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COMPUTATIONAL ANALYSIS OF MODELS FOR COTRANSPORT

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The order of substrate interaction with a cotransport carrier is studied by numerically fitting theoretical models to empirical data for a Na^+-D-glucose pathway. Our analysis is based on a least-squares minimization routine developed at CERN, and uses data derived from tracer flux of substrate under equilibrium exchange conditions. Random, Ordered mirror, and Ordered glide models are considered and the applicability of both Random and Glide systems to the experimental observations is demonstrated. A more detailed study of the Glide model provides an estimation of the relative values of the individual rate constants describing each kinetic step in the mechanism. We argue that parameterization of competing models can result in tests which will distinguish between them, even when these contain many unknowns in the proposed structure of the kinetic mechanism. The essence of this means of model discrimination is the ability to find a single set of parameter values that fits a variety of experimental observations. The ability to obtain numerical estimates of individual rate constants is also a useful tool in investigation of the kinetic fine structure of the carrier. For the data at hand we conclude that a complete parameterization of the Glide model cannot be attained, possibly due to a heterogeneity in the temperature at which the experiments have been performed. Three solutions to the Glide model are presented, each of which corresponds to a fit of the Glide model to a subset of the experimental data.

Introduction

The mathematical study of reaction-rate equations is frequently used as a tool to understand complex biochemical pathways. When the reaction pathways can be described by steady-state conditions, the dynamical problem inherent in the reaction-rate equations reduces to a study of algebraic expressions that involve large numbers of individual rate constants. These rate constants represent a set of internal parameters of the system. In most cases their exact values are unknown and they are considered as variable parameters of the mathematical model. Universal access to computer facilities now makes it possible to extend the algebraic analysis through the modeling of experimental situations. In fitting the algrebraic equations of steady-state chemical kinetics to empirical data, a single model can be tested for its consistency with a range of experimental conditions. Ultimately the success or failure of the model will depend on the quantitative prediction of the individual rate constant parameters. The mathematical analysis may provide effective rejection criteria for competing models that at first seem comparable. Moreover, recent developments in the computational approach to symbolic algebra [1] raise the possibility of analyzing models in experimental situations that

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are currently too complex to be tractable via manual procedures. In this paper we use computer modeling of two relatively complex kinetic schemes for membrane transport to study the question of order of substrate transport in the Na$^+$-D-glucose cotransporter of the renal and intestinal epithelial brush border.

Five possible models of substrate interaction with a 1 : 1 stiochiometry cotransport carrier have been described [2] (Fig. 1). Most general is the Random model, in which substrates may enter or leave the carrier in either order. The remaining cases are specialized forms of the Random model in which some specified order is imposed on the system. There are two classes of ordered transport. A Mirror mechanism is one in which the first substrate to enter the carrier at the external surface is the second substrate released at the cytoplasmic surface. Such a system may imply a rotational type motion to the transport mechanism. In a Glide carrier the first substrate to enter the carrier is also the first released. This symmetry is consistent with a gated-channel mechanism. Each class is subdivided into two cases characterized by which of the two substrates enters the carrier first.

In this paper we consider the order of transport in the Na$^+$-D-glucose carrier. For this system kinetic analysis of the order of transport has used two different approaches. In one study [3,4] it was assumed that phlorizin, a competitive inhibitor of Na$^+$-dependent D-glucose transport, interacts with the carrier in place of D-glucose. Thus the order of interaction between phlorizin and Na$^+$ would be the same as D-glucose and Na$^+$. Phlorizin binds to the carrier yet is not transported; therefore it is believed that phlorizin acts as a probe of only the initial interaction of substrates with the carrier [5–7]. Through the use of inhibitor binding, the analysis of kinetic models is simplified without restricting the experimental conditions that can be considered [3]. Phlorizin binding studies have established that the order of interaction of substrates with the Na$^+$-glucose carrier of dog kidney is apparently random, with a 'Na$^+$-first' preference at Na$^+$ concentrations greater than 25 mM [4].

In another study the rate of unidirectional substrate transport was determined as a function of the concentration of cosubstrate at equilibrium

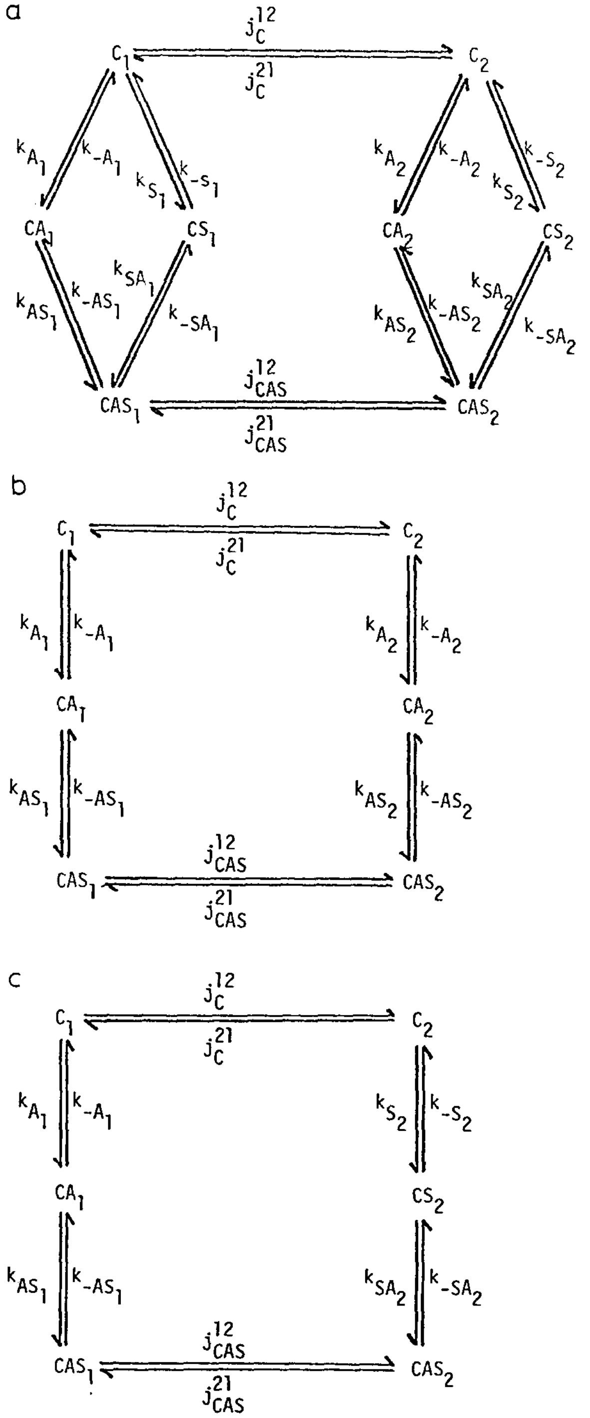


Fig. 1. Models of cotransport: (a) Random, (b) Ordered mirror, (c) Ordered glide. For the ordered models the 'A first' case is shown; the 'S first' case is obtained by exchanging A and S. Rate constants for individual steps are indicated adjacent to the appropriate step.

substrate concentrations. By restricting experimental observation to equilibrium exchange conditions the theoretical analysis is simplified, so that no assumptions about rate-limiting steps need be made. By means of this analysis, Glide, Mirror, and Random type systems can be distinguished [2]. Equilibrium exchange studies on the Na^+-D-glucose carrier of rabbit small intestine suggested an ordered model with Glide symmetry. However, the first substrate to enter the carrier could not be determined [8].

The initial response to these two studies was the suggestion that the experimental distinction between Random and Glide models was contradictory [4,9]. In this paper we consider this apparent contradiction and demonstrate that confusion in terminology has failed to distinguish between order of surface interaction and order of transport across the membrane. In further analysis we search for a single numeric solution to the algebraic description of the Na^+-first Glide model which is consistent with a series of experimental observations. In fitting the Glide model to a range of experimental data we have developed a method for testing the validity of this system.

Assumptions

To facilitate algebraic and numeric analysis of the models, a number of simplifying assumptions have been made:

(1) None of the reaction steps are assumed to be rate limiting.

(2) Carrier bound to a single substrate is not transportable.

(3) The carrier is secondary active; thus all driving forces are derived from substrate electrochemical gradients.

(4) Equilibrium exchange conditions prevail; consequently $[A_1] = [A_2] = [A]$, $[S_1] = [S_2] = [S]$, $\Delta\psi = 0$ (where $\Delta\psi$ is the transmembrane electric potential).

(5) The external volume greatly exceeds the internal volume; thus external concentrations are constant through all experiments.

(6) Total number of carriers (C_0) is constant through all experiments.

Assumption (1) actually complicates the analysis but was essential to describe the experimental results [8]. Assumption (2) was verified in equilibrium exchange experiments [8] and greatly simplifies analysis of the algebra. However, recent experiments [10] have reported carrier-dependent flux of glucose in the absence of Na^+, indicating that extensions to the model which remove this assumption may be necessary in future work. Assumptions (3) and (4) are appropriate to the carrier and experimental conditions considered, and act to constrain the final solutions to the model. Assumption (5) aids computer simulation and is appropriate for vesicle experiments due to the consistently low internal volume. Assumption (6) is based on the assumption of constant protein content through all experiments [8] and the knowledge of a linear relationship between the number of carriers and the quantity of protein [4,11]. In practice C_0 only appears during complete parameter fitting of the Glide model. In this section the actual value of C_0 is not assumed; thus all parameters are determined relative to the number of carriers present. Consequently the absolute value of each parameter is dependent on the value of C_0; however, the relative values of the parameters are independent of C_0.

Methods

Experimental data for the unidirectional rate of transport of tracer for one substrate as a function of its cosubstrate concentration, measured under equilibrium exchange conditions was reported by Hopfer and Groseclose [8] (exact values of these measurements were kindly provided by Hopfer). In this study the rates of glucose transport were measured at 15 °C and the rates of Na^+ transport at 25 °C. It has been shown [12] that the half-time to equilibration of tracer ($t_{1/2}$) is inversely proportional to the initial rate of tracer transport:

$$(t_{1/2})^{-1} = (V_A)/([A] \ln 2)$$

where $t_{1/2}$ is the half-time of equilibration of A and V_A is the initial velocity of A. Here A denotes the 'activator' (e.g., Na^+) and we will use S to denote the 'substrate' (e.g., glucose). On the basis of assumption (2) we assumed that transport at zero cosubstrate concentration was a measure of the nonspecific leak and therefore subtracted this measurement from the other data.

The best fit to the data was obtained through a minimization of the least-squares expression

$$L=\sum_i\left(V_i^{\mathrm{obs}}-V_i^{\mathrm{th}}\right)^2$$

where V_i^{obs} is the experimental value of the ith data point and V_i^{th} is the corresponding theoretical value. Minimization of L was carried out by manipulating the theoretical constants contained within the expression for V_i^{th}.

The minimization process was obtained through use of the computer program MINUIT, which is a robust, widely tested package of computer programs for function minimization developed at the European Organization for Nuclear Research (CERN) in Geneva [13]. In using MINUIT an initial seed point or approximation to the parameters is provided by the user along with a maximum range for each parameter and an initial step size for parameter variation. The minimization begins from these initial conditions. However, the algorithm is reasonably robust and one solution does not appear to be critically dependent on the initial estimates [14]. MINUIT is designed to signal convergence to a best fit when simultaneously (1), the predicted distance ρ to the true minimum is smaller than the tolerance on the minimum function value for two consecutive interactions of the algorithm and (2), the average fractional change in the diagonal elements of the error covariance matrix is smaller than the tolerance on the stability of the error matrix for two consecutive interactions, or, alternatively to (1) and (2), when the distance ρ becomes smaller than 10^{-5}-times the tolerance specified in Ref. 1.

The experimental data quantify V_{S} and V_{A} (the initial rate of uptake of tracer S or A at equilibrium exchange) as a function of the respective cosubstrate concentrations. To establish the validity of the Random and Glide models we fit the experimental results using the general algebraic functions appropriate to each model (given in detail below). To study the Glide model in more detail the general equations for the model were expanded in terms of the individual rate constants for each step in the reaction mechanism. An attempt was then made to obtain a single set of parameters for the model that simultaneously describe experimental measurements of V_{S}, V_{A} and $K_{\mathrm{m_S}}^{-1}$ (the inverse of the affinity constant for S). This latter approach which we have referred to as parameterization may provide rejection criteria for specific systems, because a model can be considered appropriate only if it is consistent with a series of experimental observations.

Results

(a) General analysis of cotransport models

Hopfer's data [8] (Fig. 2) show biphasic activation of transport for both substrates, characterized by initial stimulation of tracer flux followed by inhibition due to high cosubstrate concentration. Both the Glide model and specific solutions of the Random model can fit this data. Experimental data of this form conclusively rejects Mirror type models [2].

For the Glide model the kinetic description of the experimental data for V_{S} (Fig. 2a) as derived by others [2,14] is given by

$$V_{\mathrm{S}}=\frac{V_{\max}^{\mathrm{S}}[\mathrm{A}]}{\left(1+K_{\mathrm{m_A}}^{-1}[\mathrm{A}]\right)\left(1+K_1[\mathrm{A}]\right)} \tag{2}$$

and for V_{A} (fig. 2b) by

$$V_{\mathrm{A}}=\frac{V_{\max}^{\mathrm{A}}[\mathrm{S}]}{\left(1+K_{\mathrm{m_S}}^{-1}[\mathrm{S}]\right)\left(1+K_2[\mathrm{S}]\right)} \tag{3}$$

where the constants are functions of the rates of individual steps in the model, the total number of carriers, and the concentration of the labelled substrate. For the Random model the equation corresponding to Eqn. 2 is

$$V_{\mathrm{S}}=\frac{V_{\max}^{\mathrm{S}}[\mathrm{A}]\left(1+K_{\mathrm{A}}[\mathrm{A}]+K_{\mathrm{B}}[\mathrm{A}]^2\right)}{\left(1+K_{\mathrm{m_A}}^{-1}[\mathrm{A}]\right)\left(1+K_{\mathrm{a}}[\mathrm{A}]+K_{\mathrm{b}}[\mathrm{A}]^2\right)} \tag{4}$$

The constants K_{m} are the apparent affinity constants of either A or S measured at equilibrium exchange. They are estimated by Hopfer to have the values $K_{\mathrm{m_S}}=2.3$ mM, $K_{\mathrm{m_A}}=300$ mM.

Using these estimates the minimization routine was used to find values for the constants in each of the theoretical equations (Eqns. 2–4) so that Eqns.

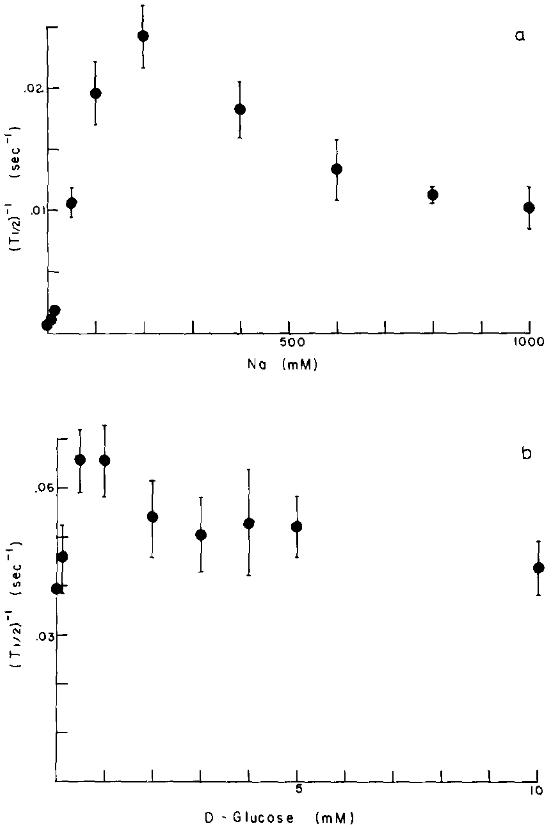


Fig. 2. (a) Half-time for equilibration, $(t_{1/2})^{-1}$ for S (i.e., V_S) as a function of [A] (Eqns. 2 and 4); (b) $(t_{1/2})^{-1}$ for A (i.e., V_A) as a function of [S] (Eqn. 3). Data from Ref. 8). Changes in Na^+ were balanced by K^+ so that total cation concentration was 1.0 M.

2 and 4 fit the experimental data in Fig. 2a and Eqn. 3 fits the data in Fig. 2b. Qualitatively both models were observed to fit the data (Fig. 3). The Glide model produces a fit which is lower than experimental values at the maximum activation of transport and greater than experimental values at high cosubstrate concentrations. In comparison to the Glide model the Random model provides a visibly better fit to all the data. The sample correlation coefficient, which was 0.97 for the Glide model, increased to 0.99 for the Random model. This improvement of the Random model over the Glide is not expected to be statistically significant (given the experimental error). Nor is it totally unexpected, since the Random model can reduce to the Glide model if specific constants are zero (i.e., $K_A = K_B = K_b = 0$, and $K_a = K_1$). However,

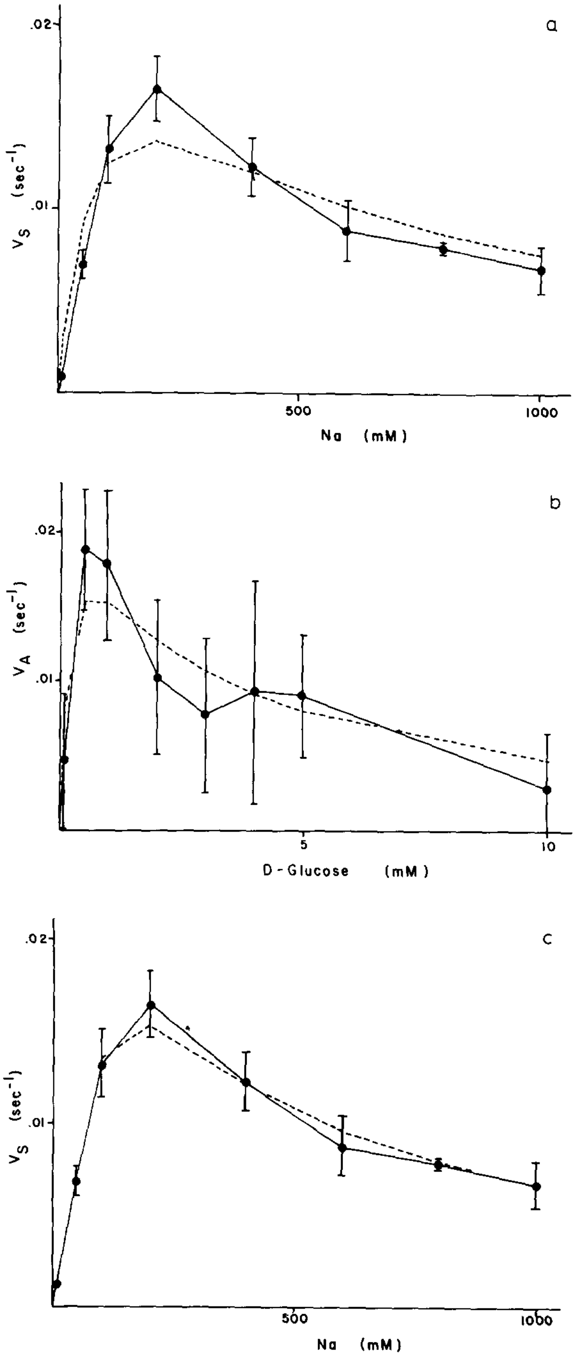


Fig. 3. Numeric fit of simplified velocity equations to the data of Fig. 2. The experimental data is given by the solid line, theoretical fit by the broken line. (a) Glide model fit to V_S (Eqn. 2 with $V_{max} = 3.056 \cdot 10^{-4}$ s^{-1}, $K_1 = 8.42 \cdot 10^{-3}$ mM^{-1}). (b) Glide model fit to V_A (Eqn. 3 with $V_{max} = 0.1282$ s^{-1}, $K_2 = 4.86$ mM^{-1}). (c) Random model fit to V_S (Eqn. 4 with $V_{max} = 2.42 \cdot 10^{-5}$ s^{-1}, $K_A = 0.167$ mM^{-1}, $K_B = 2.76 \cdot 10^{-9}$ mM^{-2}, $K_a = 5.45 \cdot 10^{-6}$ mM^{-1}, $K_b = 1.38 \cdot 10^{-4}$ mM^{-2}).

our estimation of these values gives $K_A = 0.167$ mM^{-1}, K_a a factor of 1000 less than K_1, and the values of $V_{\max}^S$ differ by a factor of 10. This solution of the Random model does not therefore appear to be merely a slightly modified Glide model. This finding serves to illustrate an important point, namely that the Hopfer results can be adequately described by a Random transport process and that these experimental results are unable to distinguish between the Glide and Random models.

(b) Parameterization of the Glide model

In the previous section we derived numerical values for the general expressions describing the data portrayed in Fig. 2. It is of interest to know if the derived solutions correspond to a single form of the Glide model. Given the relatively greater complexity of the Random model, we restricted our attention to the Glide kinetics during this phase of the investigation. In exploring the consistency of the Glide model the measurement of V_S and V_A (Fig. 2), as well as the functional dependence of $K_{m_S}^{-1}$ on the concentration of A (Fig. 4), was used. The algebraic forms of V_S and V_A corresponding to the Glide model are given in Eqns. 2 and 3. The form of K_{m_S} is

$$K_{m_S} = \frac{a + b[A]}{c + d[A]} \tag{5}$$

To consider the consistency of the Glide model in describing these three experimental measurements the constants in Eqns. 2, 3 and 5 were expanded in terms of the individual rate constants (Table I). The notation used to identify the individual rate constants is specified in Fig. 1. In this notation the rates of transmembrane reactions are given by j. Subscripts indicate the carrier complex referred to and superscripts give the direction of motion. Thus j_{CAS}^{21} is the rate at which the complex CAS passes from side 2 to side 1 (side 1 being the external and side 2 being the internal surface). Rates of surface reactions are given by k. Subscripts denote the substrate and the side it is added to with the sign indicating association (+) or dissociation (−) of substrate. The twelve parameters which describe the Glide model (Fig. 1) are constrained by assumption (3) and therefore

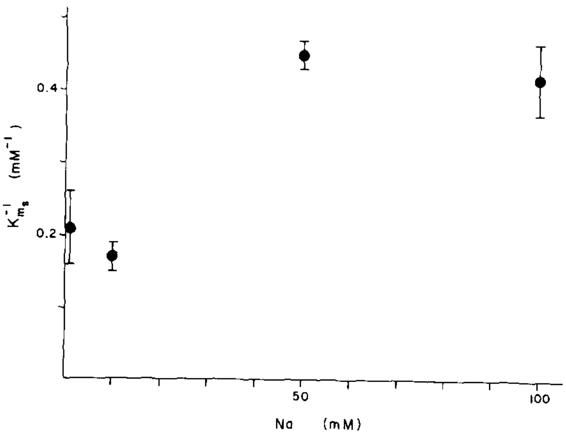


Fig. 4. Inverse of affinity constant for S, $K_{m_S}^{-1}$ as a function of [A] (Eqn. 5). Data from Ref. 8.

TABLE I

ALGEBRAIC EXPANSION OF EQUATIONS 2, 3 AND 5

$V_m^S = \dfrac{k_{AS_1} j_{CAS}^{12} k_{-SA_2} k_{A_1} C_0}{k_{-A_1}\left(j_{CAS}^{12} k_{-SA_2} + k_{-AS_1} k_{-SA_2} + j_{CAS}^{21} k_{-AS_1}\right)\left(1 + \left(j_C^{12}/j_C^{21}\right) + \left(j_C^{12} k_{S_2}[A]/j_C^{21} k_{-S_2}\right)\right)}$	$a = 1 + \dfrac{j_C^{12}}{j_C^{21}}$
$K_1 = \dfrac{j_{CAS}^{21} k_{-AS_1} k_{SA_2}}{k_{-S_2}\left(j_{CAS}^{12} k_{-SA_2} + k_{-AS_1} k_{-SA_2} + j_{CAS}^{21} k_{-AS_1}\right)}$	$b = \dfrac{k_{A_1}}{k_{-A_1}}$
$V_m^A = \dfrac{k_{AS_1} j_{CAS}^{12} k_{-SA_2} k_{A_1} C_0}{k_{-A_1}\left(J_{CAS}^{12} k_{-SA_2} + k_{-AS_1} k_{-SA_2} + j_{CAS}^{21} k_{-AS_1}\right)\left(1 + \left(j_C^{12}/j_C^{21}\right) + \left(K_{A_1}[A]/k_{-A_1}\right)\right)}$	$c = \dfrac{j_C^{12} k_{S_2}}{j_C^{21} k_{-S_2}}$
$K_2 = \dfrac{j_{CAS}^{12} k_{AS_1} k_{-SA_2}}{k_{-A_1}\left(j_{CAS}^{12} k_{-SA_2} + k_{-AS_1} k_{-SA_2} + j_{CAS}^{21} k_{-AS_1}\right)}$	$d = \dfrac{k_{A_1} k_{AS_1}}{k_{-A_1} k_{-AS_1}} + \dfrac{j_C^{12} k_{S_2} k_{SA_2}}{j_C^{21} k_{-S_2} k_{-SA_2}}$

obey the relationship

$$j^{21}_{\mathrm{CAS}} = \frac{j^{12}_{\mathrm{CAS}} k_{-\mathrm{SA}_2} k_{-\mathrm{S}_2} j^{21}_{\mathrm{C}} k_{\mathrm{A}_1} k_{\mathrm{AS}_1}}{k_{\mathrm{SA}_2} k_{\mathrm{S}_2} j^{12}_{\mathrm{C}} k_{-\mathrm{A}_1} k_{-\mathrm{AS}_1}} \tag{6}$$

In the Glide model the fluxes j^{12}_{C} and j^{21}_{C} appear only in ratio to each other; their individual values with respect to the other parameters is unknown.

In the minimization routine we determined the total sum of least squares as the sum of the least-squares difference between the experimental data for V_{S}, V_{A} and $K^{-1}_{m_{\mathrm{S}}}$ and the respective algebraic solutions (Eqns. 2, 3 and 5 as modified by Table I). From the three experiments we had a total of 21 data points to fit with 10 independent parameters. As expected from the complex form of the algebraic solutions the least-squares function was found to possess a complicated structure with many local minima. To facilitate the fitting procedure we restricted the range of our parameters to six powers of e, the base of natural logarithms. Thus the highest values are not more than 400-times greater than the lowest. In selecting this strategy we were guided by the fact that the values of rate constants for other carriers have been observed to fall within this range [16]. To search this large parameter space we used a random number generator to select initial values for the rate constants. If the selected parameter set provided a least-squares value of less than some selected value, the parameters were used as a seed point from which MINUIT searched for a mimimum least squares. The selected value was chosen so that approximately one in every fifty randomly chosen sets would be used by MINUIT. No single parameter set was found which visibly fit all three experiments. It was, however, possible to generate fits to any two experiments. The solution which best fits any pair of experiments is given in Table II; the corresponding theoretical curves are shown in Fig. 5. We note that the solution that simultaneously fits the experimental data for V_{S} and V_{A}, but fails to fit the graph of $K^{-1}_{m_{\mathrm{S}}}$ (Table IIa) has the majority of parameters at the limits of the allowed range (0.0498–20.1), implying that either we have underestimated the constraints on our parameters or these graphs do not correspond to a single solution of the parameter set. We suggest that the most appropriate solution is the simultaneous fit to V_{S} and $K^{-1}_{m_{\mathrm{S}}}$ shown in Fig. 5c, since these experiments were run at the same temperature and most of the parameter values lie within the selected range. We note that the form of V_{A} predicted by this solution (which would be the predicted flux of A at 15 °C) shows only a very small (but non zero) component of carrier-dependent transport. This would be virtually undetectable above background compared to the observed form of V_{A} at 25 °C. Unfortunately no data for V_{A} at 15 °C is available for comparison with the model predictions.

TABLE II

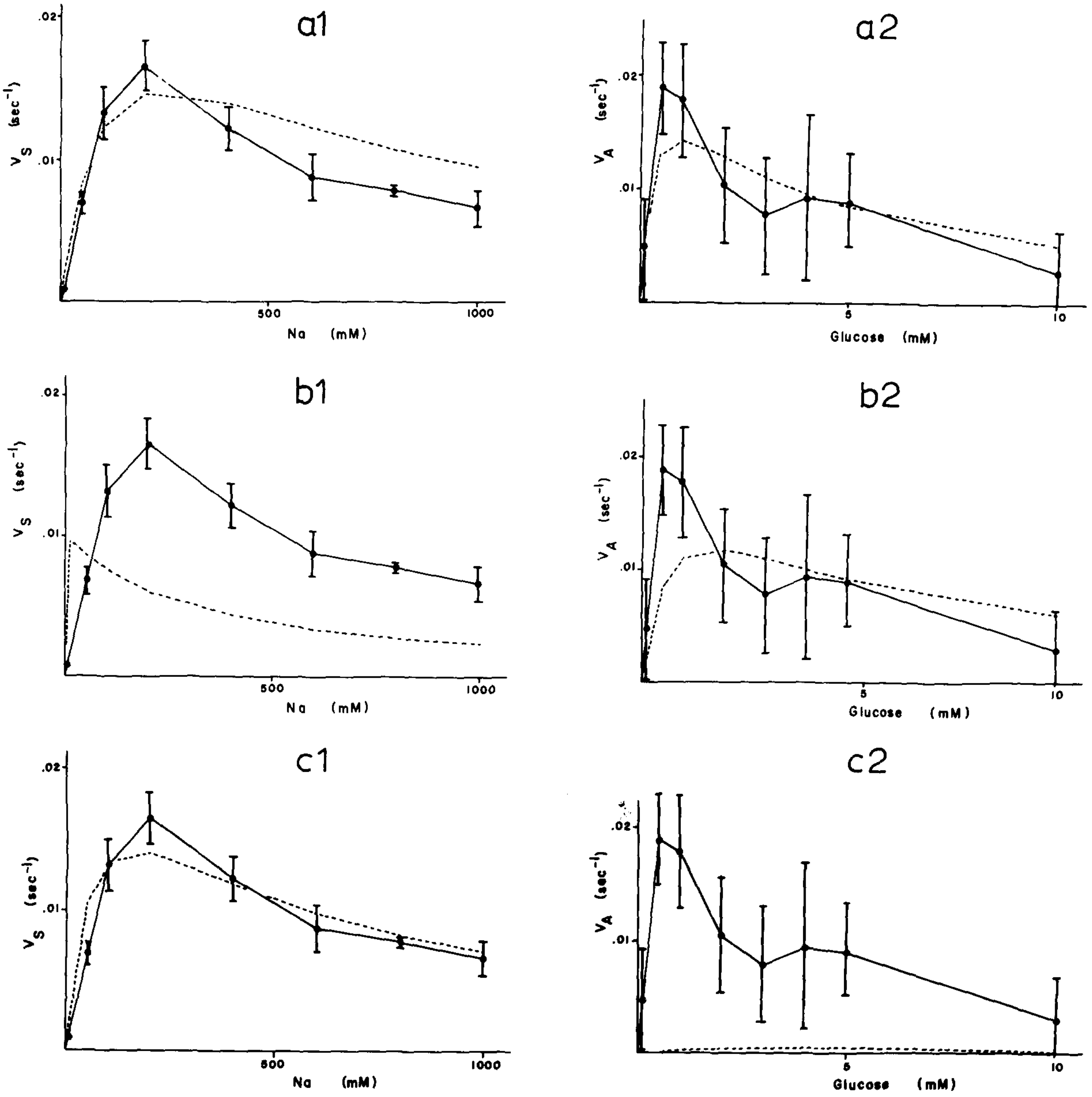
NUMERICAL SOLUTIONS TO GLIDE MODEL

Parameter	Value		
	a	b	c
Fit to	V_{S}, V_{A}	V_{A}, $K^{-1}_{m_{\mathrm{S}}}$	V_{S}, $K^{-1}_{m_{\mathrm{S}}}$
k_{S_2}	20.1	0.0518	0.0548
$k_{-\mathrm{S}_2}$	0.0498	0.691	0.171
k_{A_1}	0.847	0.159	0.0554
$k_{-\mathrm{A}_1}$	8.75	4.05	9.4
k_{AS_1}	20.1	10.5	0.616
$k_{-\mathrm{AS}_1}$	0.0498	20.1	1.39
k_{SA_1}	20.0	16.0	0.197
$k_{-\mathrm{SA}_2}$	1.79	20.1	12.2
j^{12}_{CAS}	20.1	20.1	18.2
$\frac{j^{12}_{C}}{j^{21}_{C}}$	20.1	0.243	3.32

Discussion

In this paper we have demonstrated that when compared to the Glide model the Random model will provide a visibly improved fit to the equilibrium exchange data reported by Hopfer. This improvement of fit by the Random model may not yield a statistically significant improvement over the Glide model. However, it demonstrated that the data could not distinguish between these two models. Thus one cannot claim that a contradiction exists between reported results on the order of substrate transport.

In distinguishing between Random and Glide processes potential confusion exists due partially to terminology and partially to concepts. In terminology the confusion derives from the difference between substrate entry and substrate transport. Random substrate entry means that the



initial interaction of substrates with the carrier may occur in either order. Random transport means the order in which the substrates entered the carrier has no subsequent effect on the order the substrates leave the carrier. In principle it is possible for substrates to enter a carrier randomly but leave in an order which is dependent on the order of entry. Such a case would have Random entry yet Ordered transport. Conceptually it must be remembered that a random process means that either order of entry or exit may occur but does not imply both possibilities are utilized with equal frequency. Specifically a preferred pathway, as was observed by Turner and Silverman [4], will occur. It was pointed out by Segal [15] that in a Random process the concentration of substrates will have a biasing effect on the preference of specific pathways. In essence, high concentrations of one substrate will force the reaction to proceed along the pathway in which that substrate inter-

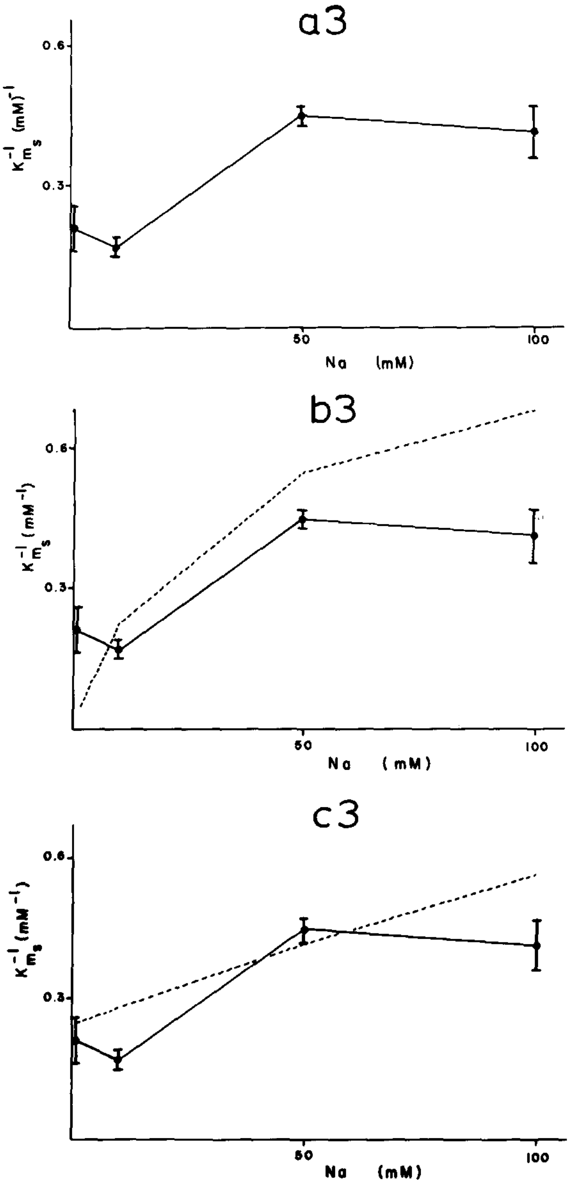


Fig. 5. Estimation of rate constants from Glide model. Figures show the fit of one set of rate constants to the data of Fig. 2a (1), Fig. 2b (2) and Fig. 4 (3). Actual values of parameters are given in Table II. (a) Fit to V_S, V_A. (b) Fit to V_A, $K^{-1}_{m_S}$. (c) Fit to V_S, $K^{-1}_{m_S}$. Experimental data is shown by the solid line, theoretical fit by the broken line. In (a) the theoretical fit to $K^{-1}_{m_S}$ is off scale; theoretical values exceed experimental by more than a factor of 20000.

acts first.

In this study we have also attempted to estimate the relative magnitude of individual steps in a Glide model consistent with experimental measurements of V_S as a function of [A], V_A as a function of [S], and $K^{-1}_{m_S}$ as a function of [A]. No such single solution was obtained although parameter sets were found to model any pair of experiments.

Does this mean that the Glide model should be rejected? We do not believe this is the case. The inability to fit all experimental data with one solution to the Glide model may be entirely due to a temperature effect in the experimental results. Experimental values for V_S and $K^{-1}_{m_S}$ were obtained at 15 °C. Thus the set of rate constants in our models which best fit the V_S and $K^{-1}_{m_S}$ data will reflect these particular operating temperatures. The only data available for V_A were obtained at 25 °C. Therefore we are forced to ask how well the model parameters specified by the 15 °C conditions are able to predict the transport rate of activator at 25 °C. Perhaps not surprisingly it turns out that the Glide model under these conditions predicts a very low (but finite) transport of substrate A (too small to be detectable above background). However, this model prediction may not be so unrealistic because at lower temperatures carrier-dependent transport of Na^+ might be significantly reduced in comparison to the leak rate. Further experimental analysis will be necessary to check this prediction. If the temperature discrepancy is not the reason for our inability to find a single solution to the experimental data then the lack of this solution will serve as a suitable rejection criterion for the Glide model.

Parameterization is a necessary goal in kinetic modeling since it allows efficient exploration of biologically significant effects of the transport mechanism, including temperature, membrane potential and protein-lipid interactions. In this paper, aided by computer simulation methods, we have focussed on another valuable use of parameterization. Specifically by considering the ability to find a single numerical solution to a kinetic model which can separately fit a number of experimental observations we have demonstrated that it is possible to obtain rejection criteria for certain kinetic models of the Na^+-D-glucose symporter. This general strategy should be readily applicable to other cotransport systems.

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