THE "PUMP-LEAK" MODEL AND EXCHANGE DIFFUSION

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ABSTRACT Steady-state concentration gradients across cell membranes have often been attributed to the associated leakage of solute down its electrochemical potential gradient, and active transport at an equal rate in the opposite direction. Several workers have evaluated the minimal energetic requirements of such a "pump-leak" model for sodium in muscle tissue, presuming that influx occurs only via the leak pathway and to no extent by way of the active transport pathway. The high energy requirements so predicted have led to the suggestions that either (*a*) sodium is not actively transported, being at equilibrium distribution across the cell surface, or (*b*) substantial sodium movement must be by means of exchange diffusion. The present treatment, based on the consideration that the active transport mechanism is bidirectional, demonstrates that the rates of influx and efflux associated with a given rate of active transport are explicit functions of two parameters: (1) the ratio of the exchange resistance of the active pathway to that of the leak pathway, and (2) the electrochemical potential difference across the cell surface. Lacking precise values for these parameters, the demonstration of a high rate of isotope flux is not compelling evidence either against active transport or for a discrete exchange diffusion mechanism. Various concepts and criteria of exchange diffusion are discussed.

GLOSSARY

f	flux ratio
F	Faraday's number
J	net flow of test species per unit area of membrane in the x direction
J_j	flow of species j
J^z	flow of isotopic tracer of test species
r_{00}	local phenomenological coefficient of test species
r_{0j}	local phenomenological coefficient relating flows of test species and species j
r_{ik}	local phenomenological coefficient relating flows of isotopes of test species
R	integral phenomenological coefficient, $\int_0^{\Delta x} r_{00}dx$
R^z	$\int_0^{\Delta x} (r_{00} - r_{ik})dx$
$-R_{0r}J_r$	integral of the metabolic force, $-\int_0^{\Delta x} r_{0r}J_r dx$
R	gas constant

T absolute temperature
X negative electrochemical potential difference
ρ specific activity
The superscripts a and p refer respectively to the active and passive pathways.
The subscripts c and m refer respectively to the interior of cells and the bathing medium.

INTRODUCTION

A fundamental problem in the analysis of the mechanisms and energetics of transport processes is the evaluation of the rate of active transport. For epithelial tissues which maintain continuous flow of various substances from one surface to another this is often readily accomplished. When such a membrane is exposed to identical solutions at each surface and the electrical potential difference across the tissue is nullified, in the absence of coupled flows of different species any net flow can be unequivocally attributed to active transport. Following Ussing and Zerahn, this "short circuit" technique has been employed in the study of a variety of epithelial membranes (1).

Many biological cells, however, are symmetrical, and therefore in the steady state the net flow of substances which are neither produced nor consumed must be zero. This need not mean, however, that there is no active transport of these species, for, despite the absence of net flow, they may not be at equilibrium distribution across the cell membrane. Such may be the case, for example, for sodium ions in red blood cells and muscle, for which the intracellular electrochemical potential appears to be less than that in the extracellular medium. Since cell membranes are permeable to a variety of substances, it has been suggested that the steady-state distribution of Na^+ in these tissues is the result of associated movement of Na^+ into the cell through a "leak" pathway and active transport out of the cell by the action of a "pump" carrier (2).

The validity of this "pump-leak" model for Na^+ in muscle tissue has been evaluated by several workers in terms of the energetic considerations (3–8). Such an analysis requires the estimation of both the electrochemical potential difference of Na^+ across the cell membrane, $-X$, and its rate of active transport, J^a. In the steady state their product, $-J^a X$, represents a minimal value for the rate of energy expenditure consistent with the model, which may be compared with estimates of the rate of supply of metabolic energy. Unfortunately, unlike the case for epithelial tissues, there are difficulties in the determination of both the above quantities. In this paper we shall consider the evaluation of one of these, the rate of active transport.

In general, the evaluation of the rate of active transport has been carried out by one of two isotopic techniques. One of these is the measurement of the rate of uptake of radioactive Na^+ from the bathing medium. If we neglect or correct for the extracellular fluid volume and back flux, the quotient of the rate of isotope flow and the specific activity of the bath evaluates the rate of "influx" of Na^+ into the cells. If we accept a commonly cited value for X/F of about -120 mv in muscle, with the cells

negative to the medium, the influx across a simple aqueous leak pathway will be far greater than the efflux (9, page 49), and will therefore closely approximate the net leakage of sodium into cells. If in addition we make the common simplifying assumption that the tracer enters the cells only by way of discrete leak pathways, and to no extent by way of the active transport mechanism, it is clear that in the steady state, when the overall net flow must be zero, J^a, the rate of active transport, will be closely approximated by the influx measured with a tracer (Fig. 1 *a*).

A second means of evaluating the rate of active transport of Na^+ is the measurement of the rate of appearance in the medium of the radioactive isotope derived from preloaded tissue. This measurement permits the calculation of the rate of "efflux." If it is assumed, as above, that the efflux occurs predominantly via the active transport pathway, and that the influx occurs only by way of the leak pathway, this value also would approximate the rate of active transport.

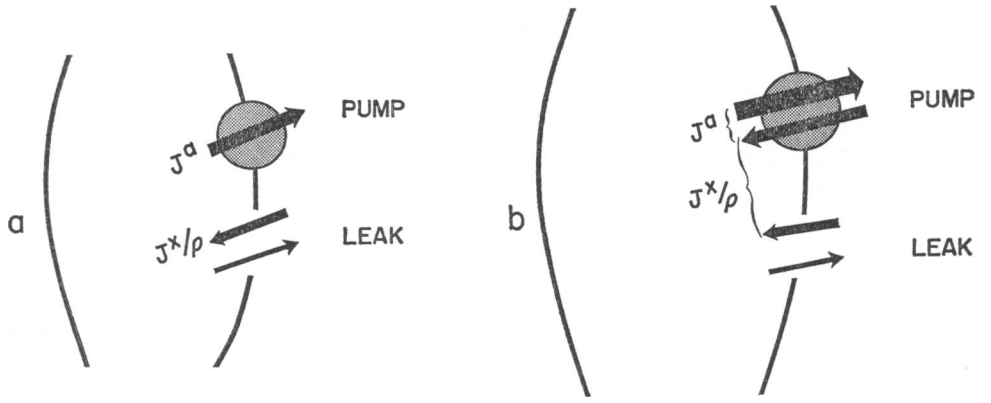

FIGURE 1 *a* "Pump-leak" model: Unidirectional active transport mechanism. 1 *b* "Pump-leak" model: Bidirectional active transport mechanism.

The application of both the above techniques to studies of muscle has led to values of J^a which, when combined with commonly cited values for X, predict impressively high minimal energy requirements, in one case well in excess of the available energy (7, 8). Ling has attributed this discrepancy to overestimation of $-X$. According to his association-induction hypothesis, sodium exists in protoplasm in a very different physical state from that in aqueous solution, so that despite its low concentration and electronegativity within the cell, it is in equilibrium with the sodium outside the cell, and there is in fact no active transport across the surface. Ussing and others have taken a very different viewpoint, suggesting that a substantial part of the isotope flow may occur by "exchange diffusion," requiring no expenditure of metabolic energy (4; 9, page 50).

In evaluating these two hypotheses it must be appreciated that the cited estimates of the rate of active transport are critically dependent on the assumption that Na^+

enters muscle cells only by way of a leak pathway, and to no extent via the active transport pathway (Fig. 1 a). However, an active transport mechanism of finite free energy permits the movement of Na^+ in either direction, depending only on the orientation of the total force acting on the particular test species (10, 11) (Fig. 1 b). In particular, a given isotopic tracer species of Na^+ would move by way of the active transport pathway in a direction opposite to that of active transport of total Na^+, provided that its electrochemical potential gradient were of appropriate magnitude and orientation to overcome the opposing metabolic forces. The implications of this consideration may be conveniently developed in terms of a recent analysis of isotope flows applicable to systems with continuity of electrochemical potential (11). It appears that a "pump-leak" mechanism may in fact be energetically compatible with the reported results.

GENERAL BACKGROUND

It is useful first to summarize certain equations derived previously which are applicable to net flow and isotope flows through a homogeneous array of parallel pathways (11). For simplicity, we shall ignore the influence of the extracellular space and consider only that component of the isotope flux associated with the intracellular region, which will be regarded as homogeneous. In practice, a multicompartmental analysis may be necessary in order to correct for the influence of series and parallel compartments.

We shall assume that during the periods employed, back flux of isotope will be slight and may be ignored. Designating the specific activity of the intracellular region by ρ_c, and the rate of isotope flow by J_c^x, the rate of "efflux" is given by J_c^x/ρ_c. Similarly, if a second tracer isotope is placed in the medium bathing the cells, $-J_m^x/\rho_m$ represents the rate of "influx." As is intuitively evident, the net flow of the total test species is given by

$$J = \frac{J_c^x}{\rho_c} + \frac{J_m^x}{\rho_m} = \text{efflux} - \text{influx}. \tag{1}$$

(In the previous paper, dealing primarily with epithelial membranes, J was defined as influx − outflux. In the present paper, directed most particularly at the transport of Na^+ in symmetrical tissues, it is convenient to conform to general usage, which considers the outward active transport of sodium as positive.)

The flux ratio f is given by definition as

$$f = \frac{-J_c^x/\rho_c}{J_m^x/\rho_m}. \tag{2}$$

Equations (1) and (2) give

$$\frac{J_m^x}{\rho_m} = \frac{J}{1-f} \tag{3 a}$$

and

$$\frac{J_c^x}{\rho_c} = \frac{fJ}{f-1}. \tag{3 b}$$

Finally, for a pathway in which the electrochemical potential is everywhere continuous the combination of equations (28) and (29) of reference (11) gives

$$JR^x = RT\ 1nf = \frac{R^x}{R}\left(X - \int_0^{\Delta x} \sum r_{0j}J_j dx\right). \tag{4}$$

Here R^x, the "exchange resistance," $= \int_0^{\Delta x} (r_{00} - r_{ik})dx$, where r_{00} is the local phenomenological resistance coefficient to the flow of the test species and r_{ik} is that introducing the coupling of flows of different isotopic forms of test species, "isotope interaction." (As is seen, R^x can be determined by measurement of the net flux and the flux ratio. Alternatively, in the absence of a net flux, R^x can be determined by measurement of the isotope flux produced by a given difference of specific activity across the membrane, as $(J^xR^x)_{J=0} = -RT\Delta\rho$. In the absence of isotope interaction $R^x = \int_0^{\Delta x} r_{00}dx = R$, the resistance to net flow.) The integral $-\int_0^{\Delta x} \sum r_{0j}J_j dx$ comprises the contribution of any coupled flows of other species, as well as that of metabolism, to the forces influencing the flux of the test species; hence equation (4) may be applied to an array of either passive or active pathways. It is emphasized that equation (4) is applicable irrespective of the dependence of the resistance coefficients on the bath concentrations or the electrical potential difference, provided that the electrochemical potential is everywhere continuous.

APPLICATION

The above development will now be applied to a model consisting of parallel arrays of active transport and leak pathways in order to derive the relationship between the rate of "influx" and the rate of net active transport. In the presence of only one tracer, J_m^x and ρ_m are represented simply by J^x and ρ; designating the active and passive pathways respectively by the superscripts a and p, the influx is given by

$$-\text{influx} = \frac{J^x}{\rho} = \frac{J^{xa}}{\rho} + \frac{J^{xp}}{\rho}$$

and, with (3 a),

$$\frac{J^x}{\rho} = \frac{J^a}{1-f^a} + \frac{J^p}{1-f^p}. \tag{5}$$

If the leak is considered to be a simple aqueous pathway without isotope interaction or significant coupling between flows of different species, $R^{xp} = R^p$ and $\int_0^{\Delta x} \sum r_{0j}J_j^p dx = 0$. Equation (4) then reduces to

$$f^p = \exp\left(\frac{X}{RT}\right) \tag{6 a}$$

and

$$J^p = \frac{X}{R^p}. \tag{6 b}$$

Here R^p represents the overall phenomenological resistance coefficient for an array of parallel leak pathways, whether homogeneous or heterogeneous. For a steady state in the absence of net flow,

$$J = J^a + J^p = 0 \tag{7}$$

and, from equations (4), (6 *b*), and (7),

$$f^a = \exp\left(\frac{J^a R^{ax}}{RT}\right) = \exp\left(-\frac{R^{ax}X}{R^pRT}\right). \tag{8}$$

Introducing equation (7) into (5),

$$\frac{J^x}{\rho} = J^a\left(\frac{1}{1-f^a} - \frac{1}{1-f^p}\right),$$

and with equations (6 *a*) and (8),

$$\frac{J^x}{\rho J^a} = \frac{1}{1-\exp\left(-\frac{R^{ax}}{R^p}\frac{X}{RT}\right)} - \frac{1}{1-\exp\left(\frac{X}{RT}\right)}. \tag{9}$$

This expression represents the value by which any observed rate of influx J^x/ρ must be divided in order to obtain the net rate of transport in the active pathway. This quantity is plotted in Fig. 2 as a function of R^{ax}/R^p, for $X/F = -120$ mv, and is tabulated in more detail in Table I. As is seen, the influx may greatly exceed the net rate of active transport at low values of $-X$ and/or R^{ax}/R^p.[1]

The efflux may be treated in similar fashion. More directly, it is seen from equation (1) that, in the absence of a net flow, the ratio of the rate of efflux to the rate of active transport is given by the negative of the expression in equation (9). In some situations the determination of the rate of efflux may be more convenient than the determination of the rate of influx. In other cases the determination of both will provide a test for internal consistency.

[1] It is emphasized that here the pertinent parameter in the active transport pathway is not the resistance to net flow, R^a, but the exchange resistance, R^{ax} (11). R^{ax} will be greater than, equal to, or less than R^a if isotope interaction is respectively positive, absent, or negative. The degree of isotope interaction for any active transport system is not yet known.

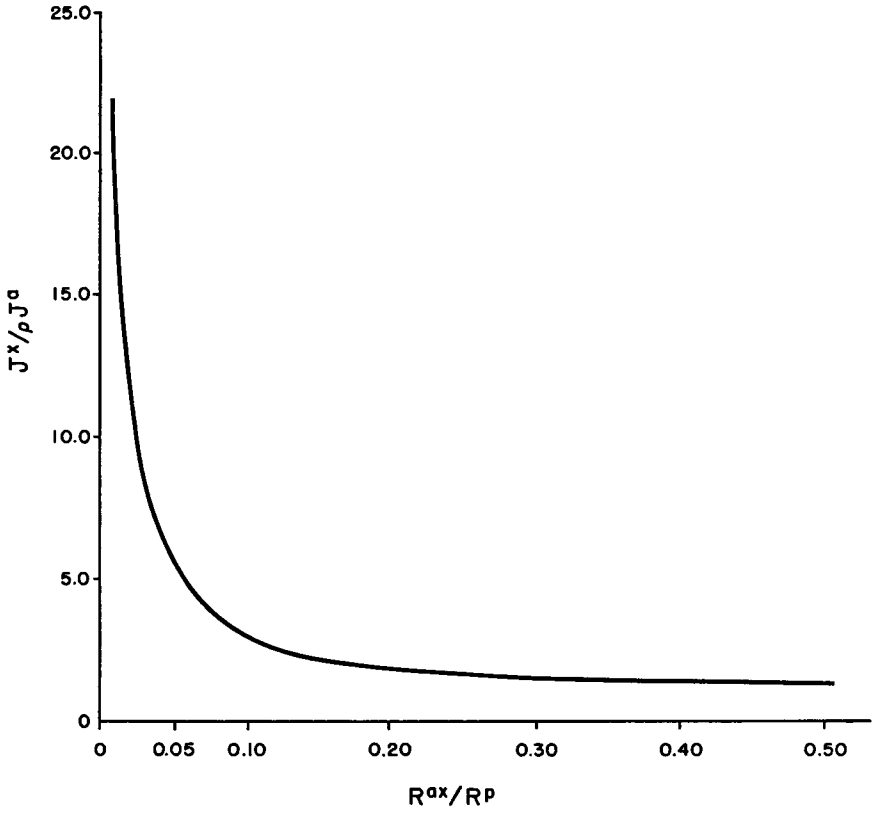

FIGURE 2 Ratio of rate of influx to rate of active transport ($-X/F$ = 120 mv).

TABLE I

RATIO OF RATE OF INFLUX TO RATE OF ACTIVE TRANSPORT

$-X/F$	R^{ax}/R^p							
	0.01	0.02	0.03	0.04	0.05	0.10	0.50	1.0
mv								
20	130	65.6	44.2	33.5	27.1	14.2	4.0	2.7
40	65.0	32.9	22.2	16.8	13.6	7.2	2.1	1.5
60	43.4	22.0	14.9	11.3	9.2	4.9	1.6	1.2
80	32.7	16.6	11.3	8.6	7.0	3.8	1.3	1.1
100	26.2	13.4	9.1	7.0	5.7	3.1	1.2	1.0
120	21.9	11.2	7.7	5.9	4.8	2.7	1.1	1.0
140	18.9	9.7	6.6	5.1	4.2	2.4	1.1	1.0
160	16.6	8.5	5.9	4.5	3.7	2.2	1.0	1.0
180	14.8	7.7	5.3	4.1	3.4	2.0	1.0	1.0
200	13.4	6.9	4.8	3.7	3.1	1.8	1.0	1.0

DISCUSSION

As the above development makes clear, the ratio of J^x/ρ to J^a is strongly dependent on both $-X$ and R^{ax}/R^p, and when the value of either is low the rate of influx may very much exceed the true rate of active transport. These relationships are readily understood. When $-X$ becomes small the flux ratio in the passive pathway approaches 1, and so the influx may much exceed the net rate of leak (which in the steady state is equal to the rate of active transport). On the other hand, when R^{ax}/R^p becomes small a large fraction of influx occurs by way of the active transport pathway rather than by way of the passive channel, and so again the influx significantly exceeds the net rate of leakage and active transport.

Unfortunately the evaluation of both X and R^{ax}/R^p is as yet highly inexact. Measurements of intracellular concentrations and electrical potentials are available, but uncertainty concerning the state of intracellular solutes, and thus their activity coefficients, makes estimates of electrochemical potential gradients suspect (7, 8). (Recent techniques employing intracellular ion-specific electrodes should permit an approach to this problem (12)). Similarly, no studies are yet available which would permit precise evaluation of R^{ax}/R^p.[2]

Lacking precise values for these parameters, it would appear that the existence of an active transport system at cell surfaces cannot be ruled out on energetic grounds simply by the demonstration of high rates of isotope flux. Similar considerations apply to the concept of exchange diffusion.

A certain confusion has been associated with the use of this term, partly owing to variability of its definition, often merely implicit, in varying circumstances. Three different mechanisms have been considered to represent exchange diffusion:

1. The concept was originally invoked in studies of sodium transport in frog sartorius muscle, where in order to explain the seeming discrepancy between high rates of Na tracer flux and energetic limitations it was suggested that active extrusion accounts for only part of the outflux, and that part of the apparent active extrusion might be due to exchange diffusion (4). It was pointed out that the hypothetical exchange system could be visualized as a Na-impermeable monolayer containing scattered anions which form a complex with Na, and which in response to thermal

[2] A rough impression of the magnitude of this quantity in epithelial tissues may be gained from the consideration that the observed flux ratio is highly sensitive to the presence of leak. Ussing reports values for the flux ratio of sodium in short-circuited frog skins ranging from 7 to 45, with a mean of 22 (9, page 118). As pointed out previously, with $R^{ax}/R^p = 0.5$ an observed f of 22 would require that the flux ratio in the active transport pathway be about 22,000 (11). In the absence of isotope interaction, observed flux ratios of 22 and 45 would correspond, in Ussing's formulation, to an E_{Na} in the active transport pathway of, respectively, about 280 and 580 mv. For $R^{ax}/R^p = 0.2$, the corresponding values would be respectively some 120 and 250 mv. Since skins may be stretched and slightly traumatized in mounting, it is possible that leak may be less significant, and thus R^{ax}/R^p lower, in vivo.

agitation move back and forth across the membrane, exchanging internal Na^{24} for external Na^{23}. In its ideal form the mechanism involved the movement of carrier molecules across the cell membrane only in combination with a given chemical species. Thus there could be no net flow, irrespective of the forces, and the process was considered to require no expenditure of metabolic energy (9, page 50).

In view of the above demonstration that a high rate of isotope flux via a simple "pump-leak" mechanism may be associated with either low or high rates of active transport, there would appear to be no compelling reason to invoke such a discrete mechanism separate from an active transport pathway.

2. As emphasized by Ussing, any real exchange system is likely to be non-ideal, and might permit passage of carriers across the cell membrane either unloaded or in combination with any of several solute species. Such a system could carry out passive net transport and countertransport.

3. The latter concept has been broadened further to permit exchange diffusion by way of the active transport system itself. The mechanism here is that assumed in the present treatment, namely bidirectional movement of solute particles across the active transport pathway, but the analysis differs in the assignment of fluxes to two categories. (See for example Heinz and Walsh's treatment of amino acid transport in Ehrlich carcinoma cells (13)). In terms of sodium transport in frog sartorius muscle, influx by way of the active transport pathway would be paired with an equivalent moiety of the efflux, thus comprising a component of 1:1 exchange diffusion; efflux over and above this value would be considered to represent active transport.

It might appear that to regard such a process as exchange diffusion, rather than simple active transport, is merely a matter of taste. However, it should be noted that such a partition of the fluxes, assigning the influx entirely to exchange diffusion, might carry the implication that the influx is independent of metabolism. However, as discussed previously, for a bidirectional active transport system this would not be the case (11). This may be seen by application of equation (3) to the active transport pathway. Introducing equation (8), and expressing the exponential as a power series, we obtain

$$\frac{-J_m^{ax}}{\rho_m} = \frac{-J^a}{1-f^a} = \frac{RT}{R^{ax}}\left(\frac{1}{1+\frac{1}{2!}\left(\frac{J^aR^{ax}}{RT}\right)+\frac{1}{3!}\left(\frac{J^aR^{ax}}{RT}\right)^2+\cdots}\right),$$

showing that measures which decrease J^a (without alteration of R^{ax}) would also increase the rate of influx via the active pathway. (Influx by way of leak pathways would of course be decreased by diminution of the magnitude of the electrochemical potential difference.)

Just as definitions of exchange diffusion have differed, so also have various criteria been cited as evidence for the process:

1. As has been mentioned, exchange diffusion was first inferred on the basis of

high rates of isotope flux considered otherwise incompatible with energetic considerations. As shown above, such findings are in fact compatible with a "pump-leak" mechanism, provided only that R^{ax} be sufficiently less than R^p. This could be the case, for example, either as a consequence of a high degree of negative coupling of isotope flows, "isotope interaction," with $R^{ax} < < R^a \leq R^p$, or, in the absence of significant coupling, with $R^{ax} \simeq R^a < < R^p$. (It should be noted that R^a can be arbitrarily small without violation of energetic considerations, since for a given value of X, R^a and the rate of metabolism are related directly. Representing the integral of the metabolic force by $-R_{0r}J_r$, equation (4) gives

$$J^a R^a = X - R_{0r}J_r - \int_0^{\Delta x} \sum_{j \neq r} r_{0j}J_j \, dx.$$

Rearranging, and introducing equations (6 *b*) and (7),

$$R_{0r}J_r = X\left(1 + \frac{R^a}{R^p}\right) - \int_0^{\Delta x} \sum_{j \neq r} r_{0j}J_j \, dx.$$

Since R_{0r} and X are both negative, J_r and R^a are related directly.)

2. Another experimental finding which is often considered to indicate the existence of exchange diffusion is the observation that the rates of influx and efflux are more nearly equal than would be predicted from the magnitude of the total forces. This criterion is not as yet useful in the presence of active transport, for in this case the total force promoting transport is unknown. For passive transport, however, in the absence of coupled flows the forces are well defined and the criterion has been expressed formally (9, page 49); in the symbols of the present paper it is given by

$$|\, \mathrm{R}\, T \ln f\,| < |\, X\,|. \tag{10}$$

Consideration of equation (4) shows that this criterion is equivalent to the requirement that there be a pathway across the cell surface for which the ratio of the exchange resistance to the resistance for net flow, R^x/R, is less than 1. To the extent that R^x is less than R it would explain also the observation of high rates of isotope flux without corresponding large values for membrane conductance (14).

One possible mechanism which would produce this result is of course ideal, i.e. 1:1, exchange diffusion, as in the first category above. Since such a system permits no net flow, whatever the forces, R is infinite. R^x remains finite, however, for isotope flow persists in the absence of net flow (see reference (11), equation (31)); thus R^x/R is zero. Such ideal exchange diffusion, in parallel with a leak pathway, has often been invoked to explain "abnormal" flux ratios in studies of passive transport, for instance in the case of chloride transport across frog intestine (14). Again, though such a mechanism is possible, it is not necessary in order to explain the data, for the value of R^x/R in the transport pathway need not be zero, but merely less than 1, as would be the case for the second mechanism above. Finite values of

R^x/R less than 1 are predictable on theoretical grounds for a variety of passive carriers (15–17), and are consistent with the observed kinetics of sugar transport in red blood cells (18). A calculation of the degree of isotope interaction as a function of kinetic constants has been carried out for a simple carrier in reference (17), model 5.

3. Still another criterion often cited as evidence for exchange diffusion is the enhancement of a tracer flux on the addition of unlabeled test substance or congeners to the "trans" side. Although such a phenomenon is quite consistent with countertransport via a carrier, it has been pointed out that other explanations should be considered as well (19, 20). One possibility is that the enhancement of the tracer flux might simply reflect the concentration dependence of the permeability of a membrane completely lacking in carriers. For instance, a charged "ion exchange" membrane, which would effectively exclude co-ions at low solution concentrations, might lose much of its permselectivity at higher concentrations (21). For such reasons more impressive evidence for exchange via a carrier would appear to be the demonstration of both trans-stimulation and cis-inhibition of transport by congeners, as shown for example for amino acids in Ehrlich carcinoma cells (13).

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