Models of Ion and Substrate Cotransport and the Effect of the Membrane Potential

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ABSTRACT

The implications of a carrier model of ion and substrate cotransport are worked out. Each carrier is assumed to have one ion and one substrate binding site. The model includes features that have not been included in previously published models. These features are the effect of the membrane potential and of the assumption that *all* carrier forms, with or without bound substrate and with or without various bound ions, can cross the membrane. The model is of a two-state (gate-type) carrier with transition rate constants. In one state the carrier interacts with outer bulk phase; in the other state it interacts with the inner bulk phase. Equilibrium in the reactions between ion, substrate, and carrier is assumed at each surface.

INTRODUCTION

Over the past decade extensive evidence has been accumulated which shows that the uptake of sugars and amino acids is accompanied by an uptake of sodium in a variety of cell types. Much of this has been reviewed by Stein [1], Mitchell [2], Rothstein [3], and Jacquez and Schafer [4]; Schultz and Curran [5] have written a detailed review. The evidence on the stoichiometry of sodium–amino acid cotransport is less extensive. It is of two kinds: (a) measurement of the dependence of initial flux of amino acid uptake on the extracellular sodium concentration; and (b) direct measurement of simultaneous fluxes of sodium and amino acid and comparison of the increment in Na^+ influx with the concomitant amino acid influx. As to the first of these, Vidaver [6] found that the glycine influx in pigeon red cells showed a second-order dependence on extracellular sodium. This was confirmed by Wheeler and Christensen [7], but these authors also found that alanine influx in pigeon red blood cells showed a first-order dependence on extracellular sodium. A first-order dependence on extracellular sodium has been reported for AIB uptake by rat diaphragm [8] and Ehrlich ascites cells [9], for alanine uptake by rabbit reticulocytes [10] and rabbit ileum [11], and for glycine uptake by LS mouse ascites cells [12].

As to the evidence on simultaneous fluxes of sodium and amino acid, Schafer and Jacquez [13] found an approximately 1 : 1 ratio of sodium to AIB uptake, over a wide range of concentrations of sodium and AIB, in Ehrlich ascites cells. Wheeler and Christensen [7] found ratios of 1.53, 2.52, and 0.96 for glycine, alanine and β-alanine, respectively, in pigeon red cells. Eddy [14] found a ratio of 0.9 for glycine uptake in LS cells. The evidence for involvement of sodium in sugar transport is equally impressive [15–20]. Furthermore, there have been a number of reports of the involvement of potassium since the early work of Christensen *et al.* [21] and Riggs *et al.* [22]. Schafer [23] found a ratio of K^+ efflux to AIB influx of 0.6 in Ehrlich ascites cells and Eddy [14] also found a ratio of 0.6 for glycine uptake in LS cells.

Many enzymes are activated by cations. A favored explanation for this activity of cations is that the binding of cation to the enzyme stabilizes the proper conformation of the enzyme molecule. A similar explanation seems plausible for the action of cations involved in cotransports. The role of monovalent cations in enzyme activation has recently been reviewed by Suelter [24].

For the remainder of this article we need a standard terminology for carrier models. Referring to Fig. 1, I distinguish between reaction rate constants, such as α, β, γ, δ, and transition rate constants, such as k_1, k_{-1}, k_0, k_{-0}. S is substrate, C carrier. The membrane carriers are viewed as

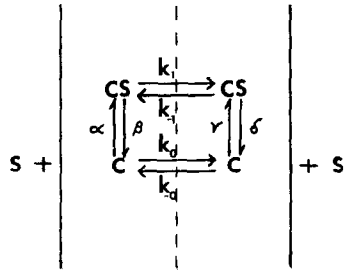

FIG. 1. Schematic of a simple two-state carrier model. C is carrier in the membrane; S is substrate. C and CS each have two states, one on the left side (outer surface) of the membrane, the other on the right side.

occurring in two possible states, one on the inner surface, the other on the outer surface of the membrane, with transition rates between the two states. In an *equilibrium carrier model* the carrier–substrate complex is assumed to be in equilibrium with the substrate in the adjacent bulk phase, whereas in a *reaction carrier model* the equilibrium assumption is not invoked. A model is said to be symmetric in the reactions if the corresponding reaction rate constants are the same at the two surfaces

(i.e., $\alpha = \gamma$, $\beta = \delta$), and symmetric in the transition rates if $k_i = k_{-i}$. These definitions extend to carrier systems that bind more than one substrate to give coupled transports. In Mitchell's terminology [2], coupled transport of two molecules in the same direction is a symport, in opposite directions, an antiport.

The concept of a carrier has been the germinative idea in the study of transport systems in living cells. Irwin [25] used the term carrier in 1931–1932 to describe the hypothetical role of proteins in the penetration of dyes into cells. In 1933 Osterhout [26] described a model (real, not mathematical) for movement of potassium across a lipid phase that was clearly a carrier model, although he did not use this term. In 1937 Lundergårdh [27] proposed a hypothesis to explain anion uptake by roots that was a carrier hypothesis. In his review of renal tubular excretion in 1939 Shannon [28] proposed a carrier-type model and showed that it predicts the occurrence of a T_m for tubular excretion, again without using the term carrier. Höber [29] uses the term carrier and discusses the carrier hypothesis in the 1945 edition of his book, and by the time of Ussing's review of ion transport in 1949 [30], the idea of carriers was clearly one of the basic ideas in active transport. The early work of Hodgkin, Huxley, and Katz [31] was inspired by a carrier model, which they present in detail in their 1949 paper [31], although in the final paper [32] of their 1952 series they conclude that the specific model that had originally guided their thinking did not account for their findings.

Carrier models have also played a basic role in the work on sugar and amino acid transport, although the models published up to about 1965 did not include ion cotransport as an explicit feature. In 1952 in a discussion of sugar transport, LeFevre and LeFevre [33] proposed an adsorption transport model, that is, one in which substrate binds to a fixed membrane site, which may dissociate and deliver substrate to either side of the cell membrane. Widdas [34] proposed a carrier model for glucose transport that was an equilibrium model with equal transition rate constants for all carrier forms with symmetry in the transition rate constants and the reaction rate constants. Rosenberg and Wilbrandt [35] also examined implications of a number of equilibrium carrier models, again symmetric in transition rate and reaction rate constants and with the assumption of equal transition rate constants for all carrier forms, but they also examined some enzyme–carrier models in which the carrier–substrate binding was enzymatically mediated. Patlak [36, 37] pointed out that a mobile carrier in the strict sense was not necessary and that all that is necessary is a membrane combining site, which he called a gate, that has two states. In one state the site is accessible to bulk phase on one side of the membrane only, in the other state it is accessible to bulk phase on the

other side of the membrane. Such a model is perhaps better represented diagrammatically by Fig. 2 than by Fig. 1. Given the transition rate constants for conversion between these states, such a gate-type model shows kinetics indistinguishable from those of what used to be called a mobile carrier model, which in its usual formulation is also only a two-state

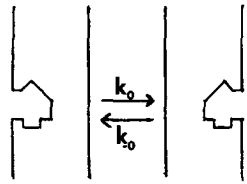

FIG. 2. Diagram of two-state or gate-type model.

system with transition rates. Mitchell [38] also proposed what appeared to be a two-state carrier model at about the same time, although he did not explicitly differentiate between a two-state carrier (gate) and a true mobile carrier model. Later, Vidaver [39] examined some special cases of the gate-type model. This distinction is generally recognized now, and the term carrier model is really used in this restricted sense by most workers when talking about transport across cell membranes. The distinction is important because the term mobile carrier really applies to an important situation in which there is simultaneous diffusion of substrate, carrier, and the substrate–carrier complex and reaction between substrate and carrier, as in the facilitated diffusion of oxygen through thick layers (1–1000 μ) of hemoglobin or myoglobin [40, 41]. Mitchell [2] has reviewed the older models used to describe this true mobile carrier transport; all assume equilibrium between carrier and substrate at all points in the diffusion path. Friedlander and Keller [42] used the techniques of irreversible thermodynamics to linearize the reaction terms in the diffusion equations. Kutchai *et al.* [43] and Kreuzer and Hoofd [44] have shown that these are poor assumptions and have solved the exact equations numerically on a computer. Kreuzer [45] and Wittenberg [46] have recently reviewed the experimental work on facilitated diffusion of oxygen in solutions of hemoglobin and myoglobin. In this article we are concerned only with the two-state type of model, and I use the term carrier model in this restricted sense.

Jacquez [47] extended the discussion to reaction carrier models without symmetry in reaction rates or in transition rate constants. Heinz and Patlak [48] calculated a lower bound for energy expenditure in a transport model that included a sequence of reactions, and Patlak [49] extended this to consideration of a linked antitransport, an antiport, and discussed the effect of multiple pathways as well as the effect of the membrane potential

on transport of a charged molecule. Jacquez [50, 51] considered kinetics of a divalent carrier transport and compared equilibrium and reaction carrier models for a univalent carrier and included the effect of exchange reactions between substrates in the bulk phase and the carrier–substrate complexes. Regen and Morgan [52] also examined a reaction carrier model without symmetry in transition rate or reaction rate constants. Finkelstein [53] used a true mobile carrier model in a discussion of active transport of sodium across a mosaic membrane but used the simplifying assumption that the reactions between substrate and carrier occurred only at the surfaces of the membrane, so he did not have to consider simultaneous diffusion and reaction in the interior of the cell membrane. Silverman and Goresky [54] proposed a carrier model in which there were two forms of the carrier with different affinities for the transported substrate and irreversible conversions between the two free forms on each side of the membrane to drive the transport. Wong [55] and later Britton [56] examined the kinetics of transport with polyvalent carriers, and Britton included consideration of the effect of the membrane potential on transport of charged particles. Hill and Kedem [57] examined the steady-state solutions for 20 different models, some of the carrier type and some that consisted of a lattice of binding sites, with use of a diagrammatic method introduced by Hill [58] for obtaining steady-state solutions of multistate transport models. The method is useful because it provides a formal approach that makes it easy to write the basic differential equations for the system and the equations for the steady state. However, it does not include leak fluxes. It is particularly interesting because the diagrams are graphs in which the nodes are states and the lines linking nodes represent allowed transitions, which makes one hopeful that the theory of graphs may contribute to the comparative study of the structures of transport models. The method has been used by Essig [59] and Essig, Kedem, and Hill [60] and by Blumenthal and Kedem [61] to examine flux ratios and the interaction of isotope fluxes in some carrier-type and lattice-type models.

Less work has been done with models of coupled transport of substrates and cations. Inui and Christensen [9] and Stein [1] assumed the sequence of reactions for Na^+–amino acid symport shown in Fig. 3, but assumed that only the complex NaCS crossed the membrane. Semenza [62] develops the Michaelis–Menten kinetics for enzyme reactions that involve a substrate and a modifier and uses the results to analyze some of the data reported on sodium-dependent transport of sugars and amino acids. Curran *et al.* [11] assume the sequence $C + S \rightleftharpoons CS + Na^+ \rightleftharpoons NaCS$ and that C, CS, and NaCS can all cross the membrane with equal transition rate constants. They do not include the effect of the membrane potential, but point out some of the required modifications if the Goldman equation [63] holds for

the flux of NaCS. Vidaver and Shepherd [64], in modeling glycine transport in pigeon red cells, assumed the formation of complexes NaC, Na_2C, and Na_2CS in that order, and that C, NaC, Na_2C, and Na_2CS all cross the cell

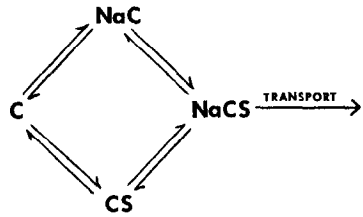

FIG. 3. Reaction mechanism with only complex NaCS transported.

membrane For a carrier model with one ion and one substrate site Eddy [14] assumed that both Na^+ and K^+ can bind at the cation binding site but that only C and NaCS can cross the membrane. The model is an equilibrium model with symmetry in reaction and transition rate constants and does not include the effect of the membrane potential. Recently Jacquez and Schafer [4] used an argument that depended only on energy considerations to show that for an obligatory 1 : 1 coupling of Na^+ and substrate for carrier movement, the steady-state concentration ratio for substrate c_i/c_e, must obey relation (1),

$$\frac{c_i}{c_e} \leqslant \frac{[\mathrm{Na}]_e}{[\mathrm{Na}]_i} \exp\left(-\frac{FV_m}{RT}\right) \tag{1}$$

where V_m is the membrane potential and $[\mathrm{Na}]_e$ and $[\mathrm{Na}]_i$ are extracellular and intracellular sodium concentrations, respectively. For an obligatory type of coupling in which NaCS moves in and KC moves out, this relation becomes (2).

$$\frac{c_i}{c_e} \leqslant \frac{[\mathrm{Na}]_e}{[\mathrm{Na}]_i} \cdot \frac{[\mathrm{K}]_i}{[\mathrm{K}]_e}. \tag{2}$$

In this brief review I have neglected the approach to an analysis of transport processes that uses strict irreversible thermodynamics in the main because this approach has contributed much less than have the kinetic models to the dialogue between experiments and theory. However, the papers by Hill [57], Hill and Kedem [58], and Essig *et al.* [60] contribute to the integration of the two approaches. A critical review can be found in the paper by Rapoport [65].

My purpose here is to examine a carrier model of substrate and cation cotransport that includes the effect of the membrane potential as well as the possibility that all carrier forms can cross the membrane. Basically this is a generalization of the model used by Eddy [14]. For the assumptions that describe obligatory coupling the model should predict the same steady-

state substrate concentration ratios that have been obtained from purely energetic considerations [4]. The possibility that Na^+, K^+, and H^+ complexes of the carrier can be formed should be included. Furthermore, we want to see if there are differences in the predictions of the models depending on whether the carrier–cation complexes are or are not charged. The present derivation is for one carrier system. There is evidence for a multiplicity of carriers in amino acid uptake in some cells. This complication would have to be considered in any application of theory.

A MODEL AND ITS BASIC ASSUMPTIONS

THE MODEL

Assume a two-state (gate-type) carrier model, each carrier having one binding site for an ion and one for a substrate. The distinction between ion and substrate is arbitrary in the general model, which we develop for any charges on ion or substrate, although we will be interested primarily in carriers that bind univalent cations and neutral substrates. The ions will

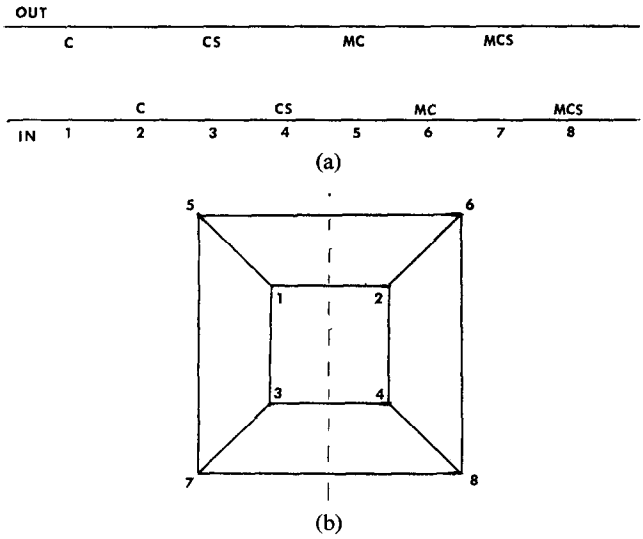

FIG. 4. (a) The numbering of the system states for the model. (b) The state graph for the model.

be indicated by the notation M_i, the substrates by S_j. In the sequel we will be concerned with Na^+, K^+, and H^+, which will be M_1, M_2, and M_3, respectively, in our notation.

If only one substrate S and one ion M are present, we can diagram the states as in Fig. 4a. The states at the outer surface have been given odd

numbers, those at the inner surface even numbers. Figure 4b shows the state graph for this model. Note that the inner cycle 1, 2, 3, 4 involves only free carrier and carrier–substrate complex. The horizontal lines represent transitions between inner and outer states, the other lines represent reactions. For each additional ion involved there is a set of states similar to 5, 6, 7, 8, which are linked to each other and to the set 1, 2, 3, 4 in the same way as are 5, 6, 7, 8. Each line represents two possible transitions; if the coefficient of the concentration term describing each transition is put above or to the left of the line for transition in one direction and below or to the right for the opposite direction, it is easy to write the differential equations from the diagram. The method for writing the steady-state solutions is given in Hill's paper [58]. We want to derive initial fluxes as well as steady-state fluxes, so we use the kinetic method directly and use the equilibrium assumption for the reactions to reduce the number of equations and then introduce a special notation to simplify the algebra.

BASIC ASSUMPTIONS

1. The binding and dissociation reactions are assumed to be rapid enough to make the equilibrium assumption valid. Thus we assume an equilibrium carrier model and symmetry in the binding reactions at the two sides of the membrane.

2. Symmetry in the transition rate constants is not assumed. Later, however, we will assume that any asymmetry is due only to the effect of the potential across the membrane. This means that we will assume no direct coupling between the carrier system and cellular metabolism, the only coupling being indirect, through effects on membrane potential and the ion gradients. The transition rate constants are not necessarily equal for different carrier species. Thus if only one ion M_i and one substrate S_j are present, the carrier species are C, M_iC, CS_j, and M_iCS_j; and for each of these there are distinct transition rate constants for the transitions between the inner and outer states in the membrane.

TERMINOLOGY

We use a double subscript notation for transition rate constants and dissociation constants, the first subscript referring to the ion, the second to the substrate, as shown in Fig. 5. A zero subscript in the first position means that no ion is present, in the second position, that no substrate is present.

1. Transition rate constants.

The asymmetry in the transition rate constants is indicated as shown in Fig. 5.

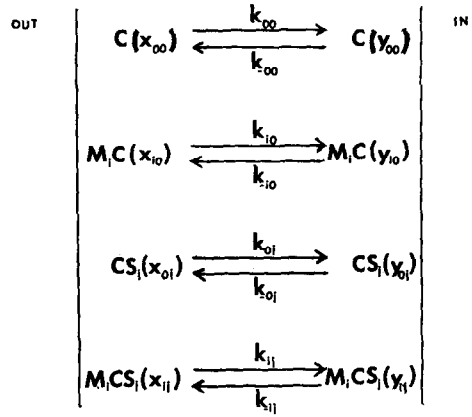

FIG. 5. Schematic diagram giving the notation for transition rate coefficients and concentrations of carrier species.

2. Dissociation constants.

Equations (3) define the dissociation constants for binding of one of the ligands.

$$K_{i0} = \frac{[M_i][C]}{[M_iC]}, \qquad K_{0j} = \frac{[C][S_j]}{[CS_j]}. \tag{3}$$

For the binding of the second ligand, parentheses around subscripts indicate the second ligand bound. Thus $K_{i(j)}$ and $K_{(i)j}$ are the dissociation constants defined by Eq. (4).

$$K_{(i)j} = \frac{[M_i][CS_j]}{[M_iCS_j]}, \qquad K_{i(j)} = \frac{[M_iC][S_j]}{[M_iCS_j]}. \tag{4}$$

Define $K_{ij} = K_{i0}K_{i(j)} = K_{0j}K_{(k)j}$. Hence, by the equilibrium assumption, Eq. (5), giving the concentrations of the different carrier complexes, apply at the inner and outer faces of the membrane.

$$\begin{aligned}
[M_iCS_j] &= \frac{[M_i][C][S_j]}{K_{ij}}, & x_{ij} &= \frac{m_i x_{00} s_j}{K_{ij}}, & y_{ij} &= \frac{n_i y_{00} t_j}{K_{ij}}; \\
[M_iC] &= \frac{[M_i][C]}{K_{i0}}, & x_{i0} &= \frac{m_i x_{00}}{K_{i0}}, & y_{i0} &= \frac{n_i y_{00}}{K_{i0}}; \\
[CS_j] &= \frac{[C][S_j]}{K_{0j}}, & x_{0j} &= \frac{x_{00} s_j}{K_{0j}}, & y_{0j} &= \frac{y_{00} t_j}{K_{0j}}.
\end{aligned} \tag{5}$$

Concentrations

As is commonly done in work on transport models, we use concentrations in place of activities to avoid having to carry a multitude of activity coefficients through the derivations, but this has to be remembered when attempting to apply the results. As is shown in Fig. 5, x and y are used to

indicate outer and inner states in the membrane. The units used for the concentration of the different carrier complexes will depend on how one expresses experimental results. The units frequently used are moles per membrane area, moles per dry weight of cells, and moles per cell water. We use m_i, s_j, and n_i, t_j for concentrations of M_i and S_j in outer and inner bulk phases, respectively. Later we translate to standard chemical notation, $[M_i]_e = m_i$, $[M_i]_i = n_i$, where subscripts e and i, *used with the standard chemical notation*, indicate external and internal bulk phases.

THE EFFECT OF THE MEMBRANE POTENTIAL

The membrane potential is assumed to have an effect only through its effect on the transition rate constants for charged complexes. As is diagrammed in Fig. 6, consider the membrane to consist of two phases, the outer one at potential ϕ_1, the inner one at potential ϕ_2. The rate of transition of M_iCS_j from phase 1 to phase 2 is $k_{ij}x_{ij}$ and the reverse is $k_{-ij}y_{ij}$.

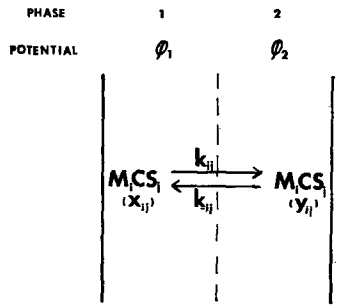

FIG. 6. Diagram giving potentials and the states for the complex M_iCS_j.

Consider such a system in a hypothetical equilibrium in which the electrochemical potentials of M_iCS_j are the same in both phases. Let z_{ij} be the charge on complex M_iCS_j. Then Eq. (6) holds.

$$RT \ln x_{ij} - z_{ij}F\phi_1 = RT \ln y_{ij} - z_{ij}F\phi_2. \tag{6}$$

Let $V_m = \phi_2 - \phi_1$ be the membrane potential. Then,

$$y_{ij} = x_{ij} \exp\left(\frac{z_{ij}FV_m}{RT}\right). \tag{7}$$

But at equilibrium, $k_{ij}x_{ij} = k_{-ij}y_{ij}$. Hence,

$$k_{-ij} = k_{ij} \exp\left(-\frac{z_{ij}FV_m}{RT}\right). \tag{8}$$

Equation (8) defines the ratio k_{-ij}/k_{ij} as a function of the membrane potential but there is no implication that one or the other of k_{ij} or k_{-ij} remains constant as V_m changes. Suppose that the two-state model is a good

picture of the actual situation, with one state at one potential, the other at another; let $k_{ij}^* = k_{-ij}^*$ be the transition rate constants at zero membrane potential. Then it is easy to show that $k_{ij} = k_{ij}^* \exp(z_{ij}FV_m/2RT)$ and $k_{-ij} = k_{ij}^* \exp(-z_{ij}FV_m/2RT)$. However, we do not need this result in what follows; we need and use only the equation for the ratio k_{-ij}/k_{ij}.

Note that the simplifying assumption that ϕ_1 and ϕ_2 are bulk phase potentials is used, and that the dissociation and binding reactions occur at these potentials. It would be of interest and more realistic to consider the bulk phases and surface phases to have different potentials and to introduce transition rate constants for movement of M_i and S_j between bulk and surface phases. It turns out that for the equilibrium type of carrier model such a model is the same mathematically as the present model; however, the dissociation constants for the reactions then really include partition coefficients within them.

BASIC EQUATIONS AND METHODS

Let C_0 be total carrier concentration and let C_{0i} be concentration of total carrier on the inside of membrane.

$$C_0 = \sum_{i=0} \sum_{j=0} (x_{ij} + y_{ij}). \tag{9}$$

In Eq. (9) and those that follow we exhibit only the index or perhaps the index and its lowest value for a particular summation, the summation being understood to be over the possible values of that index for the particular system under study. We need two basic equations, one for the rate of change of C_{0i}, one for conservation of total carrier. These are Eqs. (10) and (11).

$$\frac{dC_{0i}}{dt} = \left[k_{00} + \sum_{i=1} \frac{k_{i0}m_i}{K_{i0}} + \sum_{j=1} \frac{k_{0j}s_j}{K_{0j}} + \sum_{i=1} \sum_{j=1} \frac{k_{ij}m_i s_j}{K_{ij}}\right] x_{00} - \left[k_{-00} + \sum_{i=1} \frac{k_{-i0}n_i}{K_{i0}} + \sum_{j=1} \frac{k_{-0j}t_j}{K_{0j}} + \sum_{i=1} \sum_{j=1} \frac{k_{-ij}n_i t_j}{K_{ij}}\right] y_{00}; \tag{10}$$

$$C_0 = \left[1 + \sum_{i=1} \frac{m_i}{K_{i0}} + \sum_{j=1} \frac{s_j}{K_{0j}} + \sum_{i=1} \sum_{j=1} \frac{m_i s_j}{K_{ij}}\right] x_{00} + \left[1 + \sum_{i=1} \frac{n_i}{K_{i0}} + \sum_{j=1} \frac{t_j}{K_{0j}} + \sum_{i=1} \sum_{j=1} \frac{n_i t_j}{K_{ij}}\right] y_{00}. \tag{11}$$

These may be simplified by abbreviating the coefficients of x_{00} and y_{00} as in (12) and (13),

$$\frac{dC_{0i}}{dt} = Ex_{00} - Fy_{00}, \tag{12}$$

$$C_0 = Gx_{00} + Hy_{00}, \tag{13}$$

where E, F, G, and H are sums of positive terms.

By the pseudo-stationary-state assumption, $dC_{0i}/dt = 0$. This is the same assumption as is used in the derivation of the initial velocity of enzyme reactions. For the steady state this assumption is exact! Equations (12) and (13) then become two simultaneous linear algebraic equations that have for their solutions

$$x_{00} = \frac{C_0 F}{D}, \tag{14}$$

$$y_{00} = \frac{C_0 E}{D}, \tag{15}$$

$$D = EH + FG. \tag{16}$$

Now all fluxes can be calculated in terms of x_{00} and y_{00}. The remainder of the derivations consist of the tedious algebra of examining special cases. The results follow in the next section.

For three ions and one substrate the corresponding reaction carrier model presents a much more difficult problem. Instead of two simultaneous equations, we then have 16 simultaneous equations to solve. Even though the matrix of coefficients for these equations is rather sparse, the problem is algebraically messy. Hill's method becomes more useful in that case.

RESULTS

Before presenting the results it will help to introduce a simplifying notation for the components of E, F, G, and H. We define the following:

$$e_0 = k_{00} + \sum_{i=1} \frac{k_{i0} m_i}{K_{i0}}; \qquad e_j = \frac{k_{0j}}{K_{0j}} + \sum_{i=1} \frac{k_{ij} m_i}{K_{ij}}, \quad j \geqslant 1;$$

$$f_0 = k_{-00} + \sum_{i=1} \frac{k_{-i0} n_i}{K_{i0}}; \qquad f_j = \frac{k_{-0j}}{K_{0j}} + \sum_{i=1} \frac{k_{-ij} n_i}{K_{ij}}, \quad j \geqslant 1;$$

$$g_0 = 1 + \sum_i \frac{m_i}{K_{i0}}; \qquad g_j = \frac{1}{K_{0j}} + \sum_i \frac{m_i}{K_{ij}}, \quad j \geqslant 1;$$

$$h_0 = 1 + \sum_i \frac{n_i}{K_{i0}}; \qquad h_j = \frac{1}{K_{0j}} + \sum_i \frac{n_i}{K_{ij}}, \quad j \geqslant 1. \tag{17}$$

With this notation, E, F, G, and H become

$$E = e_0 + \sum_j e_j s_j, \qquad F = f_0 + \sum_j f_j t_j,$$

$$G = g_0 + \sum_j g_j s_j, \qquad H = h_0 + \sum_j h_j t_j. \tag{18}$$

In the main we will consider only a system with three univalent cations M_1, M_2, and M_3, which we will think of as Na^+, K^+, and H^+, respectively, and one or two substrates.

ONE SUBSTRATE

Transport fluxes

We derive the equation for the one-way transport flux of substrate S_1 and then tabulate the one-way carrier fluxes and the net carrier fluxes. To this end, let $J^{\rightarrow}(S_j)$ and $J^{\leftarrow}(S_j)$ be one-way fluxes and $J(S_j)$ the net flux The one-way carrier flux $J^{\rightarrow}(S_j)$ is given by Eq. (19).

$$J^{\rightarrow}(S_1) = k_{01}x_{01} + \sum_i k_{i1}x_{i1} = \left[\frac{k_{01}s_1}{K_{01}} + \sum_i \frac{k_{i1}m_i s_1}{K_{i1}}\right]x_{00}$$
$$= e_1 s_1 x_{00}. \tag{19}$$

Substituting Eq. (14) for x_{00} gives Eq. (20).

$$J^{\rightarrow}(S_1) = \frac{e_1 s_1 C_0(f_0 + f_1 t_1)}{(e_0 + e_1 s_1)(h_0 + h_1 t_1) + (f_0 + f_1 t_1)(g_0 + g_1 s_1)}$$
$$= \frac{e_1 s_1 C_0(f_0 + f_1 t_1)}{(e_0 h_0 + f_0 g_0) + (h_0 e_1 + f_0 g_1)s_1 + (e_0 h_1 + g_0 f_1)t_1 + (e_1 h_1 + f_1 g_1)s_1 t_1}. \tag{20}$$

This expression shows the same dependence on s_1 and t_1 as was found before the ion effects were explicitly considered [47, 50], but now the coefficients of the various terms are functions of the ion concentrations. If the intracellular concentration of substrate can be kept low, then the initial one-way flux $J_{in}^{\rightarrow}(S_1)$ is given by Eq. (21), which shows a Michaelis–Menten type of dependence on S_1 in which V_m and K_m are functions of the cation concentrations.

$$J_{in}^{\rightarrow}(S_1) = \frac{C_0 e_1 f_0 s_1}{(e_0 h_0 + f_0 g_0) + (e_1 h_0 + g_1 f_0)s_1}. \tag{21}$$

Note that since both s_1 and t_1 appear in Eq. (20), there is a *trans* effect of the substrate concentrations on the one-way fluxes. The significance of this *trans* effect depends on the relative values of f_0 and f_1 and of the coefficients of s_1 and t_1 in the denominator of Eq. (20). Table 1 gives the one-way and the net fluxes for S_1 and the rth ion M_r.

It is possible now to catalog the special cases of this model, for example, obligatory coupling of Na^+ and substrate, obligatory coupling of Na^+ and substrate and exchange for K^+. This is easily done by the reader. We will do it for the steady-state concentration ratios.

Cotransport of Ions and Substrate

It is of interest to examine the dependence of the substrate flux on the ion concentrations and the stoichiometry of ion and substrate movements.

TABLE I

FLUXES OF IONS AND SUBSTRATE PREDICTED BY EQUILIBRIUM CARRIER MODEL

Flux of	$J^{\rightarrow}$	$J^{\leftarrow}$	J_{net}
S_1	$\frac{C_0}{D} e_1(f_0 + f_1 t_1)s_1$	$\frac{C_0}{D} f_1(e_0 + e_1 s_1)t_1$	$\frac{C_0}{D}(e_1 f_0 s_1 - e_0 f_1 t_1)$
M_r	$\frac{C_0}{D}(f_0 + f_1 t_1)\left[\frac{k_{r0}}{K_{r0}} + \frac{k_{r1}}{K_{r1}} s_1\right]m$	$\frac{C_0}{D}(e_0 + e_1 s_1)\left[\frac{k_{-r0}}{K_{r0}} + \frac{k_{-r1}}{K_{r1}} t_1\right]n_r$	$\frac{C_0}{D}\left\{(f_0 + f_1 t)\left[\frac{k_{r0}}{K_{r0}} + \frac{k_{r1}}{K_{r1}} s_1\right]m_r - (e_0 + e_1 s_1)\left[\frac{k_{-r0}}{K_{r0}} + \frac{k_{-r1}}{K_{r1}} t_1\right]n_r\right\}$
M_{r0} (M_r in absence of substrate)	$\frac{C_0}{D_0} f_0 \frac{k_{r0}}{K_{r0}} m_r$	$\frac{C_0}{D_0} e_0 \frac{k_{-r0}}{K_{r0}} n_r$	$\frac{C_0}{D_0}\left[f_0 \frac{k_{r0}}{K_{r0}} m_r - e_0 \frac{k_{-r0}}{K_{r0}} n_r\right]$
	$D = (e_0 + e_1 s_1)(h_0 + h_1 t_1)$ $D_0 = e_0 h_0 + f_0 g_0$	$+(f_0 + f_1 t_1)(g_0 + g_1 s_1)$	

First consider the ion dependence of initial flux as given by Eq. (21). From Eq. (21) we obtain the following for the maximal flux $J^{\rightarrow}_{\text{inM}}$ and for K_m.

$$J^{\rightarrow}_{\text{inM}}(S_1) = \frac{C_0 e_1 f_0}{e_1 h_0 + g_1 f_0}, \qquad K_m(S_1) = \frac{e_0 h_0 + f_0 g_0}{e_1 h_0 + g_1 f_0}. \tag{22}$$

For illustration, suppose Na^+ (M_1) is the only ion that is involved (i.e., that $k_{i0} = k_{i1} = 0$ for $i > 1$). Then

$$J^{\rightarrow}_{\text{inM}}(S_1) = \frac{C_0\left[\frac{k_{01}}{K_{01}} + \frac{k_{11}[\text{Na}]_e}{K_{11}}\right]\left[k_{-00} + \frac{k_{-10}}{K_{10}}[\text{Na}]_i\right]}{\left[\frac{k_{01}}{K_{01}} + \frac{k_{11}}{K_{11}}[\text{Na}]_e\right]\left[1 + \frac{[\text{Na}]_i}{K_{10}}\right] + \left[1 + \frac{[\text{Na}]_e}{K_{10}}\right]\left[k_{-00} + \frac{k_{-10}}{K_{10}}[\text{Na}]_i\right]}, \tag{23a}$$

$$K_m(S_1) = \frac{\left[k_{00} + \frac{k_{10}}{K_{10}}[\text{Na}]_e\right]\left[1 + \frac{[\text{Na}]_i}{K_{10}}\right] + \left[k_{-00} + \frac{k_{-10}}{K_{10}}[\text{Na}]_i\right]\left[1 + \frac{[\text{Na}]_e}{K_{10}}\right]}{\left[\frac{k_{01}}{K_{01}} + \frac{k_{11}}{K_{11}}[\text{Na}]_e\right]\left[1 + \frac{[\text{Na}]_i}{K_{10}}\right] + \left[1 + \frac{[\text{Na}]_e}{K_{10}}\right]\left[k_{-00} + \frac{k_{-10}}{K_{10}}[\text{Na}]_i\right]}. \tag{23b}$$

Note that if $[\text{Na}]_i$ is relatively constant, then both $J^{\rightarrow}_{\text{inM}}(S_1)$ and $K_m(S_1)$ show a dependence on $[\text{Na}]_e$ that is not typically Michaelis–Menten in type because of the constant term in the numerators. To simplify the algebra, suppose $[\text{Na}]_i$ is relatively constant, so that f_0 and h_0 are constant. Then Eqs. (23) become Eqs. (24).

$$J^{\rightarrow}_{\text{inM}}(S_1) = \frac{C_0 f_0\left[\frac{k_{01}}{K_{01}} + \frac{k_{11}[\text{Na}]_e}{K_{11}}\right]}{\left[h_0\frac{k_{01}}{K_{01}} + f_0\right] + \left[h_0\frac{k_{11}}{K_{11}} + \frac{f_0}{K_{10}}\right][\text{Na}]_e}, \tag{24a}$$

$$K_m(S_1) = \frac{[h_0 k_{00} + f_0] + \left[h_0\frac{h_{10}}{K_{10}} + \frac{f_0}{K_{10}}\right][\text{Na}]_e}{\left[h_0\frac{k_{01}}{K_{01}} + f_0\right] + \left[h_0\frac{k_{11}}{K_{11}} + \frac{f_0}{K_{10}}\right][\text{Na}]_e}. \tag{24b}$$

Note from the first of Eqs. (24) that some of the initial flux may be a carrier-mediated flux that is independent of $[\text{Na}]_e$; the fraction of the total flux mediated by the complex CS_1 depends on the relative values of k_{01}/K_{01} and k_{11}/K_{11}.

Now let us consider the possibility of measuring the stoichiometry of the flux of ion M_1 and of the flux of the substrate. Consider one-way fluxes. For the ion M_1 (Na^+) there may well be modes of entry other than the carrier system specific for substrate S_1. Any measurement of the flux

of M_1 includes this other flux $J_0(M_1)$, which is presumably independent of S_1. Thus a measurement of the flux of M_1 consists of $J_0(M_1) + J(M_1)$. If one corrects the measured flux by subtracting the flux of M_1 when no substrate is present (i.e., for $[S_1] = 0$), one actually subtracts $J_0(M_1) + J(M_{10})$ where $J(M_{10})$ is the specific carrier system flux of M_1 in absence of substrate. Thus the ratio of corrected flux of M_1 to flux of S_1 would be given by Eq. (25).

$$\text{flux ratio} = \frac{J^{\rightarrow}(M_1) - J^{\rightarrow}(M_{10})}{J^{\rightarrow}(S_1)}. \tag{25}$$

This is the correction used by Eddy [14], although for initial flux rather than one-way flux. It is not always obvious in some of the reported studies of ion–substrate stoichiometry whether or not the flux or uptake of ion was corrected for the flux at zero substrate concentration. In practice probably the best strategy is to plot $J^{\rightarrow}(M_1)$ as a function of $J^{\rightarrow}(S_1)$ and examine how the slope of such a graph changes with S_1. It should be obvious from the equations for the fluxes that for this type of model one cannot expect fixed, near-integral stoichiometries unless there is *tight coupling* between substrate and ion movement; that is, complexes M_1C and CS_1 should not contribute appreciably to the flux of M_1 or S_1. If that is so, $k_{10} = k_{01} = 0$ and $J(M_{10}) = 0$. The flux ratio is then given by Eq. (26):

$$\frac{J^{\rightarrow}(M_1) - J^{\rightarrow}(M_{10})}{J^{\rightarrow}(S_1)} = \frac{J^{\rightarrow}(M_1)}{J^{\rightarrow}(S_1)} = 1. \tag{26}$$

Otherwise it is given by Eq. (27):

$$\frac{J^{\rightarrow}(M_1) - J^{\rightarrow}(M_{10})}{J^{\rightarrow}(S_1)} = \left(\frac{k_{10}}{K_{10}}\frac{1}{s_1} + \frac{k_{11}}{K_{11}}\right)m_1 \Big/ \left(\frac{k_{01}}{K_{01}} + \frac{k_{11}}{K_{11}}m_1\right) - \frac{D}{D_0}\frac{f_0}{(f_0 + f_1 t_1)e_1}\frac{k_{10}}{K_{10}}\frac{m_1}{s_1}. \tag{27}$$

The second term in Eq. (27) is difficult to evaluate in the general case. However, if $k_{10}/K_{10} \simeq 0$ (i.e., M_1C cannot cross the membrane or M_1C cannot be formed), then the flux ratio is independent of substrate concentration but does depend on the concentration of the ion M_1 and is less than 1 but increases toward 1 with increase in m_1 as shown by Eq. (28).

$$\frac{J^{\rightarrow}(M_1) - J^{\rightarrow}(M_{10})}{J^{\rightarrow}(S_1)} = \frac{(k_{11}/K_{11})m_1}{(k_{01}/K_{01}) + (k_{11}/K_{11})m_1} = \frac{m_1}{(k_{01}/k_{11})(K_{11}/K_{01}) + m_1}. \tag{28}$$

On the other hand, if the complex CS_1 cannot cross the membrane, but

M_1C can, the first term of Eq. (27) becomes independent of m_1 but the second term is an involved algebraic expression in m_1, n_1, t_1, and s_1.

$$\frac{J^{\rightarrow}(M_1) - J^{\rightarrow}(M_{10})}{J^{\rightarrow}(S_1)} = 1 + \frac{k_{10}}{k_{11}}\frac{11}{K_{10}}\frac{1}{s_1} - \frac{D}{D_0}\frac{f_0}{(f_0 + f_1 t_1)e_1}\frac{k_{10}}{K_{10}}\frac{m_1}{s_1}. \quad (29$$

Steady State

For the steady state, Eq. (30) holds:

$$J^{\rightarrow}(S_1) - J^{\leftarrow}(S_1) = k_s(t_1 - s_1). \quad (30)$$

That is, the net carrier-mediated flux must equal the noncarrier-mediated leak of substrate across the cell membrane. If the term $k_s(t_1 - s_1)$ is appreciable in comparison with the one-way fluxes, the implications for the steady state are rather difficult to unearth from relation (30). It is common practice to assume that $k_s(t_1 - s_1)$ is small compared to the one-way fluxes [57, 58] and we follow that here. Then one may assume that $J^{\rightarrow}(S_1) \simeq J^{\leftarrow}(S_1)$. This assumption is not always valid, but it is probably a fair assumption for amino acids for Ehrlich ascites cells, and many other cells for low values of S_1. From this assumption we obtain the steady-state distribution ratio for various special cases of this model. We assume that the substrate is concentrated, so that $t_1 > s_1$ and $k_s(t_1 - s_1)$ is a small positive number. Then

$$(C_0/D)f_1(e_0 + e_1 s_1)t_1 = (C_0/D)e_1(f_0 + f_1 t_1)s_1 - k_s(t_1 - s_1), \quad (31)$$

from which we obtain

$$\left.\frac{t_1}{s_1}\right|_{ss} = \frac{e_1 f_0}{f_1 e_0} - \frac{Dk_s}{C_0 e_0 f_1}\left[\frac{(e_1 f_0/e_0 f_1) - 1}{1 + (k_s D/C_0 e_0 f_1)}\right]. \quad (32)$$

By our assumption the second term in Eq. (32) is only a small correction term, so that $e_1 f_0/f_1 e_0$ is a close upper bound for t_1/s_1. If the assumption is false and $t_1 > s_1$ in the steady state, then the equations that are developed below all overestimate the steady-state concentration ratios. In the remainder we neglect the small correction term and develop the implications for special cases from Eq. (33)

$$\left.\frac{t_1}{s_1}\right|_{ss} = \frac{e_1 f_0}{f_1 e_0} = \frac{\left[\frac{k_{01}}{K_{01}} + \sum_i \frac{k_{i1} m_i}{K_{11}}\right]\left[k_{-00} + \sum_i \frac{k_{-i0} n_i}{K_{i0}}\right]}{\left[\frac{k_{-01}}{K_{01}} + \sum_i \frac{k_{-i1} n_i}{K_{11}}\right]\left[k_{00} + \sum_i \frac{k_{i0} m_i}{K_{i0}}\right]}. \quad (33)$$

For the more interesting special cases that follow we write $[Na]_e = m_1$, $[Na]_i = n_1$, $[K]_e = m_2$, $[K]_i = n_2$, $[H]_e = m_3$, $[H]_i = n_3$, and $c_i = t_1 = [S_1]_i$, $c_e = s_1 = [S_1]_e$, so as to put the results in a notation commonly used in transport work. In looking at the special cases it is also worth

considering two possibilities for the charge on the carrier. If the carrier carries a unit negative charge, C and CS_j are both negatively charged, but M_iC and M_iCS_j are neutral if M_i is a univalent cation. In that case, Eqs. (34) hold, where $\xi = FV_m/RT$.

$$\begin{aligned} k_{-oo} &= k_{oo}e^{-\xi}, \qquad k_{-io} = k_{io}, \\ k_{-io} &= k_{o1}e^{-\xi}. \qquad k_{-ij} = k_{ij}. \end{aligned} \tag{34}$$

On the other hand if C is neutral, then M_iC and M_iCS_j carry unit positive charges for M_i a univalent cation and relations (35) hold.

$$\begin{aligned} k_{-oo} &= k_{oo}, \qquad k_{-io} = k_{io}e^{\xi}, \\ k_{-o1} &= k_{o1}, \qquad k_{-ij} = k_{ij}e^{\xi}. \end{aligned} \tag{35}$$

Note that the equations have been derived for any charges on the various carrier complexes. The results for any particular assumed charges on the different carrier complexes are readily found by substituting $k_{-ij} = k_{ij}\exp(-z_{ij}V_mF/RT)$ in Eq. (33).

Free Carrier Cannot Cross Membrane. In this case $k_{oo} = k_{-oo} = 0$ and Eq. (36) gives the steady-state distribution ratio.

$$\frac{c_i}{c_e} = \frac{[(k_{o1}/K_{o1}) + \sum_i (k_{i1}m_i/K_{i1})][\sum_i (k_{-io}n_i/K_{io})]}{[(k_{-o1}/K_{o1}) + \sum_i (k_{-i1}n_i/K_{i1})\ [\sum_i (k_{io}/K_{io})m_i]}. \tag{36}$$

Carrier–Substrate Complex CS_1 Cannot Cross Membrane. Then $k_{o1} = k_{-o1} = 0$ and Eq. (37) describes the situation.

$$\frac{c_i}{c_e} = \frac{[\sum_i (k_{i1}/K_{i1})m_i][k_{-oo} + \sum_i (k_{-io}/K_{io})n_i]}{[\sum (k_{-i1}/K_{i1})n_i][k_{oo} + \sum_i (k_{io}/K_{io})m_i]}. \tag{37}$$

Ion–Carrier Complexes M_iC Cannot Cross Membrane. If the ions cross only in combination with the carrier and substrate, $k_{io} = k_{-io} = 0$. Then

$$\frac{c_i}{c_e} = \frac{k_{-oo}}{k_{oo}} \frac{\left[\dfrac{k_{o1}}{K_{o1}} + \dfrac{k_{11}[\mathrm{Na}]_e}{K_{11}} + \dfrac{k_{21}[\mathrm{K}]_e}{K_{21}} + \dfrac{k_{31}[\mathrm{H}]_e}{K_{31}}\right]}{\left[\dfrac{k_{-o1}}{K_{o1}} + \dfrac{k_{-11}[\mathrm{Na}]_i}{K_{11}} + \dfrac{k_{-21}[\mathrm{K}]_i}{K_{21}} + \dfrac{k_{-31}[\mathrm{H}]_i}{K_{31}}\right]}. \tag{38}$$

If the terms in potassium and hydrogen can be neglected, then Eq. (39) holds whether the carrier is neutral or negatively charged.

$$\frac{c_i}{c_e} = e^{-\xi}\frac{[(k_{o1}/K_{o1}) + (k_{11}[\mathrm{Na}]_e/K_{11})]}{[(k_{o1}/K_{o1})e^{-\xi} + (k_{11}[\mathrm{Na}]_i/K_{11})]}. \tag{39}$$

Free Carrier and Carrier–Substrate Complex Cannot Cross. If only

carrier with cation on it can cross the membrane, $k_{00} = k_{-00} = k_{01} = k_{-01} = 0$.

$$\frac{c_i}{c_e} = \frac{\begin{array}{l}[(k_{11}[\mathrm{Na}]_e/K_{11}) + (k_{21}[\mathrm{K}]_e/K_{21}) + (k_{31}[\mathrm{H}]_e/K_{31})] \\ \times[(k_{-10}[\mathrm{Na}]_i/K_{10}) + (k_{-20}[\mathrm{K}]_i/K_{20}) + (k_{-30}[\mathrm{H}]_i/K_{30})]\end{array}}{\begin{array}{l}[(k_{-11}[\mathrm{Na}]_i/K_{11}) + (k_{-21}[\mathrm{K}]_i/K_{21}) + (k_{-31}[\mathrm{H}]_i/K_{31})] \\ \times[(k_{10}[\mathrm{Na}]_e/K_{10}) + (k_{20}[\mathrm{K}]_e/K_{20}) + (k_{30}[\mathrm{H}]_e/K_{30})]\end{array}}. \tag{40}$$

Suppose $k_{11}/K_{11} \gg k_{21}/K_{21}, k_{31}/K_{31}$ and $k_{20}/K_{20} \gg k_{10}/K_{10}$, and $k_{30}/K_{30} \gg k_{10}/K_{10}$. This is then a tightly coupled pump in which substrate can enter only in association with Na^+ and carrier can return without substrate only in a K^+ or H^+ form. Then, whether the carrier is negatively charged or neutral, Eq. (41) holds:

$$\frac{c_i}{c_e} = \frac{[\mathrm{Na}]_e}{[\mathrm{Na}]_i}\frac{\left[\dfrac{k_{20}[\mathrm{K}]_i}{K_{20}} + \dfrac{k_{30}[\mathrm{H}]_i}{K_{30}}\right]}{\left[\dfrac{k_{20}[\mathrm{K}]_e}{K_{20}} + \dfrac{k_{30}[\mathrm{H}]_e}{K_{30}}\right]}. \tag{41}$$

If the terms in [H] are negligible,

$$\frac{c_i}{c_e} = \frac{[\mathrm{Na}]_e}{[\mathrm{Na}]_i}\frac{[\mathrm{K}]_i}{[\mathrm{K}]_e}. \tag{42}$$

For this sort of tight coupling of ion and substrate fluxes, Eq. (42) can be derived from strictly energy considerations [4] without referring to any model.

Tight Coupling with Only Free Carrier or Cation–Carrier–Substrate Complex Able to Cross. For this situation $k_{i0} = k_{-i0} = k_{01} = k_{-01} = 0$. Then Eq. (33) becomes (43).

$$\frac{c_i}{c_e} = \frac{k_{-00}}{k_{00}}\frac{\left[\dfrac{k_{11}[\mathrm{Na}]_e}{K_{22}} + \dfrac{k_{21}[\mathrm{K}]_e}{K_{21}} + \dfrac{k_{31}[\mathrm{H}]_e}{K_{31}}\right]}{\left[\dfrac{k_{-11}[\mathrm{Na}]_i}{K_{11}} + \dfrac{k_{-21}[\mathrm{K}]_i}{K_{21}} + \dfrac{k_{-31}[\mathrm{H}]_i}{K_{31}}\right]}. \tag{43}$$

If the terms in K^+ and H^+ are negligible, so that only the sodium–carrier–substrate complex crosses, then whatever the charge on the free carrier, Eq. (43) reduces to (44).

$$\frac{c_i}{c_e} = \frac{[\mathrm{Na}]_e}{[\mathrm{Na}]_i}\exp\left[-\frac{FV_m}{RT}\right] \tag{44}$$

Again, this can be derived from energy considerations alone [4]. In this example of tight coupling, only the full ion–carrier–substrate complex contributes to the net substrate flux and only free carrier can return.

TWO SUBSTRATES PRESENT

The algebra becomes increasingly difficult as the number of substrates is increased. It is important, however, to examine the initial fluxes for a number of special cases in which two substrates are present. These are the situations that would correspond to the experiments of competitive inhibition and exchange diffusion.

Competition

We examine how the initial flux of one substrate depends on the concentration of another. We can imagine two extreme types of substrates. One readily forms complex CS, which also crosses easily; this type of substrate forms complex M_iCS primarily via CS as intermediate. The other type of substrate takes the alternate route primarily in formation of M_iCS_j. The flux of S_1, $J^{\rightarrow}(S_1)$, in the presence of S_2 is given by Eq. (45):

$$J^{\rightarrow}(S_1) = \frac{C_0 s_1 e_1 (f_0 + f_1 t_1 + f_2 t_2)}{(e_0 + e_1 s_1 + e_2 s_2)(h_0 + h_1 t_1 + h_2 t_2) + (f_0 + f_1 t_1 + f_2 t_2)(g_0 + g_1 s_1 + g_2 s_2)}. \tag{45}$$

To simplify matters somewhat, consider the initial flux, for which $t_1 = t_2 = 0$.

$$J^{\rightarrow}_{\text{in}}(S_1) = \frac{C_0 f_0 e_1 s_1}{(e_1 h_0 + f_0 g_0) + (h_0 e_1 + f_0 g_1) s_1 + (h_0 e_2 + f_0 g_2) s_2}. \tag{46}$$

This predicts that the carrier initial flux of S_1 should be completely inhibitable by S_2 provided the concentration of S_2 can be raised sufficiently, no matter what types of substrate S_1 and S_2 are. The effect of S_2 depends on the relative values of $(h_0 e_2 + f_0 g_2)$ and $(h_0 e_1 + f_0 g_1)$. However, it is almost impossible to keep t_1 and t_2 negligible, so that in actual experiments Eq. (45) should be considered, and then it is difficult to predict the full effect of changing S_2 because t_1 and t_2 will also change and exchange diffusion may come to play an important role.

Exchange Diffusion

Now let us consider the initial flux of S_1, $J^{\rightarrow}_X(S_1)$, in cells that have been loaded with S_2 so that $s_2 = 0$, $t_1 = 0$. The flux is then

$$J^{\rightarrow}_X(S_1) = \frac{C_0 e_1 s_1 [f_0 + f_2 t_2]}{(e_0 + e_1 s_1)(h_0 + h_2 t_2) + (f_0 + f_2 t_2)(g_0 + g_1 s_1)}. \tag{47}$$

This is to be compared with initial flux for the same S_1 but for $t_2 = 0$ for which Eq. (48) holds.

$$J^{\rightarrow}_{\text{in}}(S_1) = \frac{C_0 e_1 s_1 f_0}{(e_0 + e_1 s_1) h_0 + f_0 (g_0 + g_1 s_1)}. \tag{48}$$

The ratio of the two fluxes is

$$\frac{\vec{J_X}}{\vec{J_{in}}} = \frac{[f_0 + f_2 t_2]}{f_0} \frac{[h_0(e_0 + e_1 s_1) + f_0(g_0 + g_1 s_1)]}{[(e_0 + e_1 s_1)(h_0 + h_2 t_2) + (f_0 + f_2 t_2)(g_0 + g_1 s_1)]}. \quad (49)$$

The difference of the two is

$$\vec{J_X} - \vec{J_{in}} = \frac{C_0 e_1 s_1 (e_0 + e_1 s_1) t_2 [h_0 f_2 - f_0 h_2]}{D_X \cdot D_{in}}. \quad (50)$$

DISCUSSION

Some of the implications of the equations developed so far are not immediately obvious; it may help to put them in words. A significant role is played by the relative contributions of complex CS_j and M_iCS_j to the flux of substrate. Consider two extreme types of substrate. The first readily forms complex CS_j, which can cross the membrane so that considerable substrate can cross the cell membrane independently of the ions. We talk primarily in terms of Na^+ cotransport. This is a leak around the concentrating capability of the ion-mediated transport, so we do not expect high concentration ratios. For such a system we would expect that a portion of the initial flux is sodium independent and that the ready movement of CS_j provides a high exchange capability that is not dependent on the presence of Na^+. On the other hand, the other type moves only in the complex M_iCS_j, and so we expect high concentrating ability. Furthermore, if primarily the Na^+ complex is involved in substrate movement, there can be little exchange flux unless the intracellular Na^+ concentration is appreciable. Thus at the usual low intracellular Na^+ concentrations this is a poorly exchanging substrate that is concentrated to high distribution ratios. These descriptions of the possible behavior of two types of substrates in this model come close to those given by Christensen [66] for the A and L systems for amino acid transport. One might also conceive of substrates intermediate in type. For example, one type might form NaCS via NaC as intermediate, but also form CS, which plays a minor role in transport. Thus we see at least the possibility of different behaviors for different substrates moving by the same system.

Much of the work on amino acids and the sugars has been done with initial fluxes or with near steady-state concentration ratios. It is time to emphasize the need for detailed studies of the one-way fluxes in the steady state. We could then examine the ion dependencies of the coefficients of s_1 and t_1 in Eq. (20). Initial fluxes would appear to be far less suited to this sort of estimation problem because not only is intracellular substrate concentration changing rapidly during initial flux measurements, but the intracellular ion concentrations may also be changing.

Finally, I emphasize that the foregoing model is but one example of a

two-state type of carrier model. It is possible to consider both multistate and polyvalent models, and some such probably should be examined in more detail. Not only might we consider that carrier can exist in two states, but it might be worthwhile examining models in which carrier exists in a succession of states across the membrane with transitions between adjacent states; in the limit as the number of states increases this approaches a true mobile carrier model. Hill and Kedem [57] have examined a number of models of these types. Another interesting possible group of models are basically two-state models in which the carrier is polyvalent with binding sites on both sides of the membrane. For example, consider a model that has a substrate binding site and an ion binding site on each side of the membrane per carrier molecule and assume that a transition consists of an interchange of the sites from the two sides, such as by a rotation of the molecule. One example of a model of this type has been published by Lief and Stein [67], who proposed a tetramer model of a carrier to explain findings on glucose transport in red blood cells. The molecule they propose has one low-affinity and one high-affinity binding site for sugar on each side of the membrane.

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