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A GENERAL KINETIC ANALYSIS OF TRANSPORT

TESTS OF THE CARRIER MODEL BASED ON PREDICTED RELATIONS AMONG EXPERIMENTAL PARAMETERS

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Summary

The analysis of transport kinetics has lacked both a unified treatment in which general rate equations are written entirely in terms of experimental parameters, and a convention by which these parameters may be designated in a concise yet immediately recognizable manner. Such a treatment is presented here in an easily accessible form, and a simple system of nomenclature is proposed resembling that in use in enzyme kinetics. The treatment is independent of assumptions about rate-limiting steps in transport, and applies to both active and facilitated systems, including obligatory exchange. A single substrate is characterized by twelve different parameters, only five of which are required in theory to calculate the others. If a second substrate is present on the *trans* side of the membrane there are six more parameters. All eighteen parameters are linked by multiple relationships which provide a complete set of rejection criteria for the generalized form of the mobile carrier. Relationships among parameters are also defined that give information on the rate-limiting steps in transport. Equations governing any individual experiment, involving only experimental parameters, are easily written out from the general expressions, for example under conditions of zero *trans* and infinite *trans* flux, equilibrium exchange, or competitive inhibition.

Introduction

In any kinetic analysis of a transport system, the requirement for consistency among the parameters determined in different experiments, such as

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substrate exit or entry, provides the basis for a stringent test of the hypothetical mechanism. When this approach was first applied to the classical carrier the analysis was limited by certain restrictions imposed upon the model, and later, when these had been removed, the unabridged equations appeared to be extremely complicated and therefore not easy to apply. In the present treatment the problem is greatly simplified. Though general, the new rate equations involve only experimental parameters and are therefore comparatively simple. In order to bring all relevant parameters into a single rate expression, a new and simple convention for naming the constants is proposed. The system of interrelationships shown to hold among these parameters allows the model to be rigorously tested.

As we have suggested, certain simplifying assumptions had originally been introduced into the kinetic treatment of the mobile carrier. First, there was the assumption of symmetry, the substrate affinities on the two sides of the membrane being regarded as identical and the rates of reorientation of loaded and unloaded carrier as equal; and second, the assumption of rapid breakdown of the carrier-substrate complex, with carrier reorientation the sole rate-limiting step in transport. Evidence gradually accumulated, however, to show that real systems may be unsymmetrical, as in the example of the glucose carrier of erythrocytes [1—10], and difficulties in interpreting experimental observations on the same system suggested that rapid substrate dissociation could not be assumed either without proof.

In the more general treatment which became necessary and which was presented by various authors [5,6,9,11—16], the equations were difficult to read and difficult to handle, since they involved a great many rate constants. Often, therefore, they were simplified by the introduction of new symbols in place of aggregates of rate constants. The composite constants did not necessarily correspond to individual parameters actually determined through experiment however, making their application uncertain; and given this latitude it became possible to present the final rate equations in arbitrarily different forms. An additional difficulty was a lack of agreement as to how composite constants should be named.

Indeed there is a special problem of nomenclature in the analysis of transport, for maximum rates of substrate movement and affinities can have different values in the many different experimental arrangements which are possible, such as those involving substrate entry or substrate exit into solutions either free of substrate or containing substrate at various concentrations; and it would undoubtedly be preferable for the proposed symbols to specify the experiment in which they were measured.

That a substrate is characterized by many experimental parameters might be viewed at first as an obstacle to the writing of reasonably simple rate expressions. On the contrary, it is found to be an advantage, since the most general equation for transport in the presence of two substrates reduces to an expression containing only these constants, and since all the constants are linked by predictable relationships.

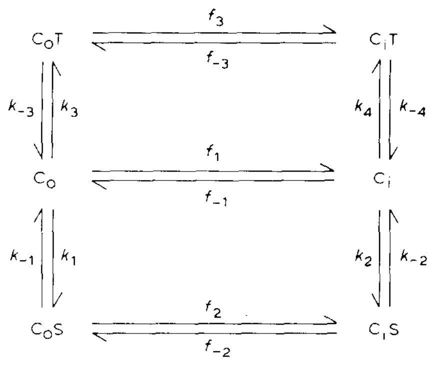

Fig. 1.

Kinetic analysis

General rate equations are now presented, expressed entirely in terms of experimental paramaters *. In naming the latter, a terminology has been adopted which is closely related to that accepted in enzyme kinetics, where V represents a maximum velocity and K, with a subscript or superscript, represents the half-saturating concentration of a substrate or reversible inhibitor. As in previous treatments, the transport model corresponds to the classical carrier, whose essential features are as follows. Two forms of free carrier and two forms of carrier-substrate complex ** are distinguished, and the assumption is made that a substrate site in the carrier is presented at either the external or internal surface of the membrane but not both simultaneously. Rate equations are derived for a transport scheme involving two different substrates (Fig. 1). The advantage in doing so is that with the appropriate assumptions as to the relative magnitudes of the rate constants and substrate concentrations, the equations may represent either competition between different substrates, exchange of different substrates, exchange of a single substrate (one pool being labeled), inhibition of substrate movement by a non-transported substrate analog on either side of the membrane, or an obligatory exchange system.

With the aid of the King-Altman schematic method [19], equations may be written without any assumption as to the rate-limiting steps in the process; the detailed procedure has been described by Schachter [13] and by Wong and Hanes [20].

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* A treatment of this problem having a similar aim, but limited to the case of zero *trans* flux, was presented earlier by Cuppoletti and Segel [17].

** Stein and Lieb [14] derived rate equations in which the two consecutive steps of formation of the substrate complex and its migration in the membrane are represented as a single process. It was shown that steady-state measurements of substrate flux alone give no information as to the relative rates of these steps, and it was therefore argued that the carrier mechanism should include only one form of carrier-substrate complex, depicted as intermediate between theoretical inner and outer forms. However, experiments with irreversible inhibitors can distinguish, both in theory and practice, two forms of complex [18]. It is therefore essential, in our view, to base any general treatment on the two-complex formulation of the carrier mechanism.

TABLE I

EXPERIMENTAL PARAMETERS FOR SUBSTRATE AFFINITY, EXPRESSED IN TERMS OF INDIVIDUAL RATE CONSTANTS FOR THE TRANSPORT SCHEME IN FIG. 1

Equilibrium constants for carrier-substrate complex formation are defined as follows: $K_{So} = k_{-1}/k_1$; $K_{Si} = k_{-2}/k_2$; $K_{To} = k_{-3}/k_3$; $K_{Ti} = k_{-4}/k_4$. The symbols G and H stand for the following combinations of constants: $G = k_{-3}f_{-3} + k_{-3}k_{-4} + k_{-4}f_3$ $H = k_{-1}f_{-2} + k_{-1}k_{-2} + k_{-2}f_2$

Experimental parameter	Experiment	Labeled substrate (*cis*)	Unlabeled analog		General expression	Rapid dissociation
			trans	*cis*		
$\bar{K}_{So}$	Zero *trans* entry entry	S_o	—	—	$\frac{(f_1 + f_{-1})H}{k_1\{k_{-2}(f_{-1} + f_2) + f_{-1}(f_2 + f_{-2})\}}$	$\frac{K_{So}(f_1 + f_{-1})}{(f_{-1} + f_2)}$
$\bar{K}_{To}$	Zero *trans* entry	T_o	—	—	$\frac{(f_1 + f_{-1})G}{k_3\{k_{-4}(f_{-1} + f_3) + f_{-1}(f_3 + f_{-3})\}}$	$\frac{K_{To}(f_1 + f_{-1})}{(f_{-1} + f_3)}$
$\bar{K}_{Io}$	Zero *trans* entry	S_o	—	I_o	$K_{Io}(1 + f_1/f_{-1})$	$K_{Io}(1 + f_1/f_{-1})$
$\bar{K}_{Si}$	Zero *trans* exit	S_i	—	—	$\frac{(f_1 + f_{-1})H}{k_2\{k_{-1}(f_1 + f_{-2}) + f_1(f_2 + f_{-2})\}}$	$\frac{K_{Si}(f_1 + f_{-1})}{(f_1 + f_{-2})}$
$\bar{K}_{Ti}$	Zero *trans* exit	T_i	—	—	$\frac{(f_1 + f_{-1})G}{k_4\{k_{-3}(f_1 + f_{-3}) + f_1(f_3 + f_{-3})\}}$	$\frac{K_{Ti}(f_1 + f_{-1})}{(f_1 + f_{-3})}$
$\bar{K}_{Ii}$	Zero *trans*	S_i	—	I_i	$K_{Ii}(1 + f_{-1}/f_1)$	$K_{Ii}(1 + f_{-1}/f_1)$

$\tilde{K}^{S}_{So}$	Infinite *trans* entry	S_o	S_i	—	$\dfrac{k_{-1}(f_1+f_{-2})+f_1(f_2+f_{-2})}{k_1(f_2+f_{-2})}$	$\dfrac{K_{So}(f_1+f_{-2})}{(f_2+f_{-2})}$
$\tilde{K}^{T}_{To}$	Infinite *trans* entry	T_o	T_i	—	$\dfrac{k_{-3}(f_1+f_{-3})+f_1(f_3+f_{-3})}{k_3(f_3+f_{-3})}$	$\dfrac{K_{To}(f_1+f_{-3})}{f_3+f_{-3})}$
$\tilde{K}^{T}_{So}$	Infinite *trans* entry	S_o	T_i	—	$\dfrac{\{k_{-3}(f_1+f_{-3})+f_1(f_3+f_{-3})\}H}{k_1\{k_{-3}f_{-3}(k_{-2}+f_2+f_{-2})+k_{-2}f_2(k_{-3}+f_3+f_{-3})\}}$	$\dfrac{K_{So}(f_1+f_{-3})}{(f_2+f_{-3})}$
$\tilde{K}^{S}_{To}$	Infinite *trans* entry	T_o	S_i	—	$\dfrac{\{k_{-1}(f_1+f_{-2})+f_1(f_2+f_{-2})\}G}{k_3\{k_{-1}f_{-2}(k_{-4}+f_3+f_{-3})+k_{-4}f_3(k_{-1}+f_2+f_{-2})\}}$	$\dfrac{K_{To}(f_1+f_{-2})}{(f_{-2}+f_3)}$
$\tilde{K}^{S}_{Io}$	Exit	S_i	I_o	—	$K_{Io}\dfrac{k_{-1}(f_1+f_{-2})+f_1(f_2+f_{-2})}{k_{-1}f_{-2}}$	$K_{Io}(1+f_1/f_{-2})$
$\tilde{K}^{S}_{Ii}$	Entry	S_o	I_i	—	$K_{Ii}\dfrac{k_{-2}(f_{-1}+f_2)+f_{-1}(f_2+f_{-2})}{k_{-2}f_2}$	$K_{Ii}(1+f_{-1}/f_2)$
$\bar{\bar{K}}_{So}$	Equilibrium exchange	S_o	S_i	—	$K_{So}\dfrac{(1+f_1/f_{-1})}{(1+f_2/f_{-2})}$	$K_{So}\dfrac{(1+f_1/f_{-1})}{(1+f_2/f_{-2})}$
$\bar{\bar{K}}_{Si}$	Equilibrium exchange	S_i	S_o	—	$K_{Si}\dfrac{(1+f_{-1}/f_1)}{(1+f_{-2}/f_2)}$	$K_{Si}\dfrac{(1+f_{-1}/f_1)}{(1+f_{-2}/f_2)}$

General rate equation for the transport of one substrate in the presence of another

The rate of transport of substrate S in the presence of a second substrate T is found to be governed by an expression having the following form:

$$\frac{-\mathrm{d}[\mathrm{S_i}]}{\mathrm{d}t}=\frac{\dfrac{\bar{V}_{\mathrm{Si}}}{\bar{K}_{\mathrm{Si}}}([\mathrm{S_i}]-\alpha(\mathrm{S_o}])+\dfrac{\tilde{V}^{\mathrm{T}}_{\mathrm{Si}}}{\tilde{K}^{\mathrm{S}}_{\mathrm{To}}\bar{K}_{\mathrm{Si}}}([\mathrm{S_i}][\mathrm{T_o}]-\dfrac{\alpha}{\beta}[\mathrm{S_o}][\mathrm{T_i}])}{1+\dfrac{[\mathrm{S_o}]}{\bar{K}_{\mathrm{So}}}+\dfrac{[\mathrm{S_i}]}{\bar{K}_{\mathrm{Si}}}+\dfrac{[\mathrm{T_o}]}{\bar{K}_{\mathrm{To}}}+\dfrac{[\mathrm{T_i}]}{\bar{K}_{\mathrm{Ti}}}+\dfrac{[\mathrm{S_o}][\mathrm{S_i}]}{\tilde{K}^{\mathrm{S}}_{\mathrm{So}}\bar{K}_{\mathrm{Si}}}+\dfrac{[\mathrm{T_o}][\mathrm{T_i}]}{\tilde{K}^{\mathrm{T}}_{\mathrm{To}}\bar{K}_{\mathrm{Ti}}}+\dfrac{[\mathrm{S_o}][\mathrm{T_i}]}{\tilde{K}^{\mathrm{T}}_{\mathrm{So}}\bar{K}_{\mathrm{Ti}}}+\dfrac{[\mathrm{T_o}][\mathrm{S_i}]}{\tilde{K}^{\mathrm{S}}_{\mathrm{To}}\bar{K}_{\mathrm{Si}}}} \tag{1}$$

In Eqn. 1 the concentrations of substrates S and T outside the cell are designated as $[\mathrm{S_o}]$ and $[\mathrm{T_o}]$ and their concentrations inside as $[\mathrm{S_i}]$ and $[\mathrm{T_i}]$. The constants α and β are the ratios of the final concentrations of substrate achieved inside and outside the cell:

$$\alpha=\left(\frac{[\mathrm{S_i}]}{[\mathrm{S_o}]}\right)_{\mathrm{final}}=\frac{\bar{V}_{\mathrm{So}}/\bar{K}_{\mathrm{So}}}{\bar{V}_{\mathrm{Si}}/\bar{K}_{\mathrm{Si}}}=\frac{f_{-1}f_2k_1k_{-2}}{f_1f_{-2}k_{-1}k_2} \tag{2}$$

$$\beta=\left(\frac{[\mathrm{T_i}]}{[\mathrm{T_o}]}\right)_{\mathrm{final}}=\frac{\bar{V}_{\mathrm{To}}/\bar{K}_{\mathrm{To}}}{\bar{V}_{\mathrm{Ti}}/\bar{K}_{\mathrm{Ti}}}=\frac{f_{-1}f_3k_3k_{-4}}{f_1f_{-3}k_{-3}k_4} \tag{3}$$

In an equilibrating system α and β equal unity, while in active transport they may be very large. All the other constants are experimental parameters whose definitions are given below, and whose equivalent expressions in terms of individual rate constants are listed in Tables I and II. Not all possible constants are given but others are readily obtained by analogy, through the substitution of corresponding rate constants in the appropriate expressions in the tables.

The rate equation for the transport of substrate T is as follows:

$$\frac{-\mathrm{d}[\mathrm{T_i}]}{\mathrm{d}t}=\frac{\dfrac{\bar{V}_{\mathrm{Ti}}}{\bar{K}_{\mathrm{Ti}}}(\mathrm{T_i}-\beta\mathrm{T_o})+\dfrac{\tilde{V}^{\mathrm{S}}_{\mathrm{Ti}}}{\tilde{K}^{\mathrm{T}}_{\mathrm{So}}\bar{K}_{\mathrm{Ti}}}([\mathrm{T_i}][\mathrm{S_o}]-\dfrac{\beta}{\alpha}[\mathrm{T_o}][\mathrm{S_i}])}{1+\dfrac{[\mathrm{S_o}]}{\bar{K}_{\mathrm{So}}}+\dfrac{[\mathrm{S_i}]}{\bar{K}_{\mathrm{Si}}}+\dfrac{[\mathrm{T_o}]}{\bar{K}_{\mathrm{To}}}+\dfrac{[\mathrm{T_i}]}{\bar{K}_{\mathrm{Ti}}}+\dfrac{[\mathrm{S_o}][\mathrm{S_i}]}{\tilde{K}^{\mathrm{S}}_{\mathrm{So}}\bar{K}_{\mathrm{Si}}}+\dfrac{[\mathrm{T_o}][\mathrm{T_i}]}{\tilde{K}^{\mathrm{T}}_{\mathrm{To}}\bar{K}_{\mathrm{Ti}}}+\dfrac{[\mathrm{S_o}][\mathrm{T_i}]}{\tilde{K}^{\mathrm{T}}_{\mathrm{So}}\bar{K}_{\mathrm{Ti}}}+\dfrac{[\mathrm{T_o}][\mathrm{S_i}]}{\tilde{K}^{\mathrm{S}}_{\mathrm{To}}\bar{K}_{\mathrm{Si}}}} \tag{4}$$

It is to be noted that the denominators of the two equations (1 and 4) are identical.

General rate equation for the transport of a substrate in the presence of a competitive inhibitor

Assuming that T does not undergo transport ($f_3 = f_{-3} = 0$), but that because of a structural similarity to the substrate it becomes bound at the substrate site in the carrier, Eqn. 1 is readily converted to the general rate equation for transport of a substrate in the presence of a competitive inhibitor. The equation is now re-written, with substitution of the symbol I (inhibitor) for T:

$$\frac{-\mathrm{d}[\mathrm{S_i}]}{\mathrm{d}t}=\frac{\dfrac{\bar{V}_{\mathrm{Si}}}{\bar{K}_{\mathrm{Si}}}([\mathrm{S_i}]-\alpha[\mathrm{S_o}])}{1+\dfrac{[\mathrm{S_o}]}{\bar{K}_{\mathrm{So}}}+\dfrac{[\mathrm{S_i}]}{\bar{K}_{\mathrm{Si}}}+\dfrac{[\mathrm{I_o}]}{\bar{K}_{\mathrm{Io}}}+\dfrac{[\mathrm{I_i}]}{\bar{K}_{\mathrm{Ii}}}+\dfrac{[\mathrm{S_o}][\mathrm{S_i}]}{\tilde{K}^{\mathrm{S}}_{\mathrm{So}}\bar{K}_{\mathrm{Si}}}+\dfrac{[\mathrm{S_o}][\mathrm{I_i}]}{\bar{K}_{\mathrm{So}}\tilde{K}^{\mathrm{S}}_{\mathrm{Ii}}}+\dfrac{[\mathrm{S_i}][\mathrm{I_o}]}{\bar{K}_{\mathrm{Si}}\tilde{K}^{\mathrm{S}}_{\mathrm{Io}}}} \tag{5}$$

TABLE II

EXPERIMENTAL PARAMETERS FOR RATES OF TRANSPORT IN TERMS OF RATE CONSTANTS IN THE SCHEME IN FIG. 1

Ct is defined as the total concentration of the carrier in all forms.

Experimental parameter	Experiment	Labeled substrate (*cis*)	Unlabeled analog (*trans*)	General expression	Rapid dissociation
$\bar{V}_{Si}$	zero *trans* exit	S_i	—	$\frac{k_{-1}f_1f_{-2}C_t}{k_{-1}(f_1+f_{-2})+f_1(f_2+f_{-2})}$	$\frac{f_1f_{-2}C_t}{(f_1+f_{-2})}$
$\tilde{V}^T_{Si}$	infinite *trans* exit	S_i	T_o	$\frac{k_{-1}k_{-4}f_{-2}f_3C_t}{f_3k_{-4}(k_{-1}+f_2+f_{-2})+f_{-2}k_{-1}(k_{-4}+f_3+f_{-3})}$	$\frac{f_{-2}f_3C_t}{(f_3+f_{-2})}$
$\bar{V}_{To}$	zero *trans*	T_o	—	$\frac{k_{-4}f_{-1}f_3C_t}{k_{-4}(f_{-1}+f_3)+f_{-1}(f_3+f_{-3})}$	$\frac{f_{-1}f_3C_t}{(f_{-1}+f_3)}$
$\bar{\bar{V}}_S$	equilibrium exchange	S_o S_i	S_i S_o	$\frac{k_{-1}k_{-2}f_2f_{-2}C_t}{(f_2+f_{-2})H}$	$\frac{f_2f_{-2}C_t}{f_2+f_{-2}}$
$\left(\tilde{v}^T/\bar{v}\right)_{S_i\to 0}$	exit	S_i	T_o	$\frac{f_3k_{-4}(1+f_{-1}/f_1)}{k_{-4}(f_{-1}+f_3)+f_{-1}(f_3+f_{-3})}$	$\frac{1+f_{-1}/f_1}{1+f_{-1}/f_3}$
$\left(\tilde{v}^T/\bar{v}\right)_{S_o\to o}$	entry	S_o	T_i	$\frac{f_{-3}k_{-3}(1+f_1/f_{-1})}{k_{-3}(f_1+f_{-3})+f_1(f_3+f_{-3})}$	$\frac{1+f_1/f_{-1}}{1+f_1/f_{-3}}$

The expressions for the inhibition constants are listed in Table I, and their definitions are given below.

Nomenclature for transport constants

In the absence of any general agreement about naming the parameters of a transport system, we have adopted a convention which appears to have several advantages, for though concise, it is sufficiently flexible to allow any constant to be identified; this is accomplished by specifying the substrate distribution in the experiment with subscripts or superscripts attached to the constant. For example, the subscripts attached to the affinity constant K designate both the particular substrate (S or T) whose affinity is measured, and the side of the membrane (inside the cell, i, or outside, o), where it becomes bound prior to transport through the membrane. A bar drawn above the constant (—) signifies that the affinity has been determined in a zero *trans* experiment (no substrate is present on the *trans* side); while a squiggle or tilde (~) refers to an infinite *trans* experiment (an unlabeled substrate is present on the *trans* side at a saturating concentration). The superscript associated with the squiggle designates the substrate on the *trans* side. A double bar (=) denotes equilibrium exchange or exchange in the final steady state. In the absence of any symbol above the constant, K represents the true dissociation constant, that is, the ratio of dissociation and association rate constants for complex formation. Similar conventions are adopted for the maximum velocity, V. The rate of transport of a substrate present at a concentration below this saturating level is indicated by a small v, as in enzyme kinetics.

In naming individual rate constants in a kinetic scheme representing the process of transport, we have adopted a convention which differentiates constants for carrier reorientation (f) from those for association and dissociation of substrate (k). The advantage in doing so is that when general expressions are written for experimental parameters, as in Table I, the consequences of having either fast or slow dissociation steps are immediately apparent.

Experimental constants which are commonly determined in transport studies are defined below:

$\overline{K}_{\mathrm{So}}$, $\overline{K}_{\mathrm{To}}$: Half-saturation constants determined in zero *trans* entry experiments for S and T, respectively. The substrate undergoing transport is present outside the cell (o).

$\overline{K}_{\mathrm{Si}}$, $\overline{K}_{\mathrm{Ti}}$: Constants determined in zero *trans* exit experiments for S and T, respectively. The substrate undergoing transport is present inside the cell (i).

$\overline{K}_{\mathrm{Ii}}$, $\overline{K}_{\mathrm{Io}}$: Competitive inhibition constants with inhibitor restricted to one side of the membrane (inside or outside). When determined in zero *trans* exit and entry experiments, respectively, where the substrate and inhibitor are present on the same side of the membrane, the inhibition is competitive. Where substrates and inhibitors are on opposite sides, inhibition is non-competitive. In either case, these constants are determined from the substrate-dependent component of the inhibition (from slopes in reciprocal plots).

$\tilde{K}^{\mathrm{S}}_{\mathrm{So}}$, $\tilde{K}^{\mathrm{T}}_{\mathrm{To}}$: Constants determined in infinite *trans* entry experiments, for S and T, respectively. In each case the same substrate whose entry is followed is present on the *trans* side of the membrane at a saturating concentration.

$\widetilde{K}^{T}_{So}$, $\widetilde{K}^{S}_{To}$: Constants determined in infinite *trans* entry experiments for the movement of S across the membrane into a solution containing T at a saturating concentration, and for the movement of T into saturating S, respectively.

$\widetilde{K}^{S}_{Ii}$, $\widetilde{K}^{S}_{Io}$: Non-competitive inhibition contants with the inhibitor restricted to one side of the membrane (inside or outside), and with the substrate at a saturating concentration on the opposite side. The constant is determined from the substrate-independent component of the inhibition (from the intercepts in reciprocal plots).

$\overline{\overline{K}}_{Si}$, $\overline{\overline{K}}_{So}$: Constants determined under conditions of no net flux, where $[S_i] = \alpha[S_o]$. $\overline{\overline{K}}_{Si}$ is the half-saturation constant for substrate exit, and $\overline{\overline{K}}_{So}$ the same constant for entry. In equilibrating systems, where $\alpha = 1$, $\overline{\overline{K}}_{Si} = \overline{\overline{K}}_{So}$.

$\overline{V}_{Si}$, $\overline{V}_{Ti}$: Maximum rates of zero *trans* exit of substrates S and T, respectively. The substrate undergoing transport is present inside the cell (i).

$\overline{V}_{So}$, $\overline{V}_{To}$: Maximum rates of zero *trans* entry of substrates S and T, respectively. The substrate undergoing transport is present outside the cell (o).

$\overline{\overline{V}}_{S}$, $\overline{\overline{V}}_{T}$: The maximum rates of transport of substrates S or T under conditions of no net flux, where $[S_i] = \alpha[S_o]$, or $[T_i] = \beta[T_o]$, respectively.

$\widetilde{V}^{T}_{Si}$: Maximum rate of infinite *trans* exit of substrate S into a solution of substrate T at a saturating concentration.

$(\tilde{v}^{T}/\bar{v})_{S_i \to o}$: Effect of unlabeled substrate (T) outside the cells on the efflux of labeled substrate (S) present internally at a low concentration ($[S_i]/\overline{K}_{Si} << 1$); $\tilde{v}^{T}$ and $\bar{v}$ are exit rates measured with a saturating concentration of substrate outside, or in the absence of external substrate, respectively.

$(\tilde{v}^{T}/\bar{v})_{S_o \to o}$: Effect of unlabeled substrate (T) inside the cells on the influx of labeled substrate (S) present externally at a low concentration ($[S_o]/\overline{K}_{so} << 1$); $\tilde{v}^{T}$ and $\bar{v}$ are entry rates measured with a saturating concentration of substrate inside, or in the absence of internal substrate, respectively.

Relationships among experimental parameters

The parameters involved in the above rate equations are not independent; in many cases, relationships are predicted which are determined by the character of the transport model, and which therefore provide the basis for a number of rigorous tests of the mechanism. They may also be utilized in calculating unknown constants from those found by experiment. The relationships are summarized in Table III.

Information from flux ratios on rate-limiting steps and symmetry

The subject of rate-limiting steps in equilibrating systems has been discussed by Hoare [12] and by Regen and Tarpley [9]. Slow substrate dissociation was shown by Hoare to be inconsistent with $\overline{\overline{V}}_{s}/\overline{V}_{so}$ ratios greater than unity, and in

TABLE III

RELATIONSHIPS AMONG EXPERIMENTAL PARAMETERS

A. Relationships for one substrate

$$\bar{K}_{\mathrm{Si}}/\bar{K}_{\mathrm{So}} = \tilde{K}^{\mathrm{S}}_{\mathrm{Si}}/\tilde{K}^{\mathrm{S}}_{\mathrm{So}} \tag{6}$$

$$\bar{V}_{\mathrm{Si}}/\bar{K}_{\mathrm{Si}} = \bar{\bar{V}}_{\mathrm{S}}/\bar{\bar{K}}_{\mathrm{Si}} \tag{7}$$

$$\bar{V}_{\mathrm{So}}/\bar{K}_{\mathrm{So}} = \bar{\bar{V}}_{\mathrm{S}}/\bar{\bar{K}}_{\mathrm{So}} \tag{8}$$

$$\bar{\bar{K}}_{\mathrm{Si}}/\bar{\bar{K}}_{\mathrm{So}} = \alpha \tag{9}$$

$$(\tilde{v}^{\mathrm{S}}/\bar{v})_{\mathrm{S_o}\rightarrow\mathrm{o}} = \bar{\bar{K}}_{\mathrm{So}}/\tilde{K}^{\mathrm{S}}_{\mathrm{So}} \tag{10}$$

$$(\tilde{v}^{\mathrm{S}}/\bar{v})_{\mathrm{S_i}\rightarrow\mathrm{o}} = 1 + \alpha\bar{K}_{\mathrm{So}}/\bar{K}_{\mathrm{Si}} - \bar{K}_{\mathrm{So}}/\bar{\bar{K}}_{\mathrm{So}} \tag{11}$$

$$\tilde{V}^{\mathrm{S}}_{\mathrm{Si}} = \tilde{V}^{\mathrm{S}}_{\mathrm{So}} = \bar{\bar{V}}_{\mathrm{S}} \tag{12}$$

B. Additional relationships for one substrate derived from Eqns. 6—11

$$\frac{\bar{V}_{\mathrm{So}}\bar{K}_{\mathrm{Si}}}{\bar{V}_{\mathrm{Si}}\bar{K}_{\mathrm{So}}} = \alpha \tag{13}$$

$$(\tilde{v}^{\mathrm{S}}/\bar{v})_{\mathrm{Si}\rightarrow\mathrm{o}} = \bar{\bar{K}}_{\mathrm{Si}}/\tilde{K}^{\mathrm{S}}_{\mathrm{Si}} \tag{14}$$

$$\frac{(\tilde{v}^{\mathrm{S}}/\bar{v})_{\mathrm{Si}\rightarrow\mathrm{o}}}{(\tilde{v}^{\mathrm{S}}/\bar{v})_{\mathrm{S_o}\rightarrow\mathrm{o}}} = \frac{\bar{V}_{\mathrm{So}}}{\bar{V}_{\mathrm{Si}}} \tag{15}$$

$$(\tilde{v}^{\mathrm{S}}/\bar{v})_{\mathrm{S_o}\rightarrow\mathrm{o}} = 1 + \bar{K}_{\mathrm{Si}}/\alpha\bar{K}_{\mathrm{So}} - \bar{K}_{\mathrm{Si}}/\bar{\bar{K}}_{\mathrm{Si}} \tag{16}$$

C. Relationships for two substrates

$$\tilde{K}^{\mathrm{S}}_{\mathrm{Ti}}/\tilde{K}^{\mathrm{T}}_{\mathrm{So}} = \bar{K}_{\mathrm{Ti}}/\bar{K}_{\mathrm{So}} \tag{17}$$

$$\tilde{K}^{\mathrm{T}}_{\mathrm{Si}}/\tilde{K}^{\mathrm{S}}_{\mathrm{To}} = \bar{K}_{\mathrm{Si}}/\bar{K}_{\mathrm{To}} \tag{18}$$

$$\tilde{V}^{\mathrm{T}}_{\mathrm{So}} = \tilde{V}^{\mathrm{S}}_{\mathrm{Ti}} \tag{19}$$

$$\tilde{V}^{\mathrm{S}}_{\mathrm{To}} = \tilde{V}^{\mathrm{T}}_{\mathrm{Si}} \tag{20}$$

$$(\tilde{v}^{\mathrm{T}}/\bar{v})_{\mathrm{S_i}\rightarrow\mathrm{o}} = \tilde{V}^{\mathrm{T}}_{\mathrm{Si}}\bar{K}_{\mathrm{Si}}/(\bar{V}_{\mathrm{Si}}\tilde{K}^{\mathrm{T}}_{\mathrm{Si}}) \tag{21}$$

$$\frac{(\tilde{v}^{\mathrm{T}}/\bar{v})_{\mathrm{S_i}\rightarrow\mathrm{o}}}{(\tilde{v}^{\mathrm{T}}/\bar{v})_{\mathrm{S_o}\rightarrow\mathrm{o}}} = \frac{\bar{V}_{\mathrm{To}}}{\bar{V}_{\mathrm{Ti}}} \tag{22}$$

$$(\tilde{v}^{\mathrm{S}}/\bar{v})_{\mathrm{S_o}\rightarrow\mathrm{o}} = \bar{K}_{\mathrm{Io}}/\tilde{K}^{\mathrm{S}}_{\mathrm{Io}} \tag{23}$$

$$(\tilde{v}^{\mathrm{S}}/\bar{v})_{\mathrm{S_i}\rightarrow\mathrm{o}} = \bar{K}_{\mathrm{Ii}}/\tilde{K}^{\mathrm{S}}_{\mathrm{Ii}} \tag{24}$$

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this case, with carrier movement rate limiting, the relative rates of reorientation of free and loaded carrier were shown to be given by

$$\frac{\bar{\bar{V}}_{\mathrm{S}}}{\bar{V}_{\mathrm{So}}} + \frac{\bar{\bar{V}}_{\mathrm{S}}}{\bar{V}_{\mathrm{Si}}} - 1 = \frac{1/f_1 + 1/f_{-1}}{1/f_2 + 1/f_{-2}} \tag{25}$$

The numerator of this expression represents the mean resistance to inward and outward movement for free carrier, and the denominator the mean resistance for the carrier-substrate complex.

Experimentally, both these questions are more conveniently answered from the flux ratios, $(\tilde{v}^{\mathrm{S}}/\bar{v})_{\mathrm{S_i}\rightarrow\mathrm{o}}$ and $(\tilde{v}^{\mathrm{S}}/\bar{v})_{\mathrm{S_o}\rightarrow\mathrm{o}}$. These are found to be remarkably informative parameters, with the advantage of being easily measured. If the magnitude of either is greater than unity, or if both equal unity, then reorientation of free carrier must be slow relative to the rate at which the carrier-substrate complex breaks down. In the first case,

$$\left(\frac{\tilde{v}^{S}}{\bar{v}}\right)_{S_o\to o} = \frac{k_{-2}f_2(1+f_{-1}/f_1)}{k_{-2}(f_{-1}+f_2)+f_{-1}(f_2+f_{-2})} > 1 \tag{26}$$

from which $f_2/f_1 > 1 + (f_2 + f_{-2})/k_{-2}$. If $k_{-2} \leqslant f_1$, then $f_2/f_1 > 1 + (f_2 + f_{-2})/f_1$, and this is not possible. The equation is therefore satisfied only if $k_{-2} > f_1$. Similarly, if

$$\left(\frac{\tilde{v}^{S}}{\bar{v}}\right)_{S_i\to o} = \frac{k_{-1}f_{-2}(1+f_1/f_{-1})}{k_{-1}(f_1+f_{-2})+f_1(f_2+f_{-2})} > 1 \tag{27}$$

then $k_{-1} > f_{-1}$

In the second case, where both flux ratios equal unity, we find from Eqns. 26 and 27 that $f_2k_{-2}/f_1 = k_{-2} + f_2 + f_{-2}$ and $f_{-2}k_{-1}/f_{-1} = k_{-1} + f_2 + f_{-2}$. If $k_{-2} \leqslant f_1$ and $k_{-1} \leqslant f_{-1}$, the former relationship could hold, approximately, only if $f_2 >> f_{-2}$, but in this case the second relationship becomes impossible. It is therefore to be concluded that $k_{-2} > f_1$ and $k_{-1} > f_{-1}$.

The relative reorientation rates for the free carrier and the complex with substrate are given directly by a sum of flux ratios. If carrier reorientation is rate limiting, so that $k_{-2} >> f_1$ and $k_{-1} >> f_{-1}$, then the flux ratios reduce to the following expressions:

$$(\tilde{v}^{S}/\bar{v})_{S_o\to o} = (1+f_{-1}/f_1)/(1+f_{-1}/f_2) \tag{28}$$

$$(\tilde{v}^{S}/\bar{v})_{S_i\to o} = (1+f_1/f_{-1})/(1+f_1/f_{-2}) \tag{29}$$

The sum of the reciprocals of these ratios is

$$\frac{1}{(\tilde{v}^{S}/\bar{v})_{S_o\to o}} + \frac{1}{(\tilde{v}^{S}/\bar{v})_{S_i\to o}} = 1 + \frac{1/f_2 + 1/f_{-2}}{1/f_1 + 1/f_{-1}} \tag{30}$$

where as before, $1/f_2 + 1/f_{-2}$ is equal to the resistance to reorientation of the carrier-substrate complex, and $1/f_1 + 1/f_{-1}$ is the corresponding value for the free carrier. In the general case, where no assumptions are made about the rate-limiting steps, Eqns. 26 and 27 yield

$$\frac{1}{(\tilde{v}^{S}/\bar{v})_{S_o\to o}} + \frac{1}{(\tilde{v}^{S}/\bar{v})_{S_i\to o}} - 1 = \frac{1/f_2 + 1/f_{-2} + (1+f_{-2}/f_2)/k_{-2} + (1+f_2/f_{-2})/k_{-1}}{1/f_1 + 1/f_{-1}} \tag{31}$$

The numerator of the latter expression represents the total resistance to substrate flux, in both directions, including that of the dissociation steps.

A measure of the inherent asymmetry in a transport system is directly given by the ratio of inward and outward flux ratios. Asymmetry causes the system to act as a valve, allowing the substrate to move more rapidly in one direction than the other even in equilibrating systems where $\alpha = 1$ [21]. It is therefore a fundamental property with regard to both mechanism and function in the cell. If carrier reorientation is rate limiting, then it is found from Eqns. 28, 29 and 15 that

$$\frac{(\tilde{v}^{S}/\bar{v})_{S_o\to o}}{(\tilde{v}^{S}/\bar{v})_{S_i\to o}} = \frac{\bar{V}_{Si}}{\bar{V}_{So}} = \frac{1/f_1 + 1/f_{-2}}{1/f_{-1} + 1/f_2} \tag{32}$$

This expression represents the resistance to substrate exit relative to entry. In the general case, when no assumptions are made as to the rate-limiting steps (Eqns. 26, 27 and 15), the ratio becomes

$$\frac{(\tilde{v}^{S}/\overline{v})_{S_o\to o}}{(\tilde{v}^{S}/\overline{v})_{S_i\to o}} = \frac{\overline{V}_{Si}}{\overline{V}_{So}} = \frac{1/f_1 + 1/f_{-2} + (1 + f_2/f_{-2})/k_{-1}}{1/f_{-1} + 1/f_2 + (1 + f_{-2}/f_2)/k_{-2}} \tag{33}$$

Finally, it may be noted that a flux ratio constant, R_s, was devised by Regen and Tarpley [9] for equilibrating systems ($\alpha = 1$). This constant differs from the flux ratios treated here, the relationship being

$$R_S = \overline{V}_{So}\tilde{K}^{S}_{Si}/\overline{\overline{V}}_S = \overline{V}_{Si}\tilde{K}^{S}_{So}/\overline{\overline{V}}_S = \overline{K}_{So}/(\tilde{v}^{S}/\overline{v})_{S_i\to o} = \overline{K}_{Si}/(\tilde{v}^{S}/\overline{v})_{S_o\to o} \tag{34}$$

R_S has two disadvantages compared to the present ratios. One is that experimentally it is less easily determined. The other is that ratios for inward and outward flux are identical, so that the information obtained from a combination of flux ratios is lacking.

Individual experiments on net transport systems

Zero trans entry and exit

The assignment of contants in Eqns. 1 and 4 may be verified by writing rate expressions for individual experiments, including only those terms that are pertinent in a given situation (see columns 3—5 in Tables I and II and definitions of the constants given above). For example $\overline{K}_{So}$ is determined from the rates of entry of substrate S into cells free of substrate ($[S_i] = [T_i] = [T_o] = 0$). The rate equation may be written down directly from Eqn. 1:

$$\frac{-d[S_o]}{dt} = \frac{\overline{V}_{So}[S_o]/\overline{K}_{So}}{1 + [S_o]/\overline{K}_{So}} = \frac{\overline{V}_{So}}{1 + \overline{K}_{So}/[S_o]} \tag{35}$$

$\overline{K}_{So}$ is clearly the half-saturation constant in this experiment, and $\overline{V}_{So}$ the maximum rate of entry. Similarly the rate expression for zero *trans* exit (with $[S_o] = [T_i] = [T_o] = 0$) is found to be:

$$\frac{-d[S_i]}{dt} = \frac{\overline{V}_{Si}}{1 + \overline{K}_{Si}/[S_i]} \tag{36}$$

Corresponding rate equations for transport of T may be written from Eqn. 4.

Infinite trans entry and exit

The rate of transport of substrate S is measured in the presence of T at a saturating concentration on the *trans* side of the membrane. Influx is governed by the following expression, found by setting $[S_i] = [T_o] = 0$, and $[T_i]/\overline{K}_{Ti} >> 1$:

$$\frac{-d[S_o]}{dt} = \frac{\alpha\tilde{V}^{T}_{So}/\beta}{1 + \tilde{K}^{T}_{So}/[S_o]} \tag{37}$$

The equation for efflux is found under the condition that $[S_o] = [T_i] = 0$, and $[T_o]/\overline{K}_{To} >> 1$:

$$\frac{-d[S_i]}{dt} = \frac{\tilde{V}^{T}_{Si}}{1 + \tilde{K}^{T}_{Si}/[S_i]} \tag{38}$$

Equilibrium exchange (or exchange in the final steady state in active systems)

Here the same substrate is present on both sides of the membrane, but one pool is labeled, and rates are determined under conditions where no net flux occurs. By setting $[\mathrm{S_o}] = [\mathrm{T_i}] = 0$, and $[\mathrm{S_i}] = \alpha[\mathrm{T_o}]$, Eqn. 1 yields

$$v = \frac{\dfrac{\overline{V}_{\mathrm{Si}}}{\overline{K}_{\mathrm{Si}}}[\mathrm{S_i}] + \dfrac{\widetilde{V}^{\mathrm{T}}_{\mathrm{Si}}}{\widetilde{K}^{\mathrm{S}}_{\mathrm{To}}\overline{K}_{\mathrm{Si}}}[\mathrm{S_i}][\mathrm{T_o}]}{1 + \dfrac{[\mathrm{S_i}]}{\overline{K}_{\mathrm{Si}}} + \dfrac{[\mathrm{T_o}]}{\overline{K}_{\mathrm{To}}} + \dfrac{[\mathrm{S_i}][\mathrm{T_o}]}{\overline{K}_{\mathrm{Si}}\widetilde{K}^{\mathrm{S}}_{\mathrm{To}}}} \tag{39}$$

To simplify this expression, we first replace the symbols for T by those for S:

$$v = \frac{\dfrac{\overline{V}_{\mathrm{Si}}}{\overline{K}_{\mathrm{Si}}}[\mathrm{S_i}] + \dfrac{\widetilde{V}^{\mathrm{S}}_{\mathrm{Si}}}{\widetilde{K}^{\mathrm{S}}_{\mathrm{So}}\overline{K}_{\mathrm{Si}}}[\mathrm{S_i}][\mathrm{S_o}]}{1 + \dfrac{[\mathrm{S_i}]}{\overline{K}_{\mathrm{Si}}} + \dfrac{[\mathrm{S_o}]}{\overline{K}_{\mathrm{So}}} + \dfrac{[\mathrm{S_i}][\mathrm{S_o}]}{\overline{K}_{\mathrm{Si}}\widetilde{K}^{\mathrm{S}}_{\mathrm{So}}}} \tag{40}$$

When Eqns. 7, 10 and 12 from Table III are introduced into Eqn. 40, it is found that

$$v = \frac{\dfrac{\overline{\overline{V}}_{\mathrm{S}}}{\overline{\overline{K}}_{\mathrm{Si}}}[\mathrm{S_i}] + \dfrac{\overline{\overline{V}}_{\mathrm{S}}[\mathrm{S_i}][\mathrm{S_o}]}{\overline{K}_{\mathrm{Si}}\overline{\overline{K}}_{\mathrm{So}}}(\widetilde{v}^{\mathrm{S}}/\overline{v})_{\mathrm{S_o}\to\mathrm{o}}}{1 + \dfrac{[\mathrm{S_i}]}{\overline{K}_{\mathrm{Si}}} + \dfrac{[\mathrm{S_o}]}{\overline{K}_{\mathrm{So}}} + \dfrac{[\mathrm{S_i}][\mathrm{S_o}]}{\overline{K}_{\mathrm{Si}}\widetilde{K}^{\mathrm{S}}_{\mathrm{So}}}} \tag{41}$$

Finally, after substitution of Eqn. 16 (Table III) and of the relationship $[\mathrm{S_i}] = \alpha[\mathrm{S_o}]$, Eqn. 41 yields

$$v = \frac{\overline{\overline{V}}_{\mathrm{S}}}{1 + \overline{\overline{K}}_{\mathrm{Si}}/[\mathrm{S_i}]} \tag{42}$$

By use of the equality $\overline{\overline{K}}_{\mathrm{Si}} = \alpha\overline{\overline{K}}_{\mathrm{So}}$, Eqn. 42 is readily converted to a form containing $[\mathrm{S_o}]$ instead of $[\mathrm{S_i}]$:

$$v = \frac{\overline{\overline{V}}_{\mathrm{S}}}{1 + \overline{\overline{K}}_{\mathrm{So}}/[\mathrm{S_o}]} \tag{43}$$

In equilibrating systems, where $\alpha = 1$, Eqns. 42 and 43 are identical, since $[\mathrm{S_i}] = [\mathrm{S_o}]$, and $\overline{\overline{K}}_{\mathrm{Si}} = \overline{\overline{K}}_{\mathrm{So}}$.

Sen-Widdas exit experiment (infinite cis efflux)

In this case, the initial rate of net exit is determined under conditions where the internal substrate concentration is saturating ($[\mathrm{S_i}]/\overline{K}_{\mathrm{Si}} >> 1$), and where the same substrate is present in the external solutions at a far lower concentration which is varied ($[\mathrm{S_o}] << [\mathrm{S_i}]$). The expression governing the rate is found from Eqn. 1 by setting $[\mathrm{T_o}] = [\mathrm{T_i}] = 0$

$$\frac{-\mathrm{d}[\mathrm{S_i}]}{\mathrm{d}t} = \frac{\overline{V}_{\mathrm{Si}}}{1 + [\mathrm{S_o}]/\widetilde{K}^{\mathrm{S}}_{\mathrm{So}}} \tag{44}$$

General rate equation in an obligatory exchange system

If reorientation of the free carrier is prohibited ($f_1 = f_{-1} = 0$), the system promotes the exchange of one substrate molecule for another on the *trans* side of the membrane, but net transport is not possible, even where the concentration gradient would favor diffusion of substrate through the membrane. The equation for the rate of exchange is readily derived from Eqn. 1. When f_1 and f_{-1} equal zero, zero *trans* flux cannot occur, and in consequence the zero *trans* constants $\bar{V}_{\mathrm{Si}}$, $\bar{K}_{\mathrm{Si}}$, $\bar{K}_{\mathrm{So}}$ etc., all become equal to zero (see Tables I and II). It is therefore necessary to eliminate these constants from Eqn. 1, and this is accomplished through substitution of ratios of infinite *trans* constants for ratios of zero *trans* constants, the required relationships being found in Table III. When these substitutions have been made, the rate of exchange is found to be governed by the following expression:

$$v = \frac{\widetilde{V}^{\mathrm{T}}_{\mathrm{Si}}([\mathrm{S_i}][\mathrm{T_o}] - [\mathrm{S_o}][\mathrm{T_i}]\alpha/\beta)}{D1} \tag{45}$$

where the denominator $D1$ has the value,

$$D1 = \frac{\widetilde{K}^{\mathrm{S}}_{\mathrm{Si}}\widetilde{K}^{\mathrm{S}}_{\mathrm{To}}}{\widetilde{K}^{\mathrm{S}}_{\mathrm{So}}}[\mathrm{S_o}] + \widetilde{K}^{\mathrm{S}}_{\mathrm{To}}[\mathrm{S_i}] + \widetilde{K}^{\mathrm{T}}_{\mathrm{Si}}[\mathrm{T_o}] + \frac{\widetilde{K}^{\mathrm{T}}_{\mathrm{Si}}\widetilde{K}^{\mathrm{T}}_{\mathrm{To}}}{\widetilde{K}^{\mathrm{T}}_{\mathrm{Ti}}}[\mathrm{T_i}]$$
$$+ \frac{\widetilde{K}^{\mathrm{S}}_{\mathrm{To}}}{\widetilde{K}^{\mathrm{S}}_{\mathrm{So}}}[\mathrm{S_o}][\mathrm{S_i}] + \frac{\widetilde{K}^{\mathrm{T}}_{\mathrm{Si}}}{\widetilde{K}^{\mathrm{T}}_{\mathrm{Ti}}}[\mathrm{T_o}][\mathrm{T_i}] + \frac{\widetilde{K}^{\mathrm{S}}_{\mathrm{Si}}\widetilde{K}^{\mathrm{S}}_{\mathrm{To}}}{\widetilde{K}^{\mathrm{S}}_{\mathrm{So}}\widetilde{K}^{\mathrm{S}}_{\mathrm{Ti}}}[\mathrm{S_o}][\mathrm{T_i}] + [\mathrm{T_o}][\mathrm{S_i}]$$

It is useful to write an equivalent expression, as follows:

$$v = \frac{\widetilde{V}^{\mathrm{T}}_{\mathrm{So}}([\mathrm{S_i}][\mathrm{T_o}]\beta/\alpha - [\mathrm{S_o}][\mathrm{T_i}])}{D2} \tag{46}$$

$$D2 = \widetilde{K}^{\mathrm{S}}_{\mathrm{Ti}}[\mathrm{S_o}] + \frac{\widetilde{K}^{\mathrm{S}}_{\mathrm{Ti}}\widetilde{K}^{\mathrm{S}}_{\mathrm{So}}}{\widetilde{K}^{\mathrm{S}}_{\mathrm{Si}}}[\mathrm{S_i}] + \frac{\widetilde{K}^{\mathrm{T}}_{\mathrm{So}}\widetilde{K}^{\mathrm{T}}_{\mathrm{Ti}}}{\widetilde{K}^{\mathrm{T}}_{\mathrm{To}}}[\mathrm{T_o}] + \widetilde{K}^{\mathrm{T}}_{\mathrm{So}}[\mathrm{T_i}]$$
$$+ \frac{\widetilde{K}^{\mathrm{S}}_{\mathrm{Ti}}}{\widetilde{K}^{\mathrm{S}}_{\mathrm{Si}}}[\mathrm{S_o}][\mathrm{S_i}] + \frac{\widetilde{K}^{\mathrm{T}}_{\mathrm{So}}}{\widetilde{K}^{\mathrm{T}}_{\mathrm{To}}}[\mathrm{T_o}][\mathrm{T_i}] + [\mathrm{S_o}][\mathrm{T_i}] + \frac{\widetilde{K}^{\mathrm{S}}_{\mathrm{So}}\widetilde{K}^{\mathrm{S}}_{\mathrm{Ti}}}{\widetilde{K}^{\mathrm{S}}_{\mathrm{Si}}\widetilde{K}^{\mathrm{S}}_{\mathrm{To}}}[\mathrm{T_o}][\mathrm{S_i}]$$

The forms of these equations become greatly simplified when each substrate is confined to one side of the membrane. For example when S is present inside the cell only and T outside ($[\mathrm{S_o}] = [\mathrm{T_i}] = 0$) the rate is found from Eqn. 45 to be

$$v = \frac{\widetilde{V}^{\mathrm{T}}_{\mathrm{Si}}}{1 + \dfrac{\widetilde{K}^{\mathrm{S}}_{\mathrm{To}}}{[\mathrm{T_o}]} + \dfrac{\widetilde{K}^{\mathrm{T}}_{\mathrm{Si}}}{[\mathrm{S_i}]}} \tag{47}$$

With the substrates reversed, and both $[\mathrm{S_i}]$ and $[\mathrm{T_o}]$ zero, the rate is most simply found from Eqn. 46:

$$v = \frac{\widetilde{V}^{\mathrm{T}}_{\mathrm{So}}}{1 + \dfrac{\widetilde{K}^{\mathrm{S}}_{\mathrm{Ti}}}{[\mathrm{T_i}]} + \dfrac{\widetilde{K}^{\mathrm{T}}_{\mathrm{So}}}{[\mathrm{S_o}]}} \tag{48}$$

Conclusions

With a single substrate, twelve different experimental parameters may be determined. According to theory these are not independent, and various combinations of five constants are sufficient to calculate all the others from Eqns. 6—11. Hence each additional parameter constitutes a test of the model, through comparison with the predicted value. Even when fewer constants are known, individual relationships in Table III may be tested. The prediction of linear relationships in plots for various experiments, as given above, also provides tests of the model.

In investigations of the rate-limiting steps in transport the flux ratios, $(\tilde{v}^S/\bar{v})_{S_o \to o}$ and $(\tilde{v}^S/\bar{v})_{S_i \to o}$, were seen to be especially convenient, and they also provide information on the relative magnitudes of various other experimental parameters (Table III). Moreover such ratios for pairs of substrates (Eqns. 21 and 22) form the basis for a method in which the transport parameters of unlabeled substrate analogs may be determined from their effects on the movement of a single-labeled substrate. This approach makes it possible to study a wide range of unlabeled substrates, and will be demonstrated in a later communication.

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