used were no more than a piece of filter paper soaked in an organic solution of the carrier. However, these membranes frequently leaked during experiments longer than 24 hr. Membranes made by clamping the filter paper between two sheets of cellophane were somewhat more reliable, but experiments were tediously long. Ionic concentrations were measured by flame ion photometry or by atomic absorption, both of which gave equivalent results.

RESULTS AND DISCUSSION

The theory developed above yields four predictions which are qualitatively verified by the experiment results: (1) the concentration differences generated by pumping are large, much larger than those caused by multicomponent diffusion; (2) pumping stops when the concentration difference of the energy supplying solute is exhausted; (3) the amount pumped is directly proportional to the carrier concentration; and (4) the amount of pumping goes through a maximum when the solubility in the membrane of the solute being pumped is decreased.

Evidence for the first of these four points, that the pumping is a large effect, is shown by the sodium ion pumping data reported in Table 1. In these experiments sodium ion is pumped by a flux of protons. The top compartment of the diaphragm cell contains 0.1*n* NaOH, and the bottom compartment contains 0.1*n* HCl and 0.1*n* NaCl. The sodium ion concentration difference is thus initially zero, but becomes large during the experiment if a carrier is present. If no carrier is present, the concentration difference generated is much smaller, as shown by the last two lines in the table. This small concentration difference is a measure of electrostatic and multicomponent diffusion effects arising from the membrane potential. The maximum concentration difference which could be generated by the mechanism developed above is 0.1 mole/liter, or 1.00 on the fractional scale in Table 1, since for each sodium ion pumped a proton diffusing in the opposite direction is required (cf. Figure 1). Some of these experiments are obviously approaching this maximum.*

TABLE 1. SODIUM ION PUMPING

Bulk solvent	Membrane solvent	Membrane carrier	Sodium conc. difference°
Water	Trichloroethane	Silicones	0.40
	Octanol	Silicones	0.95
	Pentanol	Stearic acid	0.90 (*14*)
	Hexanol	Mixed fatty acids	0.75
	Hexanol	Lecithin	0.65
	Hexanol	Monensin	0.45
	Benzene	Monensin	0.07
Ethanol†	Ethanol	Monensin	0.10
Water	Hexanol	None	0.07
	Octanol	None	0.11

° Expressed as concentration difference divided by average sodium concentration.

† Average sodium concentration of 0.02 mole/liter. Monensin retained within the membrane with ultrafiltration membranes.

The second prediction from Equation (11), that pumping stops when the acid concentration difference is exhausted, is verified in Figure 2 for the sodium ion concentration difference generated using monension as a carrier. A macrocyclic antibiotic, monensin specifically complexes sodium at high *p*H, forming a clathratelike structure similar to those of other macrocyclic antibiotics and cyclic polyethers (*12*). At low *p*H monensin is protonated and releases its bound sodium ion. In Figure 2 the sodium concentration difference caused by monensin is plotted versus time as the solid curve, and the acid concentration difference is shown by the dotted curve. Beginning at 0.1 mole/liter, the acid concentration difference drops rapidly until 8 hr., when it is essentially exhausted. At this point the sodium ion concentration difference reaches its maximum value. The lower dashed curve gives the sodium concentration difference generated without carrier, and again shows that electrostatic and multicomponent diffusion effects are small, consistent with predicted values (*13*).

The third prediction of Equation (11), that the amount pumped is proportional to the amount of carrier, is verified by the following data: with 0.07 mole/liter monensin, the maximum sodium ions concentration difference generated is 0.045 mole/liter; with 0.04 mole/liter monensin, it is 0.024 mole/liter; and with 0.010 mole/liter monensin, it is 0.010 mole/liter. If these concentration differences are plotted versus monensin concentration, they fall approximately on a straight line. The intercept of this line at zero monensin concentration represents electrostatic and multicomponent diffusion effects shown in Figure 2.

The fourth prediction of the theory, that the pumping goes through a maximum as the solubility decreases, is also illustrated by the data in Table 1. The pumping with monensin in ethanol is small; that in hexanol is much larger; but that in benzene is again small. This behavior can be explained by reference to Equation (11). For benzene, NaCl is so insoluble in the membrane (that is, $k_1 << 1$)

* The author writes that he has succeeded in obtaining 0.97 sodium concentration difference with monensin.

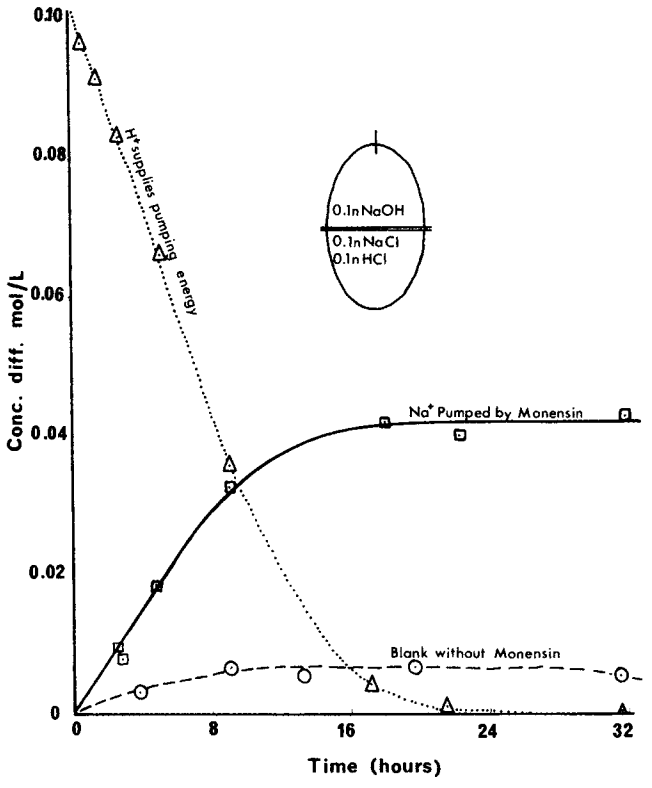

Fig. 2. Sodium pumped by monensin hexanol membrane.

that both ordinary diffusion and pumping are very small. The concentration difference generated thus remains small. In ethanol, NaCl is soluble in the membrane in the same degree as in the bulk (ethanol) solution (that is, $k_1 = 1$). While this means that the pumping is large, the ordinary diffusion is also. Thus any concentration difference generated by the pumping quickly disappears by means of ordinary diffusion in the opposite direction. The hexanol case is intermediate between the two extremes. While the solubility in the membrane is low ($k_1 < 1$), the salt complexes strongly with the carrier ($k_1K_1 > 1$). As a result, while the limited solubility produces small ordinary diffusion, the strong salt-carrier reaction gives large pumping. The most successful experiments in Table 1 are in this intermediate solubility range.

The quantitative success of the theory developed above, which will be investigated in a later paper, depends on two key assumptions: that all diffusion coefficients are constant [Equation (2)], and that the two competitive reactions with the carrier are very fast [Equation (1)]. The first assumption can be removed easily, although the results are algebraically complex. If necessary, these more complete equations will be used for the quantitative verification. The second assumption, that the carrier reacts rapidly with the diffusing solutes, is much more critical. In more exact terms, this assumption states that these reactions are faster than the diffusion, that is, the second Damköhler number is large:

$$\frac{(k_i\bar{c}_s)l^2}{D} >> 1$$

where k_ic_s is the reaction rate for the formation of one of the carrier solute complexes. Since both the weak acids and the macrocyclic antibiotics used here as carriers react extremely rapidily with both protons and cations, this condition is very easily realized for the membranes used in these experiments. However, for a biological membrane only 100 Å. thick, this condition is more stringent. For example, if the reaction rate k_ic_s is 10^7 sec.$^{-1}$, the derivation presented here is in error by about 10% (5).

In conclusion, we have investigated carrier-containing membranes which can pump a solute against its own concentration gradient. The large effects observed experimentally can be explained by a theory based on the rapid, competitive reaction of the carrier with two simultaneously diffusing solutes. One solute is that being pumped; the other supplies energy for the pumping. Since membranes of this type can separate and concentrate specific solutes, they are expected to find application both in biological modeling and in industrial processes.

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NOTATION

- $\bar{c}$ = total carrier concentration in reacted and unreacted forms
- c_i = concentration of diffusing solute; $i = 1, 2$
- c_3 = concentration of unreacted carrier
- c_{i3} = concentration of carrier-solute complex; $i = 1, 2$
- C_i = concentration of i in solution adjacent to the membrane
- D = diffusion coefficient, equal for all species
- $F\varphi$ = electrostatic potential divided by RT
- $\mathbf{j}_i$ = flux of species i relative to the volume fixed reference frame
- k_i = partition coefficient between membrane and adjacent solution
- K_i = equilibrium constant for reaction between solute and carrier
- l = membrane thickness
- R = interaction parameter, Equation (11)
- r_i = reaction rate of carrier with species i
- X = distance coordinate across membrane
- z_i = ionic charge

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