Journal of Supramolecular Structure 7:1–13 (1977)
Molecular Aspects of Membrane Transport 365–377

Kinetics of Na^+-Dependent D-Glucose Transport

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The kinetic parameters of the Na^+-dependent glucose transport system have been determined in isolated membrane vesicles for D-glucose, Na^+, and phlorhizin. The D-glucose flux measurements were carried out by the equilibrium exchange procedure at constant external and internal Na^+ concentrations and zero potential. Equations were developed to extract information about K_m and V_{max} from uptake measurements into a vesicle population that is heterogeneous with respect to size (surface to volume ratio). The K_m for D-glucose was 14 mM and independent of the Na^+-concentration, while the V_{max} was strongly Na^+-dependent and increased 15-fold between 1 and 100 mM Na^+. The K_m of Na^+ for activation of the V_{max} was 18 mM. The calculated K_I values for phlorhizin were 2.7 and 1.9 μM when determined under active and equilibrating D-glucose flux conditions, respectively.

Key words: microvillus membranes, small intestine, phlorhizin inhibition

In recent years highly purified preparations of plasma membranes have been used to investigate the mechanism of active sugar and amino acid transport. These studies have provided evidence for the existence of transport systems that catalyze coupled flow of the nonelectrolytes with Na^+. This cotransport is electrogenic, i.e., the coupled translocation of nonelectrolyte and Na^+ across the membrane is associated with the net transfer of a positive charge. In terms of mechanism, the transport reaction can be characterized as a coupled "facilitated diffusion" of 2 substrates. Because of molecular coupling the flow of Na^+ down its electrochemical gradient can support nonelectrolyte uptake by cells or isolated membrane vesicles against a gradient, thus accomplishing active nonelectrolyte transport (for reviews see Refs. 1–5). Active nonelectrolyte transport in isolated brush border membrane vesicles is consistent with the studies in intact epithelial sheets which, in addition, have provided the quantitative information of a 1:1 stoichiometry of Na^+ and nonelectrolyte (6,7).

Although the overall transport reaction of Na^+-dependent nonelectrolyte transport is now reasonably well understood, contradicting results with respect to the relevant kinetic parameters have been reported. For example, in some studies the V_{max}, but not the K_m,

Received May 25, 1977; accepted June 7, 1977

of the Na^+-dependent glucose transport system changes with Na^+ concentration (6–10) while in others the reverse is observed (11–13). In addition, the values for the K_m of D-glucose range between 80 μM and 10 mM (6–15), an unsatisfactorily high spread which has yet to be explained. Since the kinetic parameters are calculated from measurements of solute fluxes that are functions of the transport system as well as of transmembrane forces, the calculated values may not truely reflect the properties of the carrier in all studies.

It is the purpose of this paper to analyze the kinetics of the Na^+-dependent glucose transport system in isolated brush border membrane vesicles under defined transmembrane conditions of potential and Na^+ concentrations so that the K_m and V_{max} are functions only of the transport system.

MODEL AND EXPERIMENTAL DESIGN

Figure 1 shows a simple carrier model for Na^+-dependent D-glucose transport. Nonelectrolyte substrate translocation is accomplished either with or without Na^+ by formation of binary or ternary complexes, respectively, with the carrier. This mechanism of cotransport implies that the rate of D-glucose translocation is not only dependent on its own concentration, but also on the Na^+ concentration at both membrane faces and the electrical potential difference. The mutual dependence of D-glucose and Na^+ fluxes imposes certain restrictions on the experimental conditions by which the kinetic parameters can be determined. Defined K_m and V_{max} values for one substrate can only be obtained when during flux measurements all factors besides the concentration of the particular sub-

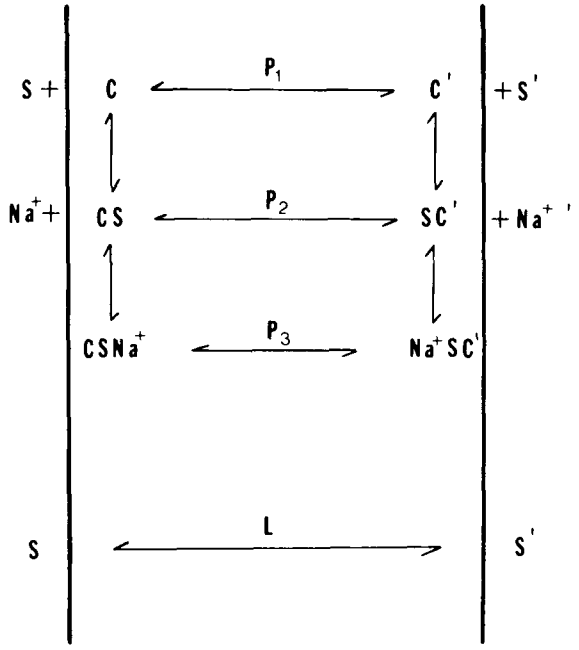

Fig. 1. Model for carrier-mediated and diffusional transport of D-glucose across brush border membrane. C) Carrier, S) nonelectrolyte substrate (D-glucose), P) apparent permeabilities of the various forms of the carrier, L) permeability coefficient of a "leak" pathway.

strate under investigation remain constant within the experimental time span and over the range of substrate concentration. Thus, when measuring the rate of D-glucose transport as a function of its concentration to determine K_m and V_{max}, the Na^+ concentration gradient across the membrane and the electrical potential difference should be kept constant. However, an experimental difficulty arises from the D-glucose flux since it is coupled to Na^+. The resulting Na^+ flux, in turn, changes the electrochemical Na^+ gradient across the membrane unless balanced by another Na^+ flux in the opposite direction via a glucose carrier-independent pathway in the membrane.

The experimental methods of maintaining a constant electrochemical Na^+ gradient across isolated membrane vesicles in spite of changing D-glucose fluxes have not been worked out. Therefore, I have used the equilibrium exchange procedure to circumvent the problem of changing electrochemical Na^+ gradient. In this method the substrate concentration is the same at the 2 membrane faces and, hence, there is no net flux. The unidirectional substrate transport is determined after addition of tracer amounts of labeled substrate. K_m and V_{max} are determined from the rate of tracer flux as a function of total substrate concentration (16, 17). The experimental conditions can be further simplified by equal Na^+ concentrations on both sides of the membrane and an electrical potential of zero. This condition is easily met in isolated vesicles by sufficiently long preincubations with unlabeled D-glucose and permeant Na^+ salts.

THEORETICAL CONSIDERATIONS

In a homogeneous cell or membrane vesicle preparation the uptake of trace amounts of labeled nonelectrolyte under equilibrium exchange conditions is described by a first order rate process which obeys the relationship (17)

$$\ln(1 - a/A) = -Vt/(S + K) \tag{1}$$

whereby a = tracer uptake at time t, A = tracer uptake at equilibrium, S = unlabeled, preequilibrated nonelectrolyte substrate concentration, $K = K_m$ = substrate concentration at half-maximal velocity, and $V = V_{max}$ = maximal velocity.

However, as shown in Figs. 2A and B, uptake of labeled D-glucose by isolated intestinal brush border membranes under these conditions is not a first-order process. An explanation for the absence of a good fit to equation 1 is heterogeneity of vesicle size (see e.g., Ref. 5) and, thereby, the surface to volume ratio, which is determinant for the rate of isotope equilibration.

Equation 1 is still useful for an analysis of the kinetics if it is assumed that the tracer uptake into an individual vesicle is described by a first-order rate process, i.e., if

$$a_i = A_i - A_i \exp[-V_i t/(S + K)]; \tag{2}$$

with i referring to an individual vesicle. The microscopic uptake into an individual vesicle is related to the macroscopically observed uptake of the entire population by the appropriate summation function

$$a = A - \Sigma A_i \exp\,[-V_i t/(S + K)], \text{ or} \tag{3}$$

$$\ln(1 - a/A) = \ln\Sigma(A_i/A) \exp\,[-V_i t/(S + K)]. \tag{4}$$

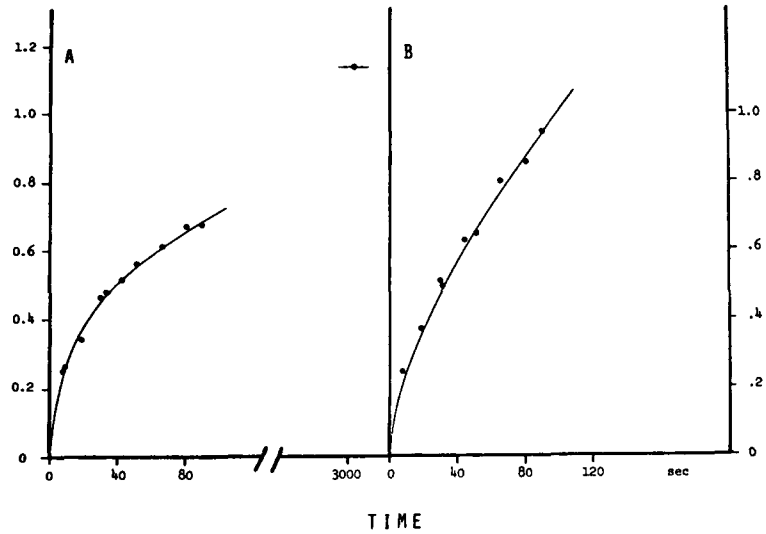

Fig. 2. Kinetics of tracer D-glucose uptake as measured with the equilibrium exchange procedure. Membrane vesicles were pre-equilibrated with 5 mM unlabeled D-glucose and 50 mM NaSCN for 1 h before labeled D-glucose was added. A) Uptake as a function of time (units of ordinate: nmole × 0.2/mg protein). B) Replot of the data from A in terms of fractional uptake with respect to equilibrium [units of ordinate: $-\ln(1 - a/A)$].

Equation 4 can be simplified if it is assumed that V_i per unit vesicle has a distribution around a macroscopic V such that $V_i = V + \Delta V_i$. Substitution of V_i simplifies equation 4 to

$$\ln(1 - a/A) = -Vt/(S + K) + \ln\Sigma(A_i/A)\exp[-\Delta V_i t/(S + K)] \qquad (5)$$

Since $t/(S + K) = -[\ln(1 - a_i/A_i)]/V_i$ (from equation 2) equation 5 can be reduced to

$$\ln(1 - a/A) = -Vt/(S + K) + \ln \Sigma i, \qquad (6)$$

$$\text{whereby} \quad \Sigma i = \Sigma(A_i/A)\,(1 - a_i/A_i)^{\Delta V_i/V_i} .$$

The summation term on the right of equation 6 is a correction factor to account for the heterogeneity of the vesicle population. It is important to note that this term is independent of unlabeled substrate concentration S. In addition, for a given membrane preparation this summation term should be constant if the macroscopic fractional uptake a/A, and thereby a_i/A_i, is kept constant.

Thus, regardless of the time course of the uptake, measurements of the times needed to give the same fractional uptake of the tracer at varying unlabeled, preequilibrated substrate concentration provide the data for calculations of the K_m and a relative V_{max}. For a/A = ½ equation 6 yields (with rearrangements):

$$t_{½} = [(S + K)/V]\ [\ln(2\Sigma i)]. \qquad (7)$$

Hence, a plot of $t_{½}$ vs S should yield a straight line whose intercept on the abscissa corresponds to $-K_m$. For V_{max} the following relationship holds:

$$V_{max} = [K/t_{½(S=0)}]\ \ln(2\Sigma i). \qquad (8)$$

It is obvious from equation 8 that V_{max} cannot be determined exactly in the absence of an evaluation of the summation term. However, as will be demonstrated in the Results section, even a relative V_{max} provides information about properties of the carrier.

Analogous equations can be derived for inhibitors (for derivations of the corresponding flux equations into a homogeneous vesicle population see Ref. 17). The equation relating $t_{1/2}$ to inhibitor concentration reads

$$t_{1/2} = [(K_I + I)/V]\ [K/K_I]\ \ln(2\Sigma i), \tag{9}$$

where K_I is the inhibitor concentration exerting half-maximal inhibition. Thus the K_I can be determined from a plot of $t_{1/2}$ vs inhibitor concentration. The experimental requirements for equation 9 to be valid are the absence of an inhibitor flux during the time span of substrate uptake and a substrate concentration well below its K_m.

Equations 7–9 are adequate to analyze transport at low D-glucose concentrations. However, it was found that the observed $t_{1/2}$ became independent of substrate concentration above ~ 50 mM suggesting the existence of another route of translocation for D-glucose across the brush border membrane. For purposes of convenience this pathway will be termed "leak" although it is realized that it may constitute another sugar transport system with a high K_m and low V_{max}. Since the Na^+-dependent uptake is saturated with unlabeled D-glucose at high concentrations, tracer D-glucose uptake proceeds predominantly through the high K_m system under these conditions.

With a second "leak" pathway present, the rate equations have to be modified slightly. Assuming that the rate of tracer uptake through the "leak" is proportional to the difference in tracer concentration and the permeability coefficient (L), the tracer uptake under equilibrium exchange conditions is described by (see also Fig. 1 for the model)

$$a_i = A_i - A_i \exp[-V_i t/(S + K) - L_i t]. \tag{10}$$

This equation can be related to the macroscopically observed uptake and rearranged in a similar manner as equation 2. An additional assumption must be made with respect to L_i, namely that L_i has a distribution around a macroscopic coefficient L. The rearranged equation reads:

$$\ln(1 - a/A) = -[Vt/(S + K)] - [Lt] + \ln\Sigma(A_i/A)\,(1 - a_i/A_i)^{\Delta V_i/V_i} \exp[t(L - L_i V/V_i)]. \tag{11}$$

Equation 11 suggests a procedure by which the carrier-related $t_{1/2}$ can be obtained. If V_i and L_i depend in the same way on vesicle parameters, e.g., surface area, then $(L - VL_i/V_i) = 0$ and equation 11 is reduced to a function in which the summation term is again independent of substrate concentration S. Any error due to the assumption of zero is likely to be small as $\exp[t(L - VL_i/V_i)]$ changes only slowly if $|t(L - VL_i/V_i)|$ remains small (e.g. $\leqslant 0.1$). Setting again $a/A = \frac{1}{2}$ the modified equation 11 can be rearranged to read:

$$(t_{1/2})^{-1} = V/[(S + K)\ln(2\Sigma i)] + L/\ln(2\Sigma i). \tag{12}$$

The term on the left is the observed $(t_{1/2})^{-1}$. The first term on the right corresponds to the carrier-related $(t_{1/2})^{-1}$ as given by equation 7 while the second can be defined as the $(t_{1/2})^{-1}$ due to the "leak." Since the "leak"-related $t_{1/2}$ can be estimated from the observed $t_{1/2}$ at a high substrate concentration, the carrier-related $t_{1/2}$ can be calculated and plotted as a function of substrate concentration according to equation 7 to yield the kinetic parameters of the relevant transport system. Equation 12 can be rewritten as

$$(t_{1/2})^{-1}_{\text{observed}} = (t_{1/2})^{-1}_{\text{carrier}} + (t_{1/2})^{-1}_{\text{"leak"}}. \tag{13}$$

EXPERIMENTAL METHODS

Brush border membranes were isolated from intestinal scrapings of Sprague-Dawley rats as described previously (18). The efficiency of the cell fractionation procedure was routinely monitored using sucrase. The enrichment of sucrase in the membrane was on the average 22-fold over the starting homogenate. The final membrane was suspended in a buffer consisting of 0.1 M D-mannitol, 1 mM Tris-Hepes buffer, pH 7.5, and 0.1 mM $MgSO_4$. Measurements of D-glucose uptake were made, after appropriate preincubations, by incubation of the isolated membranes with D-[1-^{3}H(N)]glucose, withdrawal of aliquots after predetermined periods, quenching of the transport by dilution with ice-cold buffer, and collection of the membranes on a filter. Details of the method have been given before (18). To improve the precision of time measurements a microswitch was activated at the time of the mixing of membranes with labeled D-glucose and of the quenching of the aliquots. The signals from the microswitch were recorded continuously with a strip-chart recorder.

Except where noted otherwise, experiments were carried out at 15°C in a constant temperature room. When labeled D-glucose was to be measured under equilibrating conditions, i.e., in the absence of a NaSCN gradient, membranes were preincubated with the desired concentration of unlabeled D-glucose and NaSCN as well as monactin (10 μg/ml) for ~ 1 h at room temperature. Samples were then cooled down to 15°C and uptake measurements started by addition of a small volume of labeled D-glucose to the membranes. Sufficient amounts of unlabeled D-glucose and NaSCN had been added to the labeled D-glucose so that no change in medium concentration of either D-glucose or NaSCN occurred upon addition of the tracer. Phlorhizin, when present, was added to the membranes immediately before labeled D-glucose.

$t_{1/2}$ (or occasionally $t_{0.33}$ or $t_{0.4}$) values were estimated from plots of uptake vs time as shown in Fig. 2B. Each curve had 6 time points (from 3 parallel incubations) around the actual $t_{1/2}$ value. The carrier-related $t_{1/2}$ was calculated from equation 13 whereby the "leak"-related $t_{1/2}$ was the value that would give the "best fit" (see below) to the experimental data. The "leak"-related $t_{1/2}$ was equal to or greater than $t_{1/2}$ observed at 100 mM D-glucose or 100 μM phlorhizin, respectively. K_m and relative V_{max} values were calculated using equation 7 and a weighted least-squares analysis according to the guidelines of Cleland (19). The standard deviation of the observed $t_{1/2}$ values was estimated to be proportional to the absolute value of $t_{1/2}$, i.e., $SD(t_{1/2}) \propto t_{1/2}$. The "leak"-related $t_{1/2}$ giving the best fit was found through an iterative procedure in which the "leak"-related $t_{1/2}$ was systematically increased, starting with $t_{1/2}$ observed at 100 mM D-glucose, until the correlation coefficient of the linear function of carrier-$t_{1/2}$ on substrate concentration gave a maximum. Unless otherwise indicated, data are reported as mean ± standard deviation and regression lines are calculated by the weighted least-squares method. All calculations were carried out with a programmable desk calculator (Monroe 1860).

MATERIALS

Phlorhizin was obtained from ICN-Life Science Group, (Cleveland, Ohio) and recrystallized twice from hot water before use. D-[1-^{3}H(N)]glucose was bought from New England Nuclear Corporation (Boston, Massachusetts), and D-[U-^{14}C]glucose was obtained from Schwarz-Mann (Orangeburg, New York). Monactin was a gift of Ciba-Geigy, Basel, Switzerland. Other materials were obtained from common commercial sources.

RESULTS

D-Glucose transport into brush border membrane vesicles is much slower in the absence of a Na^+ gradient than in its presence (20). Therefore, the initial experiments were designed to determine whether the observed D-glucose uptake proceeds via the Na^+-dependent glucose transport system under both experimental conditions. Phlorhizin is a relatively specific inhibitor of this system and consequently should be effective whether D-glucose uptake is actively driven by an electrochemical Na^+ gradient or is simply equilibrating. To quantitate the effectiveness of phlorhizin its K_I was determined under conditions of 1) active D-glucose transport (in the presence of a NaSCN gradient; Fig. 3), and 2) equilibrating D-glucose transport (in the presence of NaSCN, but absence of a gradient; Fig. 4).

Figure 3 shows the phlorhizin inhibition of active D-glucose uptake by membrane vesicles measured after 12 sec of incubation. D-Glucose accumulation in the vesicles in the absence of phlorhizin was 30-fold above medium concentration. The apparent K_I of 2.7 ± 0.2 μM has to be considered an upper limit of the true value for several reasons: 1) The measured uptake at 12 sec is not an initial rate. 2) Increasing phlorhizin concentrations reduced the D-glucose flux, and thereby also the Na^+ flux, so that the dissipation of the electrochemical Na^+ gradient would be retarded. Consequently, D-glucose uptake at higher phlorhizin concentrations may have been driven by higher forces. 3) The observed uptake was not corrected for "leakage" of accumulated D-glucose from the vesicles. This loss should be highest in vesicles with the highest internal D-glucose concentration, i.e., in

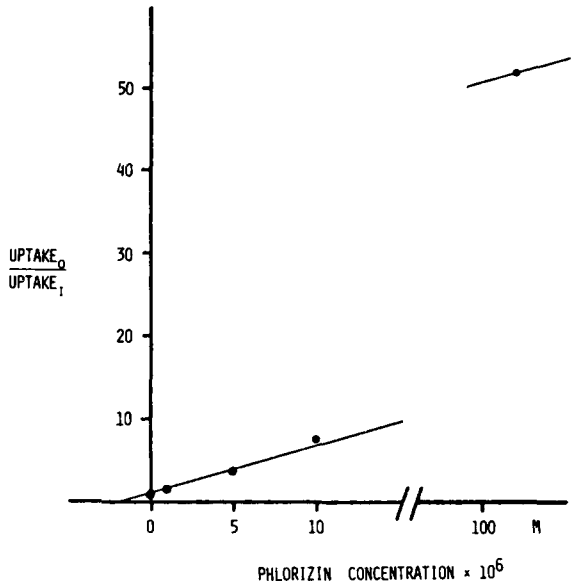

Fig. 3. Inhibition of active D-glucose transport by phlorhizin. The ordinate indicates the ratio of D-glucose uptake in absence (uptake$_0$) and presence of phlorhizin (uptake$_I$). D-Glucose uptake by brush border membranes was measured in the presence of an initial NaSCN gradient (medium: 100 mM; within vesicles: 0 mM). D-glucose concentration: 10 μM. Length of incubation: 12 sec. Temperature: 25°C. The line is calculated by linear regression assuming constant variance.

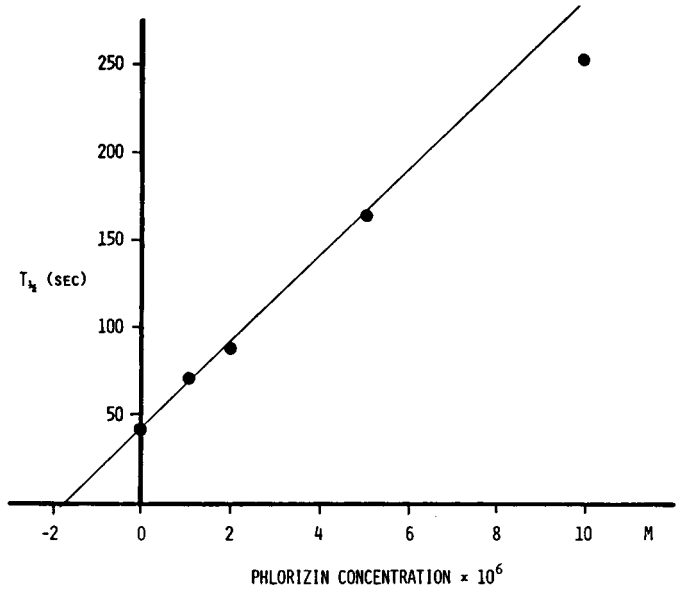

Fig. 4. Inhibition equilibrating D-glucose uptake by phlorhizin. D-Glucose uptake was measured in the presence of 100 mM NaSCN on both sides of the membrane, i.e., in the absence of a salt gradient Aliquots of the membranes were preincubated with 100 mM NaSCN, an appropriate amount of phlorhizin added, and D-glucose (10 μM) uptake measured. "Leak"-related $t_{1/2}$ used for correction was 127 sec.

vesicles exposed to the lowest phlorhizin concentrations. In other words, the rate of D-glucose transport per unit driving force may be underestimated in the absence of phlorhizin and overestimated in the presence of phlorhizin, resulting in an overestimate of the K_I.

Figure 4 demonstrates the effectiveness of phlorhizin in inhibiting equilibrating D-glucose uptake, i.e., uptake in the absence of a Na^+ gradient. The K_I under these conditions was determined to be 1.9 ± 0.2 μM which is in excellent aggreement with the above value of 2.7 μM and also those measured in intact epithelia (21). The similarity of the K_I values of phlorhizin, determined from D-glucose flux measurements under active and equilibrating transport conditions, strongly suggests that the translocation route for D-glucose is not dependent on the presence of a Na^+ gradient. This result, therefore, justifies the procedure to be employed in subsequent experiments for K_m and V_{max} determinations of D-glucose which rely on equilibrating tracer uptake and on measurements of the times necessary to achieve constant fractional uptake rather than initial rates.

Figures 5–8 summarize the information that has been obtained about the kinetic parameters of the Na^+-dependent glucose transport system using the equilibrium exchange procedure.[1] Figure 5 shows the carrier-related $t_{1/2}$ as a function of unlabeled D-glucose at

[1]Preliminary analyses of the data were presented at the ICN-UCLA symposium on "Molecular aspects of membrane transport," Keystone, Colorado, March 13–18, 1977 and the American Physiological Society Symposium on "Models for GI transport," FASEB Meetings, Chicago, Illinois, April 4, 1977. Initial calculations had been carried out by the least-squares method without weighing factors. K_m values calculated by this latter method are slightly lower. For example, at 100 mM NaSCN the K_m for D-glucose is 12.2 mM using unweighted least-squares analysis and 14.2 mM with weighing factors. The general conclusions about the effect of Na^+ on K_m and V_{max} are not influenced by the choice of statistical method.

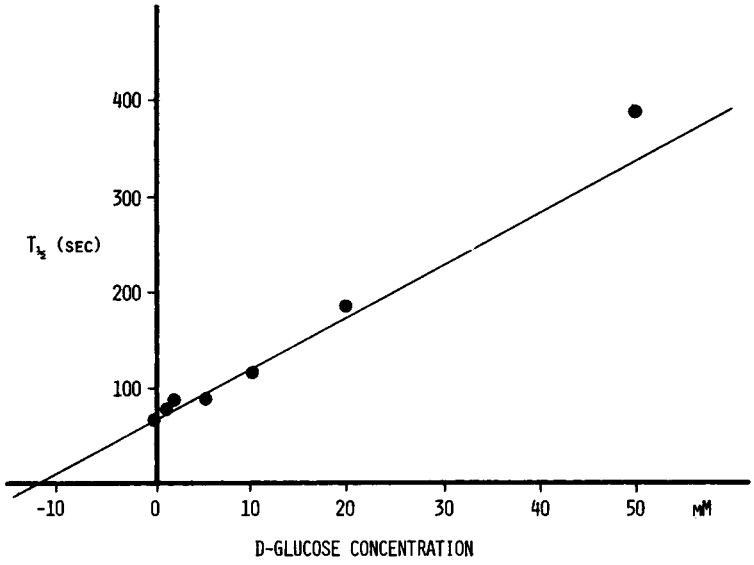

Fig. 5. Kinetics of D-glucose transport by brush border membranes as measured by the equilibrium exchange procedure. Aliquots of the membrane were preincubated with various levels of unlabeled D-glucose and 100 mM NaSCN for 1 h before addition of labeled D-glucose and 100 mM NaSCN for 1 h before addition of labeled D-glucose. "Leak"-related $t_{1/2}$ used for correction was 230 sec.

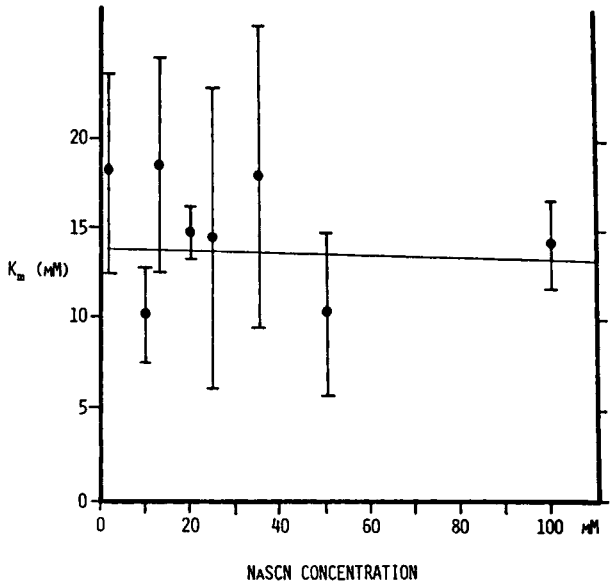

Fig. 6. Na^+ dependence of the K_m for D-glucose. Individual K_m values with standard deviation were obtained from experiments as in Fig. 5 at the various Na^+ concentrations. Bars represent 4 standard deviations. The line was calculated by weighted linear regression.

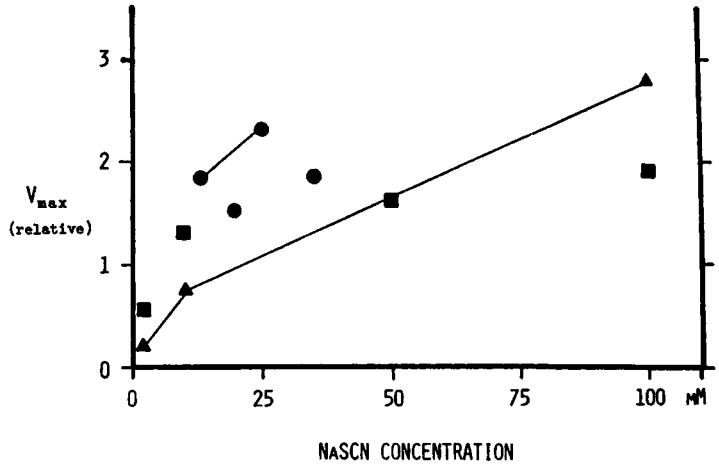

Fig. 7. Na^+ dependence of the transport rate for D-glucose. Relative V_{max} values $[K/t_{½(S=0)}] \times 10^1$ were obtained from experiments as in Fig. 5. Different symbols represent separate batches of animals. The points connected by a line were obtained with the same membrane preparation.

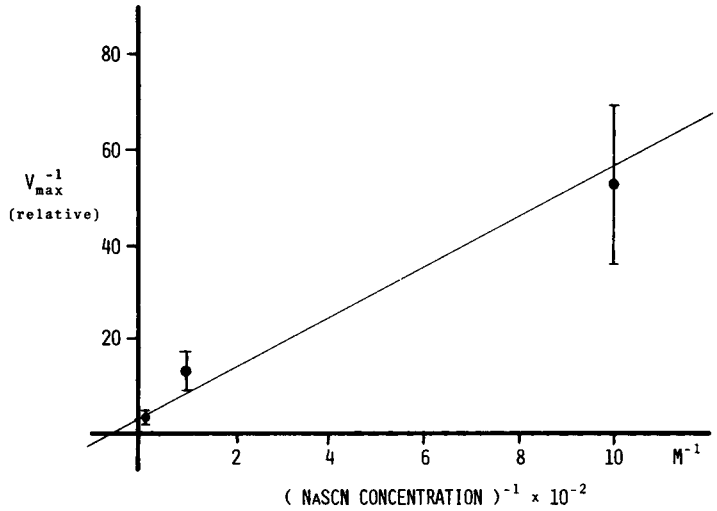

Fig. 8. Lineweaver-Burke plot of the Na^+ dependence of the transport rate of D-glucose. Data are from a single membrane preparation (replot of data from Fig. 7). Bars represent 4 standard deviations. The line was calculated by weighted linear regression.

a NaSCN concentration of 100 mM for a single experiment. The average K_m value for D-glucose at 100 mM NaSCN was 14.2 ± 1.3 mM.

To obtain some insight into the mechanism of Na^+ stimulation of D-glucose transport, K_m and relative V_{max} values were determined at several Na^+ concentrations. The effect of Na^+ on K_m is summarized in Fig. 6. Within the experimentally accessible range of 1–100 mM Na^+ the K_m for D-glucose was constant. In other words, the affinity of D-glucose for the carrier does not appear to be affected by Na^+. Uptake experiments

could not be carried out at Na^+ concentrations below 1 mM because overall D-glucose transport became dominated by D-glucose influx via a Na^+-independent, apparently non-saturable route.

As shown in Fig. 7 Na^+ increases the maximal velocity of D-glucose uptake. In initial experiments only single values were determined with each membrane preparation. Because of the possibility that the relative V_{max} was dependent on membrane preparation and not on Na^+, the same membrane preparation was used for V_{max} determinations at several Na^+ concentrations at 2 occasions. These experiments (experimental points connected by lines in Fig. 7) demonstrate a significant increase of V_{max} of about 15-fold when Na^+ is raised from 1 to 100 mM.

Figure 8 shows a Lineweaver-Burke plot of one experiment in which V_{max} was determined over a sufficiently wide enough range of Na^+ concentration. The apparent K_m for Na^+ calculated from this plot is 17.9 ± 1.2 mM.

DISCUSSION

The kinetic parameters of D-glucose for the Na^+-dependent glucose transport system have been determined in many different types of epithelial preparations, including intact epithelial tissue in vivo and in vitro isolated cells and brush border membrane vesicles (6–15). The K_m of D-glucose, the K_I of phlorhizin, and the Na^+ dependence of K_m and V_{max} presumably are expressions of certain properties of this transport system. Therefore, one would expect similar K_m and K_I values and similar Na^+ effects in the various studies. The measured K_I for phlorhizin indeed is similar in the various preparations from kidney and small intestine. However, the reported data for the K_m of D-glucose vary by 2 orders of magnitude between 80 μM and 10 mM and Na^+ affects the K_m and not the V_{max} in some studies, while the opposite is found in others. A comparison of results from different laboratories would suggest that there is no correlation of the kinetic parameters of D-glucose transport with tissue (kidney and intestine) or species when analyzed by different techniques.

Part of the reason for the divergency has been pointed out in the earlier part of this paper. A systematic error is introduced into estimates of K_m and V_{max} from net D-glucose flux measurements since corresponding Na^+ fluxes are associated with increasing D-glucose fluxes. Thus, changes in D-glucose concentration influence the electrochemical Na^+ gradient across the membrane, and, therewith, its own driving force. The drop in electrical potential across the brush border membrane due to D-glucose or L-alanine absorption has actually been measured in intact cells with microelectrodes (22–24). A mathematical consequence of this change is an underestimation of the true K_m and V_{max} for a given electrochemical Na^+ gradient. Moreover, if the transport load overwhelms the energy supply, the maximal uptake rate represents a value for transport in the absence of an electrochemical Na^+ gradient. As a consequence the V_{max} becomes independent of the Na^+ concentration over a wide range and the apparent K_m becomes Na^+-dependent.

In isolated membrane vesicles salt gradients have been used to drive active non-electrolyte transport (13, 20). Swamping of this small amount of energy by high non-electrolyte concentrations may account for the observations of Na^+ independence of V_{max} and Na^+ dependence of the K_m of D-glucose and neutral amino acid transport in isolated membrane vesicles by several investigators (13, 25–28). On the other hand, the bias introduced into estimates of K_m and V_{max} of nonelectrolytes, cotransported with Na^+, should be less pronounced in intact cells in vivo where Na^+ influx at the luminal side can be com-

pensated by active Na^+ extrusion at the serosal membrane, provided these studies are not complicated by unstirred layer effects. By this reasoning transport parameters estimated from renal microperfusion studies are least likely to be subject to artifacts. In microperfusion experiments in the rat (14) a K_m of 10.8 mM was found for D-glucose, a value relatively close to 14.2 mM as reported in this paper.

The apparent K_m of D-glucose transport in vitro is much lower than in vivo. Goldner et al. reported an in vitro K_m value of 1.4 mM for rabbit ileum (6). It must represent a lower limit since the electric potential drops about 19% under maximal D-glucose influx conditions (23).

These authors also very thoroughly investigated the effect of mucosal Na^+ concentration on K_m and V_{max} of 3-O-methyl-D-glucose transport in rabbit ileum. In agreement with the results in the isolated vesicles they observed that increasing Na^+ concentrations increased the maximal velocity, but had no effects on the apparent K_m of D-glucose. Since mucosal Na^+ depletion results in a hyperpolarization across the brush border membrane (23) the total electrochemical Na^+ gradient may have been constant in the studies of Goldner et al. (6) in spite of changing Na^+ concentrations. In this case, the agreement of results with respect to the effect of Na^+ would reflect measurements of the same carrier properties.

The main purpose for the transport studies with isolated membrane vesicles was to provide a solid data base of relevant kinetic parameters. To this end, a methodology had to be developed which utilizes the information inherent in the times necessary to obtain constant fractional solute uptake. The $t_{1/2}$ used in these studies depends on uptake of the substrate into most of the vesicles, and the transport data should therefore be less biased than in the more common initial velocity measurements. Initial velocity determinations can be misleading in biological preparations (e.g., the vesicles) that are not homogeneous because the initial velocity is determined by the elements with the fastest rates. These elements may not be representative of the transport properties of the bulk of the preparation.

Using the methodology outlined in this paper, the K_m value and the Na^+ dependence of K_m and V_{max} reflect only properties of the transport system. Application of this method to comparative kinetic studies therefore should resolve the existing discrepancies with respect to the kinetic parameters in renal and intestinal brush border membranes of the various animals.

The reported experiments were not specifically designed to test a specific model of D-glucose translocation, and the ordered binding of nonelectrolyte substrate and Na^+, shown in Fig. 1, represents only 1 of the possibilities. Nevertheless, the Na^+ dependence of K_m and V_{max} can be easily interpreted within the context of that model. The increase in V_{max} with increasing Na^+ concentration can be explained if the translocation steps across the membrane are rate limiting and $P_3 \gg P_2$. In other words, the Na^+-glucose carrier complex is transported much faster across the membrane than the carrier complex without Na^+. The presence of Na^+ shifts the equilibrium between the 2 loaded states of the carrier from the binary D-glucose carrier complex to the ternary Na^+-D-glucose-carrier complex.

Two additional points are noteworthy: 1) Transport as measured by the equilibrium exchange procedure does not necessarily involve translocation of the unloaded form of the carrier across the membrane. Therefore, the K_m and V_{max} may be different for net flux conditions. Such differences have been observed, for example, for the Na^+-independent D-glucose transport in erythrocytes (29). 2) The measured effects of Na^+ in the vesicle

system do not take into account the influence of a membrane potential. In particular, it may be possible that the permeability of the Na^+-loaded carrier, if charged, is dependent on the electrical field within the membrane. Thus, the apparent permeability of the Na^+-D-glucose loaded carrier may even be greater in intact cells where a potential of 30–50 mV (inside negative) exists across the brush border membrane (22–24).

ACKNOWLEDGMENTS

I wish to thank Ms. Clara Gulyas and Mr. Rhea Groseclose for valuable help with the experimental part, and Drs. R. Przybylski, P. Will, and K. Neet for valuable comments with regard to the manuscript. The research was supported by NIH Grant AM 18265 and PHS Career Development Award 1KO4 AM00199.

REFERENCES

1. Hopfer U, Sigrist-Nelson K, Murer H. Ann NY Acad Sci 264:414, 1975.
2. Hopfer U. In Quagliariello E, Palmieri F, Singer TP (eds): "Horizons in Biochemistry and Biophysics." Reading, Massachusetts: Addison-Wesley Publishing Company 1976, vol 2, pp 106–133.
3. Silverman M: Biochim Biophys Acta 457:303, 1976.
4. Kinne R. In Bronner F, Kleinzeller A (eds): "Current Topics in Membranes and Transport." New York: Academic Press, 1976, vol 8, pp 209–267.
5. Sacktor B. In Sanadi DR (ed): "Current Topics in Bioenergetics." New York: Academic Press 1977, vol 6, pp 39–81.
6. Goldner AM, Schultz SG, Curran PF. J Gen Physiol 53:362, 1969.
7. Schultz SG, Curran PF. Physiol Rev 50:637, 1970.
8. Kleinzeller A, Kolinska J, Benes I. Biochem J 104:843, 1967.
9. Kleinzeller A, Kolinska J, Benes I. Biochem J 104:852, 1967.
10. Vogel G, Lauterbach F, Kroger W. Arch Ges Physiol 283:151, 1965.
11. Crane RK, Forstner G, Eichholz A. Biochim Biophys Acta 109:467, 1965.
12. Bihler I. Biochim Biophys Acta 183:169, 1969.
13. Aronson PS, Sacktor B. J Biol Chem 250:6032, 1975.
14. von Baeyer H, von Conta C, Haeberle D, Deetjen P: Pfluegers Arch 343:273, 1973.
15. Turner RJ, Silverman M. Fed Proc Fed Am Soc Exp Biol (Abstract) 36:1697, 1977.
16. Eilam Y, Stein WD. In Korn ED (ed): "Methods in Membrane Biology." New York: Plenum Press, 1974, vol 2, pp 283–354.
17. Kotyk A, Janacek K. "Cell Membrane Transport, Principles and Techniques." 2nd Ed. New York: Plenum Press, 1975, pp 64–97, 159–169.
18. Sigrist-Nelson K, Murer H, Hopfer U: J Biol Chem 250:5674, 1975.
19. Cleland WW: Adv Enzymol 29:1, 1967.
20. Murer H, Hopfer U. Proc Natl Acad Sci USA 71:484, 1974.
21. Diedrich DF: Arch Biochem Biophys 117:248, 1966.
22. White JF, Armstrong W McD. Am J Physiol 221:194, 1971.
23. Rose RC, Schultz SG: J Gen Physiol 57:639, 1971.
24. Okada Y, Tsuchiya W, Irimajiri A, Inouye A: J Membr Biol 31:205, 1977.
25. Hamilton TR, Nilsen-Hamilton M. Proc Natl Acad Sci USA 73:1907, 1975.
26. Fass SJ, Hammerman MR, Sacktor B: J Biol Chem 252:583, 1977.
27. Hammerman MR, Sacktor B. J Biol Chem 252:591, 1977.
28. Lever J. J Biol Chem 252:1990, 1977.
29. Kotyk A, Janacek K. In "Cell Membrane Transport, Principles and Techniques." 2nd Ed. New York: Plenum Press, 1975, pp 391–395.