A UNIFIED KINETIC HYPOTHESIS OF CARRIER MEDIATED TRANSPORT: ITS APPLICATIONS

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ABSTRACT A model analysis of the process of carrier mediated membrane transport is presented, wherein the carrier is present in two forms of differing affinity for substrate. The two forms of carrier undergo interconversion by asymmetric metabolic reactions on each side of the membrane. From this model system expressions are derived for the steady-state distribution ratio for substrate, for the unidirectional fluxes of substrate and hence for the initial velocity of uptake of substrate, and for the effect of preloading cells upon the initial velocity of uptake of labeled substrate. These expressions are applied to published data for glycine transport in Ehrlich ascites tumor cells to obtain numerical values for the parameters of a concentrative membrane carrier system. Concentrative uptake is shown to be consequent to the differing affinities of the two forms of carrier. When the affinities of the two forms are equal, equilibrative uptake occurs. The model analysis is applied to the phenomena of metabolic and competitive inhibition.

The study of the transport of substances across cell membranes has led to the concept of a carrier mechanism operating within the cell membrane. This mechanism is considered, in essence, to consist of a reaction of the transport substrate with a membrane component to form a complex, and of the movement of the complex from one side of the membrane to the other, with subsequent release of substrate. Experimentally, the process of transport has been found to result either in the equilibration of substrate across the membrane or in concentration of substrate within the cell (1).

The purpose of the present communication is to construct a carrier model system from which the characteristics of both an equilibrating and a concentrating system may be predicted. The model is formulated and from this formulation expressions are derived for the initial velocity of uptake of substrate, the steady-state distribution ratio of substrate, and the time course of uptake of substrate. The methods necessary to obtain numerical estimates of the parameters of a given

transport system are developed and, as an illustrative example, the methods are applied to data on the concentrative transport of glycine by Ehrlich ascites tumor cells.

FORMULATION OF THE MODEL

The following notation will be used in developing this model:

subscript 1 denotes the outside surface of membrane or extracellular space,

subscript 2 denotes the inside surface of membrane or intracellular space.

Carrier is assumed to be present in the membrane in two mobile forms, C and C'.

$\mathbf{C}$ and $\mathbf{C}'$ are the total concentrations of the two forms of carrier in the membrane. Each expression includes both the form bound to substrate and the "free" form.

$\mathbf{A}$ is the concentration of the transport substrate.

$\mathbf{AC}$ and $\mathbf{AC}'$ represent the concentrations of carrier-substrate complex within the membrane.

$(\mathbf{C} - \mathbf{AC})$ and $(\mathbf{C}' - \mathbf{AC}')$ are the concentrations of "free" carrier within the membrane.

$\mathbf{K}$ and $\mathbf{K}'$ are the Michaelis constants of the two forms of carrier, C and C'. $\mathbf{s} = \mathbf{K}'/\mathbf{K}$.

δ is the thickness of the membrane.

$\mathbf{x}$ is the distance within the membrane, defined from the outer surface of the membrane, hence $0 < \mathbf{x} < \delta$.

$\mathbf{D}$ is the diffusion coefficient of C, C', AC, and AC'.

α is the rate constant for the transformation, $(\mathbf{C}' - \mathbf{AC}') \rightarrow (\mathbf{C} - \mathbf{AC})$.

α' is the rate constant for the transformation, $(\mathbf{C} - \mathbf{AC}) \rightarrow (\mathbf{C}' - \mathbf{AC}')$.

$\mathbf{Z}$ is the total surface area across which transport occurs.

$\mathbf{Q}$ is the total volume of membrane across which transport occurs.

$\mathbf{T}$ is the total amount of carrier in the membrane.

$\gamma = \mathbf{Q}/\mathbf{Z}$. For a plane film $\gamma = \delta$.

$\phi = \alpha'\gamma\delta/D$.

$\theta = \alpha'/\alpha$.

All other symbols are defined in the discussion as required.

The assumptions underlying the formulation of the model (Fig. 1) consist of the following:

(*a*) Substrate can traverse the membrane only if linked to a carrier molecule.

(*b*) The diffusion coefficient, $\mathbf{D}$, is taken to be small compared to the rate constants of the reaction between substrate and carrier. This implies virtually instantaneous equilibration between substrate and carrier (both C and C').

(*c*) The diffusion coefficients of free carrier and of substrate-carrier complex for both C and C' are assumed to be equal. This assumption would be reasonable if, for instance, the carrier molecules were large compared to substrate molecules and if the difference between C and C' was in the tertiary molecular structure.

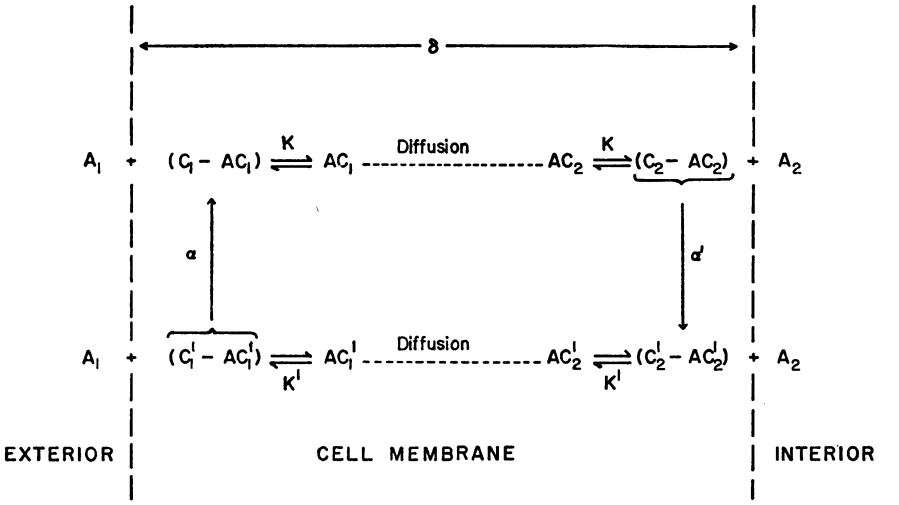

FIGURE 1 Model of a membrane carrier transport system wherein the mass reaction coefficients α and α' describe the conversion of only the free carrier species. Subscript 1 denotes extracellular space and outer surface of the membrane, and subscript 2 denotes intracellular space and inner surface of the membrane.

(*d*) The transformations of $(C - AC) \rightarrow (C' - AC')$ and $(C' - AC') \rightarrow (C - AC)$ involve only the "free" forms of each carrier.

(*e*) Unless otherwise stated, the energy for carrier transport exists at all times and is never a limiting factor in the system.

(*f*) It is assumed that the total carrier is in a steady state.

(*g*) The total concentration of carrier in a cross-section of the membrane is a constant, T.

(*h*) The membrane thickness, δ, is taken to be sufficiently small such that dC/dx may be replaced by $\Delta C/\delta$.

In this model system substrate, *A*, combines with carrier, *C*, at the outer surface to form *AC*. This complex traverses the membrane by diffusion and dissociates at the inner surface. "Free" *C* is transformed to *C'*. The new form of carrier *C'* associates with *A*, diffuses across the membrane, and releases *A* at the outer surface. *C* is then regenerated from "free" *C'*. In essence the system is a self-perpetuating closed shuttle service for substrate.

From the assumptions, expressions for the carrier concentrations at the two surfaces of the membrane may be derived (see Appendix[1]). Utilizing these, expressions for the initial unidirectional fluxes of substrate and for the steady state of substrate will be developed.

[1] Equations will be numbered in logical order, beginning with the Appendix.

Initial Rates. Let F_1 represent the unidirectional flux of substrate inwards, defined as amount per unit area per unit time.
Then

$$F_1 = \frac{D}{\delta}(AC_1 + AC_1') = \frac{D}{\delta}\left(\frac{A_1 \cdot C_1}{A_1 + K} + \frac{A_1 \cdot C_1'}{A_1 + K'}\right). \quad [5]$$

Substituting for C_1 and C_1' in equation [5]

$$F_1 = \left(\frac{aD}{2\delta}\right)\left(\frac{A_1}{A_1 + K}\right)\left(1 - \frac{K\theta(1 - 1/s)}{\theta A_1/s + A_2 + K(1 + \theta + \phi)}\right). \quad [6]$$

Now let v_o be the inward rate of substrate transport observed at zero time. Then $v_o = ZF_1$. Experimentally this quantity is measured by adding to the external medium an amount of labeled substrate $q_{1,0}$ at zero time, so that the specific activity of substrate in the medium becomes $q_{1,0}/A_1V_1$, where V_1 is the volume of the external medium. The accumulation of radioactivity in the cells under consideration is measured, and from this the initial inward rate of transport of radioactivity is deduced. Division of this initial rate by the specific activity of the external medium at zero time yields a value for the initial rate of transport of substrate, v_o, in terms of amount transported per unit time, since there can be no efflux of radioactivity at zero time.

Experimentally, the initial rate of transport has been found to be independent of the internal concentration of substrate, in an equilibrating system (1). In contrast, Heinz has demonstrated that, in the case of the concentrative transport of glycine by Ehrlich ascites carcinoma cells, preloading with glycine increases the initial velocity of transport of labelled glycine (2). Examination of equation [6] demonstrates that in the model system there is a similar dependence of v_o upon A_2. For an equilibrating system (where $s = K'/K = 1$) the expression for initial velocity reduces to

$$v_o = \left(\frac{aDZ}{2\delta}\right)\left(\frac{A_1}{A_1 + K}\right), \quad [7]$$

and v_o is independent of A_2. As A_1 increases, the initial inward rate of transport will approach a maximum value, $aDZ/2\delta$. Preloading with substrate will not affect this initial inward rate. In contrast, for a concentrating system (where $s > 1$) it may be seen that the factor

$$\left[1 - \frac{K\theta(1 - 1/s)}{\theta A_1/s + A_2 + K(1 + \theta + \phi)}\right]$$

in equation [6] will increase as A_2 increases, and hence the initial inward rate will increase as A_2 increases. From equation [6] it is therefore evident that the initial rate of transport in an equilibrating system with the same parameters (Michaelis constant K, total amount of carrier T, external substrate concentration A_1, volume V_1, initial external substrate specific activity q_1/A_1V_1, and numerical value for $aDZ/2\delta$) will be greater than that of the concentrating system by the reciprocal of the factor

$$\left[1 - \frac{K\theta(1 - 1/s)}{\theta A_1/s + A_2 + K(1 + \theta + \phi)}\right].$$

Only when the intracellular concentration A_2 is very large will the two rates approach equivalence.

Steady State of Substrate. The relation describing the unidirectional influx of substrate F_1 has been given in equation [5]. A similar expression can be written for the unidirectional efflux of substrate F_2,

$$F_2 = \frac{D}{\delta}\left(\frac{A_2 \cdot C_2}{A_2 + K} + \frac{A_2 \cdot C_2'}{A_2 + K'}\right). \qquad [8]$$

Steady state implies that $F_1 = F_2$.

Now let $p = A_2/A_1$ be the accumulation ratio of the system. Then, from equations [5] and [8]

$$p^2 + p\left[\frac{K(s + \theta + \phi)}{A_1} + (\theta - 1)\right] - \frac{K}{A_1}[\theta + s(1 + \phi)] - \theta = 0. \qquad [9]$$

Inspection of this seemingly cumbersome expression reveals the following:

(*a*) as A_1 increases, p approaches unity,

(*b*) as A_1 decreases, p approaches $(\theta + s + s\phi)/(\theta + s + \phi)$,

(*c*) if $s = 1$, $p = 1$ for any A_1 and the model is then equivalent to an equilibrating system, and

(*d*) if $s > 1$, $p > 1$ for small A_1 and as A_1 increases p asymptotes to unity.

Use of Data to Obtain the Parameters of a System. Expressions for the initial inward rate of transport and for the steady state have been developed. When these expressions are examined, it is found that the behaviour of an equilibrating system depends on two parameters, the maximal initial inward rate of transport, $aDZ/2\delta$, and the concentration of substrate leading to half maximal initial inward rate, K. When values for the initial inward rate of transport at various external substrate concentrations are available, the two parameters may be obtained easily by the method of Lineweaver and Burk. The behaviour of a concentrative system, in contrast, is found to depend upon 5 parameters: $aDZ/2\delta$, K, θ, ϕ, and s. The purpose of the present section is to develop a method for obtaining these parameters. As an illustrative example, the values for the parameters of the concentrative transport system for glycine in Ehrlich ascites tumor cells will be estimated from published data. The data come from a number of sources and so the numerical values for the parameters are, to some extent, approximate.

Data published from experiments done at 37°C were selected. The information utilized was in the following form:

(*a*) initial rate of uptake plotted as a function of the extracellular concentration with the cells empty (3),

(*b*) initial rate of uptake plotted as a function of extracellular concentration with cells preloaded (3), and

(*c*) accumulation ratio, p, plotted as a function of extracellular concentration (4, 5). Heinz attempted to wash all the glycine out of these cells without success. He found that a certain amount, somewhat less than 10 mM, always stays inside the cell (6). Christensen has studied the water uptake by Ehrlich ascites tumor cells associated with the glycine gradient, and has found that water uptake occurred only when the gradient was greater than 6 mM (7). This is taken to imply that this amount of glycine is bound within the cell and as a consequence is not freely available to the transport mechanism. Therefore the p value used for these studies was calculated by using a figure for A_2 which has been corrected for the 6 mM intracellular binding.

The maximum accumulation ratio observed for Ehrlich ascites tumor cells [the value corresponding to $(\theta + s + s\phi)/(\theta + s + \phi)$] is of the order 10:1. Also, over the experimental range employed by Heinz (3), the fit of the initial rates to a Lineweaver-Burk type plot appears adequate. It would, therefore, seem not unreasonable to make the following approximations:

(*a*) $s = K'/K \gg 1$ and therefore $(1 - 1/s) \approx 1$, and
(*b*) $A_1/s \ll A_2 + K(1 + \theta + \phi)$.

With these approximations equation [6], the expression for the initial rate of transport, reduces to

$$v_o = \left(\frac{aDZ}{2\delta}\right)\left(\frac{A_2 + K(1 + \phi)}{A_2 + K(1 + \theta + \phi)}\right)\left(\frac{A_1}{A_1 + K}\right). \quad [10]$$

Hence for the case of glycine transport by Ehrlich ascites tumor cells

$$v_o = v_{max}\left(\frac{A_1}{A_1 + K}\right),$$

where

$$v_{max} = \left(\frac{aDZ}{2\delta}\right)\left(\frac{A_2 + K(1 + \phi)}{A_2 + K(1 + \theta + \phi)}\right). \quad [11]$$

From these equations it can be seen that the effect of preloading the cells with unlabeled glycine will be to increase the v_{max}, leaving the Michaelis constant, K, unchanged. This is precisely the behaviour observed by Heinz (3). It should be stressed that the v_{max}, as defined above, will be a value derived from experimental data by a maneuver such as a Lineweaver-Burk plot and that the value is not the true v_{max} for the system.

Determination of θ, ϕ, *and* s. A method of obtaining θ, ϕ, and s from experimental data will now be described. From the data given by Heinz (3), K = 3.1 mM, and for

(*a*) A_2 = 6 mM, v_{max} per *g* cells = 31.5 μmoles/*g* 2 minutes (where *g* refers to dry cell weight), whereas for

(*b*) A_2 = 63 mM, v_{max} per *g* cells = 63.3 μmoles/*g* 2 minutes.

When the values of A_2 have been corrected for intracellular binding of 6 mM glycine,

then A_2 is zero in the first instance, and 57 mM in the second, or preloaded, case.

Now let w be the total dry weight of cells in the experimental preparation. Then substitution of the experimental values into equation [11] yields the following two identities:

$$31.5 = \left(\frac{aDZ}{2\delta w}\right)\left(\frac{1 + \phi}{1 + \theta + \phi}\right),$$

and

$$63.3 = \left(\frac{aDZ}{2\delta w}\right)\left(\frac{57 + 3.1(1 + \phi)}{57 + 3.1(1 + \theta + \phi)}\right).$$

From these,

$$\phi = -(\theta + 21.38)/2 \pm \frac{\sqrt{(\theta + 21.38)^2 - 4(19.39 - 17.21\theta)}}{2}.$$

ϕ will be real and greater than zero if θ is greater than 1.13. For θ greater than 1.13 it is then possible to construct a table of corresponding values of θ and ϕ which satisfy this expression. As A_1 approaches 0, the accumulation ratio p approaches p_o, which equals $(s + \theta + s\phi)/(s + \theta + \phi)$. Thus if p_o is known, s can be calculated for each corresponding pair of values of θ and ϕ. The data of Christensen and Riggs (4) and of Heinz and Mariani (5), illustrated in Fig. 2, were examined and from these a choice

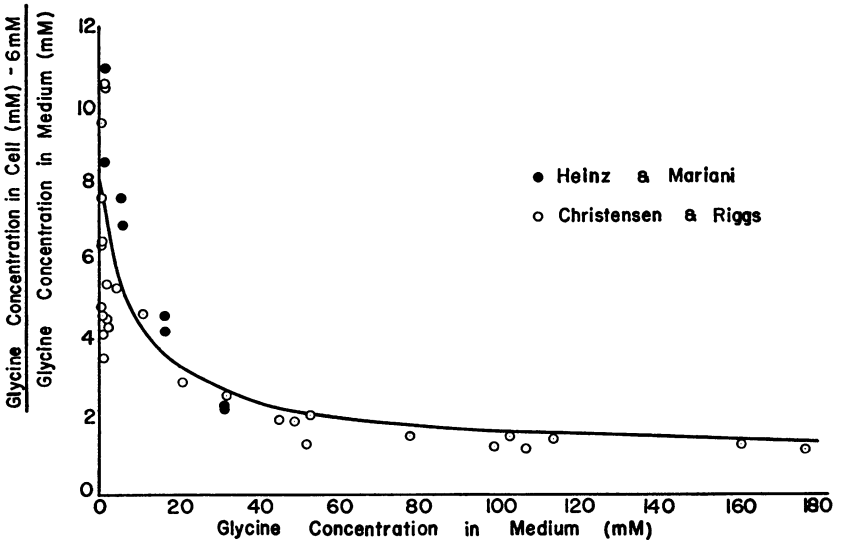

FIGURE 2 Steady-state glycine distribution ratio as a function of external glycine concentration. Complete intracellular binding of glycine up to 6mM was assumed. The data of Heinz and Mariani (5) were obtained from 15 minute incubations, those of Christensen and Riggs (4) from 1 hour incubations. The data of the former tend to be somewhat higher than those of the latter, probably because of a lower proportion of dying cells. The best fit line was selected to lie approximately midway between the two sets of data; the p_o value of this line was 8.

of a value for p_o was made which appeared to fit the data. For each p_o, a table of corresponding values of θ, ϕ, and s was constructed. The values were substituted into equation [9], and that set of values yielding the best fit to the experimental data illustrated in Fig. 2 was chosen by a process of trial and error. The numerical values of the parameters which fit the initial velocity and steady-state data were determined to be:

$\theta = 60$, $\phi = 11$, and $s = 128$.

It should be noted that in the above treatment, the average uptake values at 2 minutes were used to construct a plot of v_o *versus* A_1 (3). Heinz has pointed out (2) that the relationship among the uptake values at different concentrations is practically the same after 2 minutes as that among the extrapolated values for zero time. This has been taken to imply that the relationship between the two initial velocity curves will not be significantly altered by using average 2 minute uptake values rather than the initial rates.

Determination of $aDZ/2\delta w$. Substitution of $\theta = 60$ and $\phi = 11$ into the pair of identities above gives, from the average 2 minute uptake values, $aDZ/2\delta w = 190$ μmoles/g 2 minutes. Assuming approximate linearity of uptake rate with time, this implies $aDZ/2\delta w \approx 95$ μmoles/g minutes. In reality, however, the rate of uptake is a non-linear function of time, the rate being maximal at zero time and decreasing thereafter. For this reason the value tabulated above is an underestimate.

The value of $aDZ/2\delta w$ may also be obtained from another set of data. Heinz has examined the exchange fluxes of glycine between intra- and extracellular fluid during the steady state (8) and has defined influx and efflux coefficients. Using the present formulation the expressions for influx and efflux coefficients are, respectively, F_1Z/A_1w, and F_2Z/A_2w. The values obtained above for θ, ϕ, and s and the flux coefficients obtained from the first 3 experiments of Table I of Heinz (8) were substituted into the expressions for the flux coefficients. The values for $aDZ/2\delta w$ are listed in Table I, and the average value is 257 μM/g minutes. The values obtained in this way are much larger than the value estimated from the average 2 minute velocities. If the cells were at the same stage of growth (*i.e.*, if the surface to volume ratio was approximately the same), then the first estimate must be revised upwards. As a rough approximation,

TABLE I

FLUX COEFFICIENTS AND CORRESPONDING ESTIMATES OF $aDZ/2\delta w$

A_1	$\frac{A_2}{A_1}$	A_2	$A_2 - 6$	Influx coefficient	$\frac{aDZ^*}{2\delta w_{in}}$	Corrected efflux coefficient	$\frac{aDZ^*}{2\delta w_{out}}$
mM		mM	mM	ml/g min.	μM/g min.	ml/g min.	μM/g min.
0.75	14.7	11.0	5.0	15.0	303	2.9	420
3.90	6.1	23.8	17.8	5.3	133	1.2	192
0.75	16.0	12.0	6.0	13.1	260	1.6	234

* $aDZ/2\delta w_{in}$ is calculated using the influx coefficient; $ADZ/2\delta w_{out}$, using the efflux coefficient.

a value twice that estimated from the average 2 minute velocities, 190 μmoles/g minutes will be used in the following discussion. More suitable data will undoubtedly lead to a more precise estimate in the future.

Numerical values for the 5 parameters of the glycine transport system in Ehrlich ascites tumor cells have been found. Expressions for the time course of transport in washed and preloaded cells will be developed. If the numerical values are correct, their substitution should lead to calculated time courses for mass transport which correspond to those observed experimentally.

Time Course of Mass Transport. Consider that the suspension of tumor cells is a closed two compartment system. Let V_1 be the extracellular volume, V_2 be the intracellular volume, and R be the total amount of substrate in the system. Then at any time, t

$$A_1 V_1 + A_2 V_2 = R, \qquad [12]$$

where V_2 is assumed to be related to the cell weight by a constant factor. Now net flux into the cells is

$$F_1 - F_2 = \frac{D}{\delta}(AC_1 + AC_1' - AC_2 - AC_2'),$$

and therefore

$$\frac{dA_2}{dt} = \frac{DZ}{V_2\delta}\left(\frac{A_1 \cdot C_1}{A_1 + K} + \frac{A_1 \cdot C_1'}{A_1 + K'} - \frac{A_2 \cdot C_2}{A_2 + K} - \frac{A_2 \cdot C_2'}{A_2 + K'}\right), \qquad [13]$$

where C_1, C_1', C_2 and C_2' are known functions of A_1 and A_2 from equations [4*A*] to [4*D*].

Now let us substitute into equation [13] the numerical values already obtained for the parameters of the Ehrlich ascites tumor cell glycine transport system and the values for the volumes and cell weight used by Paine and Heinz (9), *i.e.*, V_1 = 3.1 ml, V_2 = 0.16 ml, and w = 0.042 *g*. The initial conditions (*i.e.*, the state of the system at t = 0) used by these authors were A_1 = 5mM, and A_2 = 0 mM. Then from equation [12]:

$$3.1A_1 + 0.16A_2 = 15.5.$$

Substitution of these values in equation [13] gives:

$$\frac{dA_2}{dt} = -6677\,\frac{(A_2^2 - 529A_2 + 11{,}516)}{(A_2^2 + 243A_2 - 61{,}020)(A_2 + 231)} \quad \text{mM/minute.}$$

When positive values of dA_2/dt are plotted against A_2 a straight line results. The behaviour of the system over this range thus approximates a first order system. The solution, illustrated in Fig. 3, is found to be:

$$A_2 = 22.73(1 - e^{-0.24t}),$$

and

$$A_1 = 3.80 + 1.20e^{-0.24t}.$$

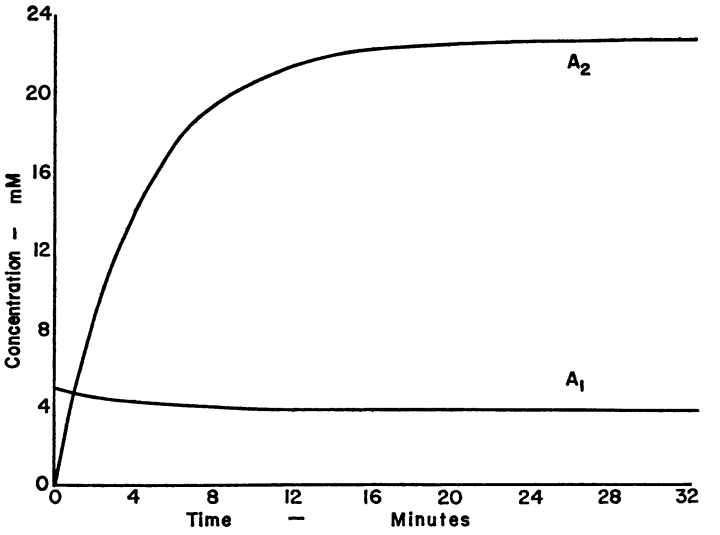

FIGURE 3 Time course of uptake of glycine by empty cells, under the conditions specified.

This solution has the same form as that found experimentally by Heinz (2), and the half time, 2.9 minutes, corresponds closely to the value reported by Heinz. Thus substitution of values for V_1, V_2, w, initial substrate concentrations, and for the numerical parameters of the system into equation [13] yields the correct results for the time course of mass transport.

Time Course of Uptake of Labeled Glycine by Cells Preloaded with Unlabeled Glycine. Heinz and Walsh (10) have observed that the ratio of intracellular to extracellular radioactivity rises more rapidly after preloading than in washed cells, reaches a peak after approximately 10 minutes, and then drops gradually to the final steady-state value, along with the corresponding ratio of total glycine. This "overshoot" was not observed in the absence of preloading. The rate of equilibration of label between the extracellular and intracellular spaces is apparently greater than the rate at which total glycine becomes distributed between the two spaces.

The following example has been chosen to show that the overshoot phenomenon may be predicted from the present model. Let the volumes and weight of cells remain as above and let the initial conditions (*i.e.*, the conditions at $t = 0$) be:

(*a*) that the medium contains glycine-C^{14}, concentration 5 mM, with a specific activity of 1000 counts/μM minute, *i.e.*, the 3.1 ml volume contains the total radioactivity, q_o.

(*b*) that the intracellular space contains unlabeled glycine, concentration 57 mM. Let q_1 be the radioactivity in the medium at any time t in CPM, and q_2 be the intracellular radioactivity at any time t in CPM. Then at any time t,

$$q_o = q_1 + q_2,$$

$$\frac{dq_1}{dt} = -\frac{q_1 F_1}{A_1 V_1} + \frac{q_2 F_2}{A_2 V_2},$$

and

$$\frac{dq_2}{dt} = -\frac{q_2 F_2}{A_2 V_2} + \frac{q_1 F_1}{A_1 V_1}.$$

Accordingly these three equations may be combined into a single first order differential equation with variable coefficients,

$$\frac{dq_1}{dt} = -q_1\left(\frac{F_1}{A_1 V_1} + \frac{F_2}{A_2 V_2}\right) + q_o\left(\frac{F_2}{A_2 V_2}\right). \qquad [14]$$

In the previous section the mass transport equation was solved for the initial conditions $A_1 = 5$ mM, $A_2 = 0$ mM, at $t = 0$. The mass transport equation may now be solved for the new initial conditions $A_1 = 5$ mM, and $A_2 = 57$ mM, by use of identical methods. The solution is found to be:

$$A_2 = 32.7 + 24.3e^{-0.17t},$$

and

$$A_1 = 6.3 - 1.3e^{-0.17t}.$$

Thus A_1 and A_2 become known functions of time. With the substitution of these known functions into equation [14], it becomes possible to solve for q_1 and hence for q_2 as functions of time, by use of a numerical technique such as the Runge-Kutta method (11).

A solution is shown in Fig. 4. It may be seen that, in this particular example, the ratio q_2V_1/q_1V_2 reaches its overshoot peak at 8.2 minutes, and subsequently slowly declines to its steady-state value. The present model thus accounts for the overshoot phenomenon found experimentally by Heinz and Walsh (10).

Metabolic Inhibition. In the present model, α and α' are the mass reaction coefficients governing the conversion of free C to C' and free C' to C, respectively. If the transformations are linked to the metabolism of the cell, then it is to be expected that α' and α will change in the presence of metabolic inhibition. This hypothesis will be examined in the following section.

Heinz (8) has studied the exchangeability of glycine accumulated in Ehrlich ascites carcinoma cells in the presence of 1 mM 2, 4-dinitrophenol plus 1 mM iodoacetate and has shown that:

(*a*) the steady-state accumulation ratio is decreased,

(*b*) the influx coefficient for labeled glycine is decreased in the steady state, and

(*c*) the efflux coefficient for labeled glycine shows little detectable change in the steady state. Christensen *et al.* (12) have found that the efflux of valine and α-aminoisobutyrate were increased following addition of 2, 4-dinitrophenol. Since the accumulation ratio was lowered the efflux coefficient for these two substances was increased.

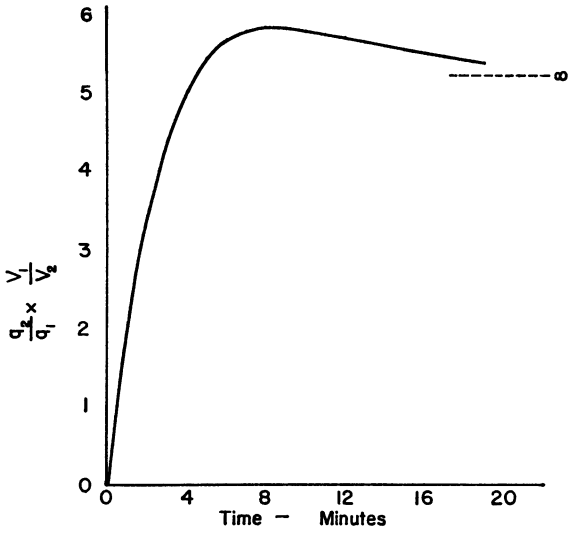

FIGURE 4 Computed illustration of the time course of the ratio, intracellular: extracellular radioactivity, after preloading.

In order to demonstrate how the model predicts the phenomenon of metabolic inhibition, let

$$\mathbf{m} = \frac{(1 + \phi)}{\theta} = \frac{\alpha}{\alpha'} + \frac{\alpha\gamma\delta}{\mathbf{D}}.$$

Substitution for $(1 + \phi)$ yields a new expression for the influx coefficient

$$\frac{\mathbf{F_1 Z}}{\mathbf{A_1 w}} = \frac{\mathbf{aDZ}}{2\delta\mathbf{w}}\left(\frac{1}{\mathbf{A_1} + \mathbf{K}}\right)\left(1 - \frac{\mathbf{K}\theta(1 - 1/\mathbf{s})}{\theta\mathbf{A_1}/\mathbf{s} + \mathbf{A_2} + \mathbf{K}\theta(1 + \mathbf{m})}\right). \quad [15]$$

In this expression it is clear that as m decreases, the influx coefficient also decreases. With a similar substitution, the expression for the efflux coefficient becomes

$$\frac{\mathbf{F_2 Z}}{\mathbf{A_2 w}} = \frac{\mathbf{aDZ}}{2\delta\mathbf{w}}\left(\frac{1}{\mathbf{A_2} + \mathbf{sK}}\right)\left(1 + \frac{\mathbf{K}(\mathbf{s} - 1)}{\theta\mathbf{A_1}/\mathbf{s} + \mathbf{A_2} + \mathbf{K}\theta(1 + \mathbf{m})}\right), \quad [16]$$

and it is evident that as m decreases, the efflux coefficient increases. With the same substitution, equation [9], the expression derived for the accumulation ratio, p, under steady-state conditions becomes

$$\mathbf{p}^2 + \mathbf{p}[\mathbf{K}(\mathbf{s} + \theta + \theta\mathbf{m} - 1)/\mathbf{A_1} + (\theta - 1)] - [\mathbf{K}(1 + \mathbf{sm})/\mathbf{A_1} + \theta] = 0. \quad [17]$$

For a given $\mathbf{A_1}$, as m decreases, the second and third terms decrease and therefore as m decreases, the steady-state accumulation ratio decreases.

Decrease in the parameter m thus reproduces qualitatively the changes produced by metabolic inhibition. Inspection of the expression for m demonstrates that a decrease in m can result when:

(*a*) α and α' decrease, the decrease in α being proportionately larger than that in α', or when

(*b*) α alone decreases.

The implication of the latter case would be that the reaction described by the mass reaction coefficient α' is spontaneous, that metabolic coupling occurred only for the reaction governed by α, and not for that governed by α'. Simple demonstration of a decrease in the size of the parameter m will not distinguish between these two cases.

Now let us examine the transport system for glycine in the Ehrlich ascites tumor cell. The numerical values of the parameters for glycine transport have been shown to be $\phi = 11$, and $\theta = 60$, and hence $m = 0.20$ under these circumstances.

Under the conditions of metabolic inhibition it should be possible to find a value of m which allows one to fit data on steady-state distribution. The data are not available. However, Heinz and Mariani (5) have published data illustrating the variation of the influx coefficient with the external glycine concentration, in the presence of 1 mM 2, 4-dinitrophenol and 1 mM iodoacetate. Using equation [15] an approximate fit of the data was achieved with $m = 0.035$ (Fig. 5). The values of m in the uninhibited and inhibited states were used in Table II to compute illustrative values for free intracellular concentration, and influx and efflux coefficients, in the steady state of substrate, when the extracellular glycine concentration was 5 mM. The decrease in the calculated influx coefficient with metabolic inhibition is large, whereas the increase in the efflux coefficient is proportionately much smaller and may not be apparent because of the variability encountered within a group of experiments.

Heinz and Mariani (5), using a "pump and leak" model, considered that the influx coefficient in Fig. 5 probably would approach a constant value as the extracellular concentration was increased. Equation [15] predicts that the influx coefficient will approach zero as the extracellular concentration increases greatly.

Competitive Inhibition. Let A and B be two substrates competing for the same carrier site on the carrier molecules C and C'; K_A and K'_A be the Michaelis constants for the reaction between substrate A and carriers C and C', and K_B and K'_B be the Michaelis constants for the reaction between substrate B and the carriers C and C'.

Expressions analogous to equations [1] and [2] were derived, and the flux coefficients were then formulated. The expression for the influx coefficient for A in the presence of competing substrate B was found to be

$$\frac{F_{1A}Z}{A_1 w} = \frac{aDZ}{2\delta w}\left(\frac{1}{A_1 + K_A(1 + B_1/K_B)}\right) \cdot\left(1 - \frac{K_A\theta(1 - 1/s_A) + (K_A\theta B_1/K_B)(1/s_B - 1/s_A)}{\theta A_1/s_A + A_2 + K_A(1 + \theta + \phi + B_2/K_B + \theta B_1/K'_B)}\right). \quad [18]$$

To elucidate the effect of B on the influx coefficient for A, consider the following cases:

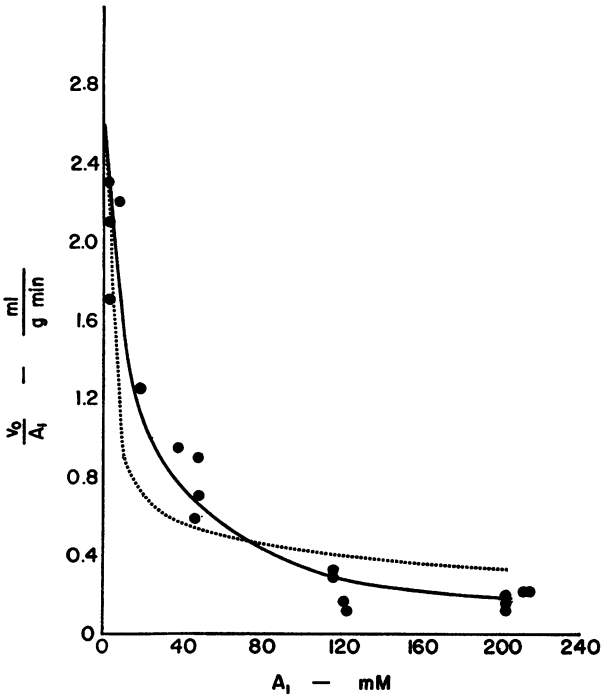

FIGURE 5 Relation of the influx coefficient to the external glycine concentration, under conditions of maximal metabolic inhibition, from Heinz and Mariani (5). The solid line was drawn arbitrarily through the points by Heinz and Mariani. The dotted line is the approximate fit, computed using the value for m of 0.035.

(*a*) $B_1 = 0$; $B_2 \neq 0$ (*i.e.*, cells preloaded with inhibitor). Then as B_2 increases, F_{1A}/A_1 increases. Thus for any A_1, A_2, and B_2, F_{1A}/A_1 will be greater than if $B_2 = 0$ (*i.e.*, preloading cells with inhibitor increases the influx coefficient and hence the relative influx of substrate). Heinz and Walsh (10) observed that preloading with sarcosine stimulated the influx of glycine.

(*b*) $B_2 = 0$; $B_1 \neq 0$ (*i.e.*, competing substrate present only extracellularly). If $s_B > s_A$, as B_1 increases, F_{1A}/A_1 decreases; and when $s_B < s_A$, as B_1 increases,

TABLE II

COMPUTED ILLUSTRATION OF THE EFFECTS OF MAXIMAL METABOLIC INHIBITION

	Uninhibited Process			Inhibited Process		
A_1	$A_2 - 6$	Influx coefficient	Efflux coefficient	$A_2 - 6$	Influx coefficient	Efflux coefficient
mM	*mM*	*ml/g min.*	*ml/g min.*	*mM*	*ml/g min.*	*ml/g min.*
5.0	27.95	6.38	1.14	9.45	2.06	1.38

F_{1A}/A_1 also decreases. Although the effect of B on A is qualitatively the same regardless of the relative magnitudes of s_B and s_A, the decrease in F_{1A}/A_1 will be more marked if $s_B > s_A$. Heinz and Walsh (11) observed that sarcosine present externally strongly depressed the initial influx of glycine. Paine and Heinz (9) observed that preaccumulated methionine left the Ehrlich cells so rapidly that when the cells were added to the glycine solution, sufficient extracellular methionine was present to compete with glycine for influx and so reduce the initial influx of label. A similar effect occurred with L-alanine. The preloading effect was abolished. Jacquez (13) found that 1 mM extracellular L-leucine increased the one-minute influx of 1 mM L-tryptophane; higher concentrations of L-leucine produced competition. The observation that 1 mM L-leucine reaches equilibrium concentrations very rapidly (14) may well underly this phenomenon. The rapid entry would produce, in essence, a preloading effect.

The expression for the efflux coefficient for A in the presence of B was found to be

$$\frac{F_{2A}Z}{A_2w} = \frac{aDZ}{2\delta w}\left(\frac{1}{A_2 + K_A'(1 + B_2/K_B')}\right) \cdot\left(1 + \frac{K_A'(1 + B_2/K_B') - K_A(1 + B_2/K_B)}{\theta A_1/s_A + A_2 + K_A(1 + \theta + \phi + B_2/K_B + \theta B_1/K_B')}\right). \quad [19]$$

Consider the same two cases:

(*a*) $B_1 = 0$; $B_2 \neq 0$. As B_2 increases, F_{2A}/A_2 decreases.

(*b*) $B_2 = 0$; $B_1 \neq 0$. As B_1 increases, F_{2A}/A_2 decreases. Stimulation of efflux of preloaded unlabeled substrate by externally added unlabeled substrate is not predicted. Experimentally, however, this phenomenon has been demonstrated to occur during the transport of one amino acid, L-leucine, by Ehrlich cells (14). In this instance the exceedingly rapid approach of L-leucine to its steady-state value (14) appears to underly this phenomenon. Since external preloading increases the total amount of substrate in the system, a steady state occurs in which, in contrast to the non-preloaded system, a larger proportion of the label is found to be extracellular (the concentration ratio is lower). The distribution of leucine between cells and medium is so rapid that the one minute efflux values reflect more closely this final partition of label, rather than an initial efflux rate.

Rosenberg and Wilbrandt (15) observed that the addition of a competitive inhibitor (unlabeled mannose or unlabeled glucose) to the suspending phase of an equilibrative system (red cells) in which a substrate (labeled glucose) had previously come to its equilibrium value transiently resulted in an efflux of the equilibrated substrate against its concentration gradient. This phenomenon was termed uphill transport induced by counterflow. The phenomenon is predicted by equations [18] and [19] and may be seen to result simply from an initially decreased unidirectional influx. Similarly, labeled substrate can be flushed into the suspending phase of a concentrative system [*e.g.*, the flush of galactose-C^{14} from the mutant of *Escherichia*

coli lacking galactokinase by unlabeled galactose (16)]. Since addition of unlabeled substrate increases the total amount of substrate in the system, a larger proportion of the label remains extracellular, once the new steady state is attained; the proportion of label present in the cells is permanently reduced.

The data of Lacko and Burger (17) may also be due simply to competitive inhibition. One minute rates of uptake of added galactose by red cells were much decreased by the presence of previously equilibrated glucose in the system. Unfortunately these values for uptake are so far from the values for initial unidirectional influx that quantitative treatment of their data is not possible.

Now let us examine the effect of competitive inhibitor upon the steady-state accumulation ratio for substrate *A*. In the presence of inhibitor

$$\mathbf{p}^2 + \mathbf{pb} - \mathbf{c} = 0, \qquad [20]$$

and

$$\mathbf{p} = [-\mathbf{b} + \sqrt{\mathbf{b}^2 + 4\mathbf{c}}]/2,$$

where

$$\mathbf{b} = \frac{\mathbf{K}_A}{\mathbf{A}_1}\left(\theta + \mathbf{s}_A + \phi + \frac{\theta \mathbf{B}_1}{\mathbf{K}_B'} + \frac{\mathbf{s}_A \mathbf{B}_2}{\mathbf{K}_B'}\right) + \frac{\theta(1 + \mathbf{B}_1/\mathbf{K}_B') - (1 + \mathbf{B}_2/\mathbf{K}_B)}{1 + \mathbf{B}_1/\mathbf{K}_B},$$

and

$$c = \frac{\mathbf{K}_A}{\mathbf{A}_1}\left(\frac{1 + \mathbf{B}_2}{\mathbf{K}_B'}\right)\left(\theta + \frac{\mathbf{s}_A(1 + \phi + \mathbf{B}_2/\mathbf{K}_B)}{1 + \mathbf{B}_1/\mathbf{K}_B}\right) + \frac{\theta(1 + \mathbf{B}_2/\mathbf{K}_B')}{1 + \mathbf{B}_1/\mathbf{K}_B}.$$

It can be shown that the steady-state accumulation ratio for substrate will be decreased by the competitive inhibitor if

$$\frac{1 + \frac{\mathbf{B}_1}{\mathbf{K}_B}}{1 + \frac{\mathbf{B}_2}{\mathbf{K}_B'}} > 1 + \frac{\frac{\mathbf{K}_A}{\mathbf{A}_1}\left(\frac{\theta \mathbf{B}_1}{\mathbf{K}_B} + \frac{\mathbf{s}_A \mathbf{B}_2}{\mathbf{K}_B}\right)}{\frac{\mathbf{K}_A}{\mathbf{A}_1}\left(\theta + \mathbf{s}_A(1 + \phi)\right) + \theta}.$$

In particular this condition will be satisfied if $\mathbf{B}_2/\mathbf{K}_B' \ll \mathbf{B}_1/\mathbf{K}_B$, *i.e.*, if the system is highly concentrative for *B* in the absence of *A*. Experimentally sarcosine and DL-alanine have been found to depress the final steady-state accumulation ratio of glycine and *vice versa* (4, 18).

Energetics. In the mathematical development of the model, substrate-carrier reactions and carrier transformations have been assumed to proceed in equilibrium. Therefore the only irreversible or energy-consuming process remaining is the diffusion of free and complexed carrier.

Let **B** be the amount of energy per unit area per unit time required to maintain the steady state. It is given by

$$\mathbf{E} = \frac{\mathbf{D}}{\delta}[(\mathbf{C}_1 - \mathbf{AC}_1) - (\mathbf{C}_2 - \mathbf{AC}_2)]\mathbf{RT} \ln\left[\frac{\mathbf{C}_1 - \mathbf{AC}_1}{\mathbf{C}_2 - \mathbf{AC}_2}\right]$$

$$+ \frac{\mathbf{D}}{\delta}(\mathbf{AC}_1 - \mathbf{AC}_2)\mathbf{RT} \ln\left[\frac{\mathbf{AC}_1}{\mathbf{AC}_2}\right]$$

$$+ \frac{D}{\delta}[(C_1' - AC_1') - (C_2' - AC_2')]RT \ln \left[\frac{C_1' - AC_1'}{C_2' - AC_2'}\right]$$

$$+ \frac{D}{\delta}(AC_1' - AC_2')RT \ln \left[\frac{AC_1'}{AC_2'}\right], \qquad [21]$$

where R is the gas constant and T is the absolute temperature.

The system is assumed to be in a steady state and therefore

$$AC_1 - AC_2 = AC_1' - AC_2'.$$

From previous assumptions $C_1 - C_2 = -(C_1' - C_2')$, and hence equation [21] becomes

$$E = \frac{D}{\delta}(C_1 - C_2)RT \ln \left[\frac{C_1C_2'(A_1 + K')(A_2 + K)}{C_2C_1'(A_1 + K)(A_2 + K')}\right]. \qquad [22]$$

From equations [4] and the numerical values of the parameters, E can be calculated for given values of A_1 and A_2.

Consider the following two cases:

(*a*) A_1 and $A_2 \ll K$ or K' (low saturation).

In terms of the parameters θ and ϕ we have from equations [4]

$$(C_1 - C_2) = (a/2)[\theta/(1 + \theta + \phi)],$$

$$C_1/C_2 = 1 + \phi,$$

and

$$C_2'/C_1' = (\theta + \phi)/\theta,$$

and therefore

$$E = \frac{aD}{2\delta} \frac{\theta}{(1 + \theta + \phi)} RT \ln \left[\frac{(1 + \phi)(\theta + \phi)}{\theta}\right].$$

(*b*) A_1 and $A_2 \gg K$ or K' (high saturation).

Then both C_1/C_2 and C_2'/C_1' approach unity and hence equation [22] approaches zero. Thus the energy requirement defined by steady-state conditions in the model system becomes maximal for low substrate concentrations and diminishes as the substrate concentrations are raised. Experimentally, the oxygen consumption of Ehrlich ascites tumor cells transporting glycine in the steady state has been found to be maximal at low external glycine concentrations and to decrease with rise in glycine concentration (5).

In the case of glycine transport by the Ehrlich ascites carcinoma cells at 37°C under low saturation conditions, substitution of the numerical values of the parameters of the system leads to the value $BZ/w = 0.053$ cal/*g* minutes for maximal energy consumption. Assuming that:

(*a*) O_2 consumption under low saturation conditions is approximately 8 μmoles/*g* minutes (5),

(*b*) aerobic glycolysis produces lactic acid at the rate of 22.5 μmoles/g minutes (19), where the weights have referred to dry cell weight, and

(*c*) 7 moles of ATP are formed when 1 mole of oxygen is consumed in respiration, 1 mole of ATP is formed when 1 mole of lactic acid is formed, and the free energy for the formation of ATP is 7000 cal/mole, then

$$\frac{\text{maximal energy requirement for transport in the steady state}}{\text{energy supplied from } O_2 \text{ consumption and aerobic glycolysis}} = 0.096.$$

Under maximal steady-state energy requirements (external substrate concentration low, accumulation ratio high), the calculated parameters of the system have lead to the prediction that approximately 10 per cent of the energy supplied by cell metabolism is used by the transport mechanism to maintain a glycine gradient. The observed increase in oxygen consumption with reduction of the glycine concentration is of this order (5).

DISCUSSION

The Model. This model of a membrane carrier process, one in which there are two forms of the same carrier of differing affinities, interconverted by metabolic processes, has been devised to simultaneously account for three experimentally observed phenomena in a highly concentrative system:

(*a*) the presence of a saturation phenomenon when the initial inward rate of substrate transport is considered as a function of the external concentration of substrate,

(*b*) the presence of an increased maximal initial inward rate of transport of labeled substrate and an unchanged Michaelis constant for initial influx, when the cells are preloaded with unlabeled substrate, and

(*c*) the presence of a steady-state accumulation ratio which is maximal for low external substrate concentrations and which approaches one as the external substrate concentration rises greatly.

A method has been devised for obtaining the numerical parameters of the system from quantitative observations of these phenomena. When the data are sufficiently accurate, the time course both of mass transport of substrate and of distribution of labeled substrate in the non-steady as well as the steady-state may be predicted from these parameters. Agreement between the predicted and the observed time course may be taken as verification of the precision of the estimates of the numerical parameters. The model also accounts for the phenomena observed during both metabolic and competitive inhibition.

The unidirectional flux of substrate inwards in a concentrative system has been shown to depend upon the internal as well as the external concentrations. The process of exchange diffusion, formerly invoked to account for this dependence (10), becomes an unnecessary conception. The model is essentially a very simple one and has been applied only to non-electrolyte transport. No attempt has been made to generalize

it to account for the phenomena of ion transport, or the phenomena whereby non-electrolyte transport is influenced by the presence of ions.

Steady State. Consider the following cases:

(*a*) $K'/K \gg 1$,

(*b*) $K'/K > 1$, and

(*c*) $K'/K = 1$.

From the analysis of steady-state conditions, it is clear that these three cases represent different degrees of cellular concentrating ability. When $K'/K \gg 1$, the accumulation ratios are very large, as exemplified by the transport of glycine by Ehrlich ascites tumor cells. At the other extreme, when $K'/K = 1$, an equilibrating system will result (for example, the transport of sugars by erythrocytes). In this manner it will be possible to characterize the transport of different substrates on a sliding scale according to the accumulation ratio attained in the steady state.

Initial Rates. The interpretation of Lineweaver-Burk plots constructed from experimental data relating initial rates of uptake to external substrate concentration may now be examined. Only when $K'/K = 1$ will such a plot yield a straight line from which correct values for v_{max} and K may be determined.

For a system in which $K'/K \gg 1$ (*e.g.*, glycine transport by Ehrlich ascites tumor cells), the deviation from linearity will depend upon the relative magnitudes of θ, ϕ, s, K, and the range over which the extracellular concentration is varied. In general, when $K'/K \gg 1$, the errors incurred in the estimation of K from a Lineweaver-Burk plot will be small over the range in which the low affinity carrier C' may be neglected. Recently Rotman and Radojkovic (20) have examined the concentrative transport of galactose by *E. coli*, and, by varying the extracellular concentrations over the appropriate range, have been able to demonstrate two K's, differing by a factor of 10.

When $K'/K > 1$, but is not too large, a system results which is intermediate between those systems which have a high concentrating ability and those which exhibit equilibration. In this instance a significant proportion of the available extracellular substrate will be transported by the carrier C'. The curve relating initial rate of uptake to extracellular substrate concentration will appear to be the sum of two components, one of which increases rapidly and saturates (C), the other of which increases slowly with rising extracellular concentration and is not easily demonstrated to approach saturation (C'). This phenomenon was observed by Akedo and Christensen (21) during their study of the entry of α-aminoisobutyrate into the isolated rat diaphragm *in vitro*. Oxender and Christensen (14) noted the same phenomenon, to a lesser degree, in a kinetic analysis of the transport of neutral amino acids by the Ehrlich cell. Under these circumstances, a Lineweaver-Burk plot of the data will not be a single straight line and any interpretation based upon the assumption of linearity may be seriously in error.

Other Models. If the present model is altered by the assumption that carrier undergoes transformation to the corresponding form of differing affinity, irrespective

of whether it is "free" or complexed with substrate, no preloading effect is encountered. Similarly, Rosenberg and Wilbrandt (22) found that a similar model, in which the kinetic characteristics of the metabolic reactions governing the interconversion of the two carrier forms were not precisely specified, exhibited no preloading effect. The unidirectional flux inwards depended only on the external concentration and a constant resembling the Michaelis constant; the flux outwards, on internal concentration and a second similar constant. If the diffusion constant for combined carrier was assumed to be larger than that for free carrier, the particular kind of "independence" of unidirectional fluxes was abolished; both fluxes became dependent on both internal and external concentrations, and a preloading effect could be demonstrated. In the model considered in this paper the assumptions that the diffusion constants of free and combined carriers are equal is preserved, and the kinetic characteristics of the interconversion of the two carrier forms are incorporated into the model. These two alterations produce the preloading effect, albeit in a different manner. Oxender and Christensen (14) have extended the present model, and have suggested that two separate concentrative systems with overlapping affinities mediate the transport of neutral amino acids in Ehrlich cells. Quantitative estimation of the parameters may aid in the evaluation of this hypothesis. Jacquez (23) has examined a one carrier system in which the membrane also possesses passive permeability to the substrate. Stationary state solutions (24) conform to data for initial uptake rates, competitive inhibition, and preloading; steady-state solutions have not been examined.

Estimation of the Carrier Present in the Membrane. In the transport of glycine by Ehrlich ascites tumor cells, $aDZ/2\delta w \approx 190$ μmoles/g minutes, where the weight is expressed as dry weight. Now $w = 0.042$ g, and if we may take $Q \approx Z\delta$, $\delta = 70$ A, and $D = 10^{-4}$ to 10^{-6} cm^2/second, and if 0.25 per cent of the dry cell weight is membrane, then the amount of carrier present per g dry membrane is 6×10^{-4} to 6×10^{-6} μmoles/g.

Effect of Metabolic Inhibitors and Pyridoxal on Glycine Transport. The transformation of variables, $(1 + \phi) = m\theta$, was introduced into the carrier model and it was shown that as m decreased, the steady-state accumulation ratio decreased, the influx coefficient decreased, and the efflux coefficient increased slightly. Metabolic inhibition has been found to produce these effects on glycine transport in the Ehrlich ascites tumor cell and hence it may be assumed that a decrease in m simulates the effects of metabolic inhibition.

In contrast, if m increases, the steady-state accumulation ratio increases, the influx coefficient increases, and the efflux coefficient decreases. Pyridoxal has been observed to increase the steady-state accumulation ratio, decrease the efflux coefficient, and increase the influx coefficient (3, 25). Quantitative assessment of the change in m which accounts for these phenomena must await the acquisition of a more complete set of data.

It is not likely that metabolic inhibitors or pyridoxal affect the diffusion constant of the carrier or the structural characteristics of the membrane of the cell. It must

be concluded that metabolic inhibitors tend to decrease the magnitude of α' and α while pyridoxal tends to increase their magnitude. In the absence of sufficient steady-state and initial rate data, it is not possible to distinguish the relative effects of metabolic inhibitors and pyridoxal on α' and α separately.

An Excluding System. If the present model were rearranged so that the polarity of the membrane is reversed (*i.e.*, the outside becomes the inside, and *vice versa*), the membrane would tend to remove substrate from the intracellular space. This case is most conveniently considered by assuming that $\mathbf{K}' < \mathbf{K}$ in the model illustrated in Fig. 1, *i.e.*, that $\mathbf{s}$ is less than one.

The expression for fluxes become

$$\mathbf{F}_1 = \frac{\mathbf{aD}}{2\delta}\left(\frac{\mathbf{A}_1}{\mathbf{A}_1 + \mathbf{K}}\right)\left(1 + \frac{\mathbf{K}\theta(1/\mathbf{s} - 1)}{\theta\mathbf{A}_1/\mathbf{s} + \mathbf{A}_2 + \mathbf{K}(1 + \theta + \phi)}\right),$$

and

$$\mathbf{F}_2 = \frac{\mathbf{aD}}{2\delta}\left(\frac{\mathbf{A}_2}{\mathbf{A}_2 + \mathbf{sK}}\right)\left(1 - \frac{\mathbf{K}\theta(1 - \mathbf{s})}{\theta\mathbf{A}_1/\mathbf{s} + \mathbf{A}_2 + \mathbf{K}(1 + \theta + \phi)}\right).$$

When $\mathbf{s} < 1$, $\mathbf{F}_1$ is larger and $\mathbf{F}_2$ is smaller than it would be for an equilibrating system ($\mathbf{s} = 1$), *i.e.*, the relative change is opposite to that for a concentrating system. Preloading the cells with substrate will diminish the initial inward rate of transport of labeled substrate in this system. When the steady-state accumulation ratio, $\mathbf{p}$, is examined, it is found that:

(*a*) as $\mathbf{A}_1$ approaches zero, $\mathbf{p} \rightarrow (\theta + \mathbf{s} + \mathbf{s}\phi)/(\theta + \mathbf{s} + \phi)$, a finite number less than one, and

(*b*) as $\mathbf{A}_1$ increases greatly, $\mathbf{p} \rightarrow 1$.

Hence the cell will exclude substrate under these circumstances.

If $\mathbf{s} \ll 1$, *i.e.*, if $\mathbf{s}$ is approximately zero, then $\mathbf{F}_1 \approx \mathbf{aD}/2\delta$, and $\mathbf{F}_2 \approx \mathbf{aD}/2\delta$. The unidirectional fluxes become maximal and are independent of both intracellular and extracellular substrate concentrations.

Oriented Cells. Many organs are made up of cells which are polarized in terms of structure and function. Under these circumstances transport of substrate into the cell may occur across an outer membrane, and then out of the cell across an inner membrane, or *vice versa*. The parameters of the transport system in each membrane may differ. For instance, the two systems may be either both equilibrative, or equilibrative and excluding, so that a simple translocation across the cell results. Alternatively they may be both concentrative, so that a large concentration difference is built up across the cell by two discrete steps. Both transport systems must be characterized before the time course of mass transport or of distribution of labeled substrate may be analyzed.

APPENDIX

Carrier Concentrations at the Surfaces of the Membrane. Consider the reaction

in which substrate combines with free carrier species to form substrate carrier complex. Since instantaneous equilibration has been assumed we have

$$AC_i = \frac{A_i \cdot C_i}{A_i + K} \quad \text{and} \quad AC_i' = \frac{A_i \cdot C_i'}{A_i + K'}, \text{ where } i = 1, 2. \qquad [1]$$

In accord with assumption (*f*), page 489, we may write

$$\frac{dC_1}{dt} = \alpha(C_1' - AC_1') - \frac{D}{\gamma\delta}(C_1 - C_2) = 0, \qquad [2A]$$

$$\frac{dC_1'}{dt} = \alpha(C_1' - AC_1') + \frac{D}{\gamma\delta}(C_2' - C_1') = 0, \qquad [2B]$$

$$\frac{dC_2}{dt} = -\alpha'(C_2 - AC_2) + \frac{D}{\gamma\delta}(C_1 - C_2) = 0, \qquad [2C]$$

and

$$\frac{dC_2'}{dt} = \alpha'(C_2 - AC_2) - \frac{D}{\gamma\delta}(C_2' - C_1') = 0. \qquad [2D]$$

The total amount of C and C′ in the membrane, T, is

$$\int_0^\delta [C(x) + C'(x)]Z\, dx.$$

From assumption (*h*), page 489,

$$\frac{dC(x)}{dx} \approx \frac{\Delta C}{\delta} = \frac{C_1 - C_2}{\delta}$$

and thus

$$C(x) = C_1 - \frac{(C_1 - C_2)}{\delta} \cdot x,$$

and

$$C'(x) = C_1' + \frac{(C_2' - C_1')}{\delta} \cdot x.$$

Addition of equations [2*A*] and [2*B*] yields

$$C_1 - C_2 = C_2' - C_1'.$$

Hence

$$T = (C_1 + C_1')/Q$$

and therefore

$$C_1 + C_1' + C_2 + C_2' = 2T/Q = a. \qquad [3]$$

Use of equations [1] to [3] leads to the following expressions for carrier concentrations:

$$C_1 = \frac{(a/2)[A_2 + K(1 + \phi)]}{\theta A_1/s + A_2 + K(1 + \theta + \phi)}, \qquad [4A]$$

$$C_1' = \frac{(a/2)(\theta/s)(A_1 + sK)}{\theta A_1/s + A_2 + K(1 + \theta + \phi)}, \qquad [4B]$$

$$C_2 = \frac{(a/2)(A_2 + K)}{\theta A_1/s + A_2 + K(1 + \theta + \phi)}, \qquad [4C]$$

and

$$C_2' = \frac{(a/2)[\theta A_1/s + K(\theta + \phi)]}{\theta A_1/s + A_2 + K(1 + \theta + \phi)}. \qquad [4D]$$

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