Infinite-Cis Kinetics Support the Carrier Model for Erythrocyte Glucose Transport†

Thomas J. Wheeler* and Joseph D. Whelan

Department of Biochemistry, University of Louisville School of Medicine, Louisville, Kentucky 40292

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ABSTRACT: It has been claimed that the K_m for infinite-cis uptake of glucose in human erythrocytes is so low that the carrier model for transport must be rejected. We redetermined this parameter for three experimental conditions and found instead that the K_m values were in good agreement with the model. For each of a variety of cis glucose concentrations, cells were preequilibrated with various concentrations of glucose, and the apparent K_m was determined as the intracellular concentration reducing the initial rate of net uptake by half. The dependence of the apparent K_m values on the cis glucose was as predicted by the carrier model; the infinite-cis K_m was determined from both this concentration dependence and the extrapolated value at infinite cis glucose. The resulting values were 15 mM for fresh blood at 0 °C, 39 mM for outdated blood at 0 °C, and 11 mM for outdated blood at 25 °C. Previous measurements of the K_m at room temperature yielded values of 2–3 mM. These earlier studies used a time course procedure that indicated rapid changes in rates during the initial 10 s of uptake but did not directly measure such changes. We examined the uptake of 60 mM glucose at 20 °C into cells containing 0 and 5 mM glucose; rapid changes in rates were not observed in the first few seconds, and the time courses were more consistent with our higher K_m values. Our new values, together with other initial rate measurements in the literature, support the adequacy of the carrier model to account for the kinetics of glucose transport in human erythrocytes.

The glucose transporter of human erythrocytes is one of the most extensively studied of all transport proteins and is the prototype of a facilitated-diffusion transporter with a single substrate. However, its kinetics remain controversial despite many investigations [for reviews see Jung (1975), Naftalin and Holman (1977), Widdas (1980), Carruthers (1984), Wheeler and Hinkle (1985), and Stein (1986)]. Some aspects of the kinetics can be accounted for by a carrier model in which no assumptions are made concerning symmetry or equal mobility of loaded and unloaded carriers (Regen & Morgan, 1964). This model is kinetically equivalent to a model involving a conformational change that exposes a single binding site alternately to the two sides of the membrane (Vidaver, 1966).

Other aspects of the kinetics appeared inconsistent with this model, particularly the low value for the infinite-cis uptake K_m [nomenclature according to Eilam and Stein (1974)]. Experiments using outdated blood gave values of 2.8 mM (Hankin et al., 1972) and 1.8 mM (Dustin et al., 1984) at 20 and 24 °C, respectively, for this parameter, much lower than expected from the carrier model. The importance of this finding was recently emphasized by Stein (1986), who, in referring to the carrier model parameter K, wrote the folllowing: "Yet neither is the system a simple carrier, since the derived values for the parameter K (which would be a single value for the system) are very different, indeed almost 15-fold different in one case. The biggest discrepancy is between the values of K derived from the zero-trans and equilibrium-exchange experiments, on the one hand, and the infinite-cis uptake experiment on the other. Just this latter experiment, which provided the first suggestion that a carrier and not a pore is operative for the system, seems to reveal sugar binding sites so effective as to force the rejection of the simple carrier for the system." The term "simple carrier" in this discussion refers to a model in which both asymmetry and unequal mobilities of loaded and unloaded carrier are possible. A recent paper also referred to the infinite-cis uptake K_m, stating "The low $K_{m(app)}$ site at the interior of the cell remains the major anomaly in red cell sugar transport" (Helgerson & Carruthers, 1987).

Because of this and other discrepancies between the observed kinetics and the predictions of the carrier model, a number of other models have been proposed, involving multiple binding sites and allosteric effects. The most recent of these involves two binding sites for glucose and modulation of the activity by ATP, and results supporting this model have been presented (Carruthers, 1986a,b; Helgerson & Carruthers, 1987). However, as discussed previously (Wheeler, 1986), most of the recent data on the properties of the purified transporter (Sogin & Hinkle, 1980; Baldwin et al., 1982; Meuckler et al., 1985; Appleman & Lienhard, 1985; Alvarez et al., 1987), as well as binding and labeling studies using erythrocyte membranes (Gorga & Lienhard, 1981; Wang et al., 1986; Holman & Rees, 1987), support the single-site carrier model and argue against the more complex models.

Recent studies from our own (Wheeler, 1986) and other (Brahm, 1983; Lowe & Walmsley, 1986) laboratories have reevaluated some aspects of the kinetics using initial rate measurements. These studies supported the adequacy of the carrier model. They suggest that some earlier results which appeared inconsistent with the model (the high K_m for zero-trans efflux and the low K_m for infinite-trans efflux) may have been due to the use of inappropriate techniques, such as analyses of the time courses of transport rather than initial rate measurements. Using some of the more recent data, we found that nearly all of the kinetic parameters observed in the various transport experiments could be accounted for by the model, both at 0 °C and at 20–25 °C [Tables III and IV, respectively, of Wheeler (1986)].

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* Author to whom correspondence should be addressed.

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Because the reported low K_m value for infinite-cis uptake remained as the only major basis for rejecting the carrier model, we decided to reinvestigate this parameter. In the infinite-cis procedure, the net uptake of glucose is measured when there is a saturating ("infinite") concentration on the extracellular (cis) side. V_{max} is the rate of uptake when there is no intracellular (trans) glucose, while the K_m (K_{ic})[1] is the concentration of intracellular glucose reducing the rate of net uptake to half of V_{max}. We had noted (Wheeler, 1986) that it was possible that the K_{ic} obtained by analyzing the time course of glucose entry into glucose-free cells (Hankin et al., 1972) might be erroneously low if the observed transport rate decreased with time for reasons other than the accumulation of intracellular glucose in an ideal manner. Accordingly, we applied initial rate techniques to this measurement. In addition to performing the experiments at room temperature with outdated blood, as by Hankin et al. (1972) and Dustin et al. (1984), we also examined rates at 0 °C using both fresh and outdated blood, conditions under which we had earlier measured some of the other kinetic parameters (Wheeler, 1986). The time course analyses used previously (Hankin et al., 1972; Dustin et al., 1984) predicted rapid changes in rates during the first 10 s of uptake, but the studies did not actually examine this time interval. We therefore examined the early time points of uptake under similar conditions. Our results at room temperature indicate a much higher value for K_{ic} than previously reported. The new results, as well as those at 0 °C, are in good agreement with predictions from the carrier model (Wheeler, 1986) and thus support the adequacy of that model to account for the kinetics. Portions of this work have been presented in preliminary form (Wheeler & Whelan, 1987).

Experimental Procedures

Materials. Sources of blood and chemicals were as listed previously (Wheeler, 1986).

Cell Washing and Glucose Equilibration. Aliquots of cells (1 mL) were washed 4 times with 5 mL of phosphate-buffered saline [150 mM NaCl/5 mM sodium phosphate (pH 7.4)] containing the desired intracellular (trans) glucose concentration and sufficient added NaCl to make the total osmolarity equal to that of the assay solution. In each wash the cells were incubated 10 min at room temperature. Aliquots (0.5 mL) were then transferred to separate tubes and washed 3 times with 2.5 mL of radioactive glucose solutions. These were of compositions identical with those of the original washes except that the glucose was derived from the assay solution and thus was of the same specific activity of isotopic label. The incubations with radioactive glucose were for $^1/_2$ min each at room temperature. The washed cells were then either placed on ice or left at room temperature for the 0 and 25 °C experiments, respectively. We verified that this washing procedure was adequate by measuring the net uptake of glucose when the intracellular and extracellular glucose concentrations were equal; this was found to be zero, as it must be at equilibrium.

Assay Procedure. Assays were carried out as described (Wheeler, 1986), with minor modifications. The volume of cell suspension was 20 μL in most experiments, although some experiments used 10 or 30 μL. That the amount of cells used was not critical was demonstrated in two ways. In one experiment, the apparent K_m for the infinite-cis (K_{app}) uptake was not significantly different when 10, 20, or 30 μL of cells was used. In addition, the K_{app} was determined for two other conditions in pairs of experiments using different amounts of cells, and the results were in good agreement. Cells, tubes, and assay solution (1 mL per assay) were maintained at the same temperature as the assays. Stopping and washing of cells and determination of intracellular radioactivity were as described (Wheeler, 1986).

Determinations of radioactive glucose content were normalized to the amount of cells in each assay using the absorbance at 540 nm of a sample of lysed cells (Wheeler, 1986). We discovered that our earlier calculations of intracellular water using this method erred in not correcting for the fraction of total cell volume occupied by intracellular water (about 63%); thus the extents and rates of glucose efflux reported previously (Wheeler, 1986) (which were expressed per liter of cell water) should be multiplied by 1.6. When the correct factor was used, the extent of glucose uptake observed in the cells preequilibrated with glucose was in good agreement with complete equilibration of the intracellular water, as observed by Lowe and Walmsley (1986).

Experimental Design. The infinite-cis uptake K_m was determined from a series of measurements of K_{app} at various cis glucose concentrations. Each experiment consisted of determinations of the apparent initial rate of net glucose uptake at a single cis glucose concentration and several trans glucose concentrations. In most experiments, five trans concentrations were measured, zero glucose and four concentrations extending both below and above the anticipated K_{app}. In some experiments only two nonzero concentrations were tested, at about 1 and 2 times the anticipated K_{app}. Typically, the uptake at zero trans glucose, to which the other uptakes were compared, was determined by using five assays at each time point, while the other concentrations were assayed in quadruplicate. In most experiments the various glucose concentrations were tested in a random order.

The glucose content of the cells was determined both for zero time assays (stopping solution added before assay solution) and for assays in which cells were mixed with the assay solution for a period of time before stopping. Since both extracellular and intracellular glucose were at the same specific activity, the change in radioactive glucose content in the cells was a measure of the net uptake of glucose. The times were selected to give 15–25% equilibration of glucose for the cells which initially contained no glucose; the same time points were used for all trans glucose concentrations in an experiment. These ranged from 20 s (1 mM cis glucose) to 120 min (120 mM) for fresh blood at 0 °C; from 5 (2 mM) to 270 min (120 mM) for outdated blood at 0 °C; and from 3 (10 mM) to 45 s (120 mM) for outdated blood at 25 °C. We verified that the choice of time points was not critical by measuring, in two experiments, the K_{app} using time intervals of 0.5, 1.0, and 1.5 times those which normally would have been tested; the results were not significantly different.

By subtracting the counts obtained at zero time for cells without intracellular glucose from the zero time counts for the other trans glucose concentrations, the amount of glucose taken up during the washes, and thus the cell water that equilibrates with extracellular glucose, could be determined. For the cells washed without glucose, the mean of the cell water determinations for the other samples was used as an estimate of the cell water. Using the value of the cell water and the concentration of radioactivity in the assay solution, the intracellular glucose concentration at the end of each time interval could be determined.

Time Course Studies. For the experiments shown in Figures 5 and 6, outdated erythrocytes were washed as above, except

[1] Abbreviations: K_{ic}, infinite-cis uptake K_m; K_{app}, apparent value of K_{ic} measured at a given cis glucose concentration.

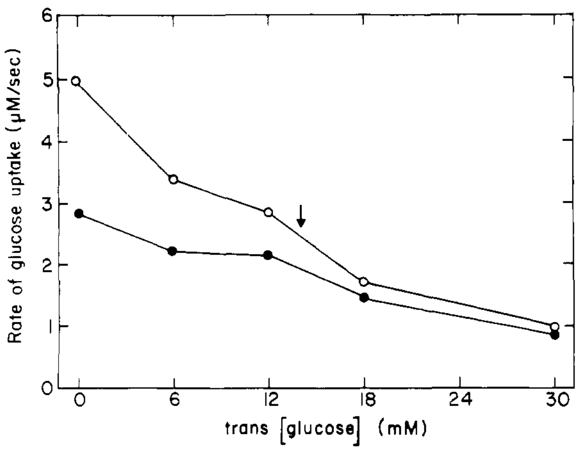


FIGURE 1: Inhibition of net glucose uptake by intracellular glucose. Fresh human erythrocytes were preequilibrated with radioactive glucose at the concentrations shown on the x axis. The net uptake of 90 mM radioactive glucose at the same specific activity was then measured at 0 °C. The apparent initial rates of uptake were determined from the difference in radioactivity between four or five assays each at 0 and 90 min for each intracellular (trans) glucose concentration (●). The corrected initial rates (○), which take into account the decrease of the uptake rates with time, were calculated by using the carrier model as described in the text and illustrated in Figure 2. The arrow indicates the trans glucose concentration at which the corrected net uptake rate was reduced to half the value observed in the absence of intracellular glucose. This concentration (14.0 ± 3.2 mM) was the apparent infinite-cis uptake K_m.

that the washing medium contained phosphate-buffered saline with no added NaCl. Assays of the uptake of 60 mM glucose were carried out at 20 °C according to the procedure described above, with five determinations at each time point. Intracellular glucose concentrations were determined by comparing the uptakes to assays that were allowed to proceed for 60 min (Hankin et al., 1972).

RESULTS

Experimental Plan. The infinite-cis uptake procedure measures the net uptake of glucose when a saturating ("infinite") concentration of glucose is present outside the cells. Since no concentration is infinite, we considered how the choice of extracellular glucose concentration tested would affect the apparent K_{ic} (Appendix). Our experiments were designed to test the carrier model, which can account for most other kinetic features of glucose transport in human erythrocytes; therefore, it was appropriate to use the model in the analysis. It was found by using values of the model parameters estimated previously (Wheeler, 1986)[2] that K_{app} is approximated by a saturable function of the cis glucose concentration, reaching half the value of K_{ic} when the cis glucose is twice K_{ic} (Appendix, eq 6). Therefore, we measured the K_{app} at various cis glucose concentrations, allowing an extrapolation of the K_{app} values to infinite glucose. Moreover, the observed dependence of K_{app} on the cis glucose concentration compared to the predictions of eq 6 would provide a further test of the model. Since we felt use of time course data to be unreliable, due to possible nonideal behavior (Wheeler, 1986), we determined the K_{app} values using initial rate measurements.

Illustration of the Method. The procedure employed to measure K_{app} is illustrated in Figure 1. In this experiment,

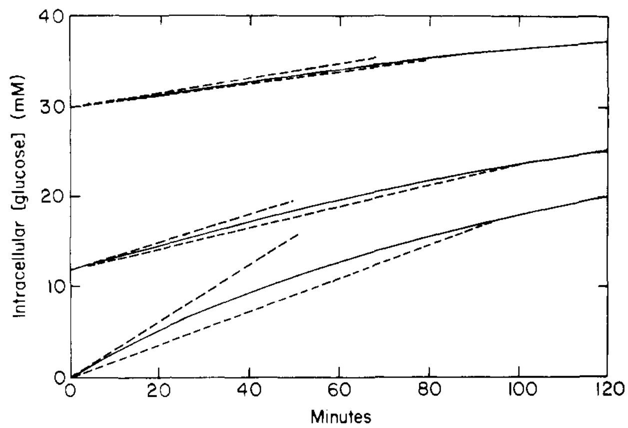


FIGURE 2: Method of correction of apparent initial rates. The figure illustrates the application of the method to three of the conditions in the experiment shown in Figure 1. The solid curves show the simulated time courses, while the broken lines above and below each time course indicate the true initial rate and the apparent initial rate, respectively. The ratio of these yields the correction factor. Details are presented in the text.

the net uptake of glucose into fresh erythrocytes was measured at 0 °C. The extracellular glucose was 90 mM, while the intracellular glucose at the beginning of the assays ranged from 0 to 30 mM. Our experiments were designed to give levels of uptake that were sufficiently high to be measured accurately; in this case, the uptake was measured by using a time interval of 90 min, which resulted in about 18% equilibration for the cells which initially contained no glucose. Thus for these cells the intracellular concentration rose to about 16 mM. At the highest trans glucose concentration tested (30 mM), the intracellular glucose rose to about 35 mM during the 90-min period.

The lower curve in Figure 1 (solid circles) plots the apparent initial rate of uptake at each trans glucose. This apparent rate is progressively reduced by increasing levels of trans glucose. However, at such a high level of extracellular glucose, the rate of net uptake will decrease significantly during the time interval due to the efflux of the accumulating intracellular glucose. Thus, the apparent initial rates are somewhat lower than the true initial rates.

Correction of Apparent Initial Rates. In order to correct the apparent initial rates, an equation for the net uptake of glucose according to the carrier model (eq 2, Appendix) was used to simulate the time course of glucose uptake for each condition. The model parameters used in the simulations were those estimated previously (Wheeler, 1986).[2] These were based on initial rate measurements in the literature, which did not include infinite-cis uptake experiments. Thus, the corrections were independent of the parameters being measured in this study. Since the cell washing solutions were formulated to balance the intra- and extracellular osmolarity at the beginning of each assay, the only movement of water should be that which accompanies the uptake of glucose. The concentration of intracellular glucose used in eq 2 was calculated by taking this water movement into account. The largest calculated cell volume changes during the assays were about 4%. Calculations performed without correcting for the water movement yielded, in most experiments, virtually identical K_{app} values as those including the corrections.

The method used to correct the apparent initial rates is illustrated in Figure 2 for three of the measurements of Figure 1. The solid curves are the simulated time courses of uptake of 90 mM glucose into cells initially containing 0, 12, and 30 mM glucose. For each of these, the broken line below the

[2] The parameters for the simulations and the derivation in the Appendix were as follows: for fresh erythrocytes at 0 °C, R_{12} = 185, R_{21} = 8.52, R_{ee} = 2.28, R_{00} = 191, and K = 0.168; for outdated erythrocytes at 0 °C, R_{12} = 250, R_{21} = 25, R_{ee} = 2.64, R_{00} = 272, and K = 0.28; for 25 °C, R_{12} = 1.67, R_{21} = 0.50, R_{ee} = 0.19, R_{00} = 1.97, and K = 0.99. Units of R were s·mmol^{-1}·L; those of K were mM.

Table I: Kinetic Parameters for Infinite-Cis Glucose Uptake in Human Erythrocytes

blood	temperature (°C)	V_{max}[a] ($mmol \cdot L^{-1} \cdot s^{-1}$)	A[b] (mM)	B[b] (mM)	slope[c]	K_{ic}[d] (mM)
fresh	0	0.0051 ± 0.0013	15.4 ± 1.3	27.5 ± 5.0	1.79 ± 0.36	14.6 ± 1.9
outdated	0	0.0020 ± 0.0005	33.2 ± 5.1	89.6 ± 19.5	2.70 ± 0.72	39.0 ± 7.4
outdated	25	0.99 ± 0.19	10.8 ± 1.4	23.4 ± 8.4	2.17 ± 0.83	11.2 ± 2.8

[a] V_{max} values are the means (±SD) of the corrected initial rates of uptake obtained at various cis glucose concentrations in the absence of intracellular glucose. [b] K_{app} values were fitted to the equation $K_{app} = A[G_{cis}]/([G_{cis}] + B)$ by using a nonlinear regression program (Duggleby, 1981) to give the indicated values (±SD) of A and B. [c] The slope of a double-reciprocal plot ($1/K_{app}$ vs $1/[G_{cis}]$) is B/A. [d] K_{ic} was determined from the average of A and $B/2$.

curve connects the initial to the final concentrations observed experimentally (16.5, 24.1, and 35.0 mM, for initial concentrations of 0, 12, and 30 mM, respectively). The slopes of these lines are the apparent initial rates (3.06, 1.87, and 1.03 μM/s, respectively) of the simulations. The upper broken line for each simulation indicates the true initial rate (5.40, 2.49, and 1.13 μM/s, respectively). Dividing the true initial rate by the apparent initial rate gives the correction factor (1.76, 1.33, and 1.11, respectively) for each condition. These factors were used to correct the experimental apparent initial rates in Figure 1, giving the upper curve (open circles).

As this example shows, the correction factors were largest for the lowest trans glucose concentration at any given cis concentration. In addition, the factors were larger when higher cis concentrations were used, because of the relatively long time intervals tested. Thus, most correction factors were lower than the largest (1.8) calculated for this example, which had a relatively high cis glucose concentration (90 mM). For the 133 corrected rates used for determinations of kinetic parameters, the mean correction factor was 1.34; only six exceeded 2.0, with the largest being 2.79.

Determination of V_{max}. For each of three experimental conditions, uptake was measured at various cis glucose concentrations. These ranged from 1 (fresh erythrocytes, 0 °C), 2 (outdated erythrocytes, 0 °C), and 10 mM (outdated erythrocytes, 25 °C) to 120 mM for each condition. The literature K_m values for zero-trans uptake under these conditions are about 0.2, 0.5, and 1.6 mM, respectively. The cis concentrations were therefore at least 4-fold higher than these values, and in 37 of the 41 experiments they were at least 10-fold higher. Thus, the rates of uptake in the absence of intracellular glucose give the V_{max} for zero-trans uptake, which is equal to the V_{max} for infinite-cis uptake. Figure 3 shows the rates obtained by using outdated erythrocytes at 25 °C. While the uncorrected apparent initial rates (solid circles) decrease with increasing cis glucose concentrations, the corrected rates (open circles) are independent of the cis glucose despite the large range in correction factors. Similar results were obtained with the two other conditions. These results indicate the validity of the correction procedure. The mean values of the corrected rates are listed in Table I as the experimental V_{max} values for infinite-cis uptake. In the case of fresh erythrocytes at 0 °C, the V_{max} value (5.1 μM/s) is in excellent agreement with that reported by Lowe and Walmsley (1986) for zero-trans uptake (5.5 μM/s).

Determination of K_{app} and K_{ic}. We defined K_{app} as the trans glucose concentration reducing the net uptake by 50% for any given cis concentration. We determined this concentration by linear interpolation between those concentrations that bracketed a 50% reduction in the corrected initial uptake rate obtained at zero trans glucose. In the experiment shown in Figure 1, that concentration was 14.0 ± 3.2 mM (indicated by the arrow). The standard deviation of the resulting K_{app} was determined from the appropriate arithmetic manipulations of the errors of the rates used in the interpolation. This procedure was used to determine K_{app} for various trans glucose concentrations for fresh erythrocytes at 0 °C, outdated erythrocytes at 0 °C, and outdated erythrocytes at 25 °C. The results are shown in Figure 4 (panels A, B, and C, respectively).

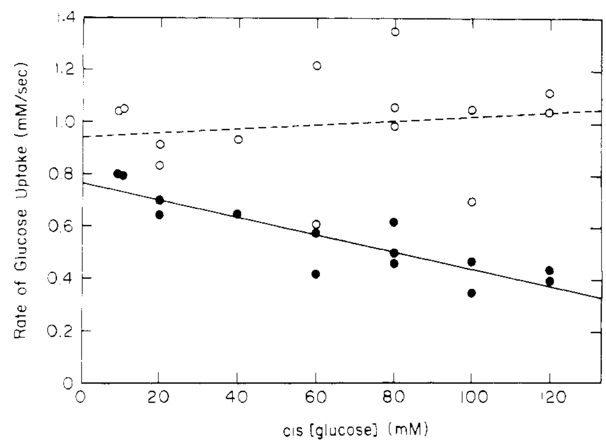


FIGURE 3: Initial rates of glucose uptake into glucose-free outdated erythrocytes at 25 °C. Apparent initial rates (●) and corrected initial rates (○) are plotted vs the cis glucose concentration. The data were fitted by linear regression to give slopes of $-0.0032\ s^{-1}$ (uncorrected rates, solid line) and $0.0008\ s^{-1}$ (corrected rates, broken line). The mean (±SD) corrected rate, 0.99 ± 0.19 mM/s, is the V_{max} for inifinite-cis uptake.

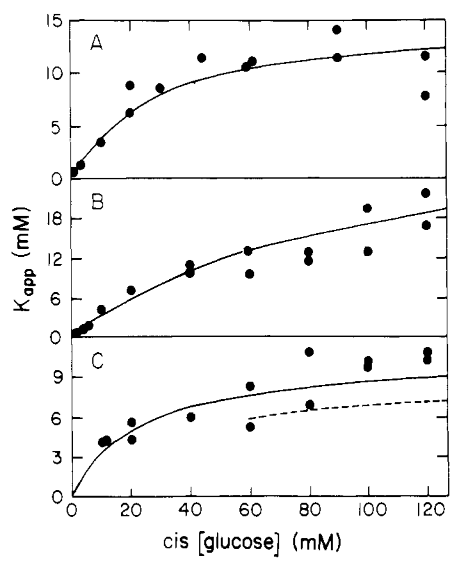


FIGURE 4: Apparent infinite-cis uptake K_m values obtained at various cis glucose concentrations. K_{app} values were determined at the indicated concentrations of extracellular (cis) glucose by the method described in the text and illustrated in Figure 1. (A) Fresh erythrocytes, 0 °C; (B) outdated erythrocytes, 0 °C; (C) outdated erythrocytes, 25 °C. The data were fitted to the equation $K_{app} = A[glucose_{cis}]/([glucose_{cis}] + B)$, resulting in the parameters listed in Table I. The solid lines were drawn according to the equation and these parameters. The broken line in (C) indicates the K_{app} values expected if K_{ic} were 2.8 mM (see text).

The data in Figure 4 show that K_{app} is a saturable function of the cis glucose concentration, as predicted by the carrier model (see Appendix). The model also predicts (eq 6) that K_{app} should reach half its maximum value when the cis glucose is twice K_{ic}. At infinite cis glucose, $K_{app} = K_{ic}$. We analyzed the data shown in Figure 4 using eq 7, which treated the two appearances of K_{ic} in eq 6 as independent parameters, A and B. According to the model, B should be twice A. The data were fitted to eq 7 by using a nonlinear regression program (Duggleby, 1981); the resulting parameters are listed in Table I. The slope of the double-reciprocal plot ($1/K_{app}$ vs $1/[\text{glucose}_{cis}]$) is B/A according to eq 7, which should be 2. Table I also lists the value of B/A for each condition, which was observed to be within experimental error of 2. Thus the concentration dependence of K_{app} confirms the predictions of the model.

We also tested an alternate fitting procedure, in which the data were treated as if they were enzyme rate data. In this procedure a least-squares fit was made of $1/K_{app}$ vs $1/[\text{glucose}_{cis}]$, with data weighted according to ${K_{app}}^4/SD^2$ (Cleland, 1967). When this procedure was used, the resulting parameters and their ratios were not significantly different than those listed in Table I.

Since the parameters A and $B/2$ each measure K_{ic} (from the extrapolated maximum value of K_{app} and from the dependence of K_{app} on the cis glucose concentration, respectively), we used the mean of these two as our experimental value of K_{ic}. The resulting K_{ic} values are listed in the last column of Table I.

For the experiments at room temperature we simulated the results that would have been expected if the uptake followed eq 26 of Hankin et al. (1972) with a K_{ic} of 2.8 mM.[3] These simulations indicate that if the K_{ic} were truly that low, our corrected rates would have underestimated the initial rates and we would have obtained K_{app} values in excess of the true K_{ic}. The calculated K_{app} values for these simulations, shown by the broken curve in Figure 4C, would have risen from 5.9 mM at 60 mM cis glucose to 7.1 mM at 120 mM cis glucose. The experimental K_{app} values are clearly in excess of these, particularly at the higher cis glucose concentrations. Thus the results support a K_{ic} value much higher than 2.8 mM.

Time Course Studies at Room Temperature. In considering why there should be a discrepancy between our results and previous determinations of the K_{ic} at room temperature (Hankin et al., 1972; Dustin et al., 1984), we noted that the time course method used previously depends critically upon the estimate of the initial rate of uptake. However, that rate was not determined directly but was based on an extrapolation using relatively late time points. For example, in the experiment shown in Figure 2 of Hankin et al. (1972) [raw data are given by Lieb and Stein (1977)], the first time point was 10 s, by which time the intracellular glucose was 3.9 mM (giving an apparent initial rate of 0.39 mM/s). The initial rate calculated by fitting the time course at 10–40 s to a theoretical equation was much higher, 1.1 mM/s. On this basis it was concluded that K_{ic} had already been exceeded by the time the first measurement was made.

In order to test whether such rapid changes in rate actually occurred in the first 10 s, we performed the experiment shown in Figure 5. Uptake of 60 mM glucose into glucose-free outdated erythrocytes was determined at 20 °C. The time course of uptake at 10–40 s was consistent with that observed by Hankin et al. (1972). However, we also measured the uptake at 2, 3, 4, 6, and 8 s. It was observed that the uptake was essentially linear in the first 10 s; the apparent initial rates using the first six time points ranged from 0.42 to 0.48 mM/s. Thus the data do not support rapid changes in the uptake rate in this time interval.

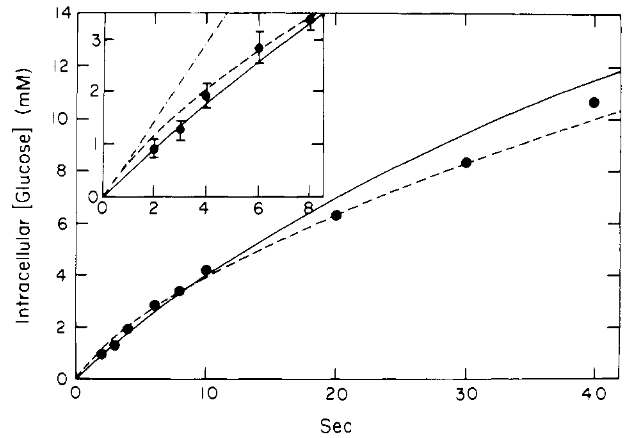


FIGURE 5: Time course of uptake of 60 mM glucose. Uptake of 60 mM glucose into glucose-free outdated erythrocytes was determined at 20 °C. The solid line is a simulation for $K_{app} = 9.7$ mM, the broken line for $K_{ic} = 2.8$ mM (see text). The earliest time points are shown with expanded scales in the inset (upper left); the bars indicate standard errors of the determinations. The upper, straight line in the inset indicates the initial rate (0.74 mM/s) predicted by the simulation for $K_{ic} = 2.8$ mM.

We fitted the data in Figure 5 using eq 26 of Hankin et al. (1972).[3] In this equation the parameter K (which should be distinguished from the parameter K of the carrier model as used in eq 1 of the Appendix) is the operational saturation constant at the internal surface, which would be K_{ic} according to Hankin et al. (1972) or K_{app} according to our nomenclature. For this fit the values used for K were 2.8 mM (Hankin et al., 1972) or 9.7 mM [the expected K_{app} at 60 mM glucose according to the carrier model and a K_{ic} of 14.3 mM, corrected to 20 °C[4] from the value of 11.2 mM at 25 °C (Table I)]. In

[3] The equation is

$$dN/dt = vK/[K + N(P + C_0)/(P + N)]$$

where C_0 and N are the extracellular and intracellular glucose concentrations, respectively, v is the V_{max}, K is the infinite-cis uptake K_m (assuming C_0 is "infinite"), and P is the osmolarity of nonpenetrating solutes in both the medium and cells.

[4] V_{max} and K_m values were converted from 25 to 20 °C by using temperature data of Brahm (1983) and Lowe and Walmsley (1986). For the zero-trans (and, therefore, infinite-cis) uptake V_{max} a factor of 0.26 was used [Figure 2 of Lowe and Walmsley (1986)]. For zero-trans efflux V_{max}, the factor was 0.56 [Figure 7 of Brahm (1983)]. For the exchange V_{max}, an average of the factors inidicated by the same two figures was 0.63. For the zero-trans efflux K_m, interpolations [on a graph of ln K vs $1/T$ (Lowe & Walmsley, 1986)] between K_m values for 10 and 25 °C (Brahm, 1983) yielded a factor of 0.93. For the exchange K_m, an average of the results of Brahm (1983) and Lowe and Walmsley (1986) (Figure 4) gave a factor of 1.05. We estimated from these changes that the model parameter K has a value at 20 °C of 52% of its value at 25 °C; from the relationship $K_{ic\,uptake} = K(V_{exchange}/V_{zero\text{-}trans\,uptake})$ and the factors of 0.63 and 0.26 for the exchange and uptake V_{max} values, respectively, a factor of 1.28 was calculated for K_{ic}. We did not correct the kinetic parameters for differences between fresh and outdated blood. Weiser et al. (1983) reported a 1.26-fold higher exchange V_{max} for fresh vs outdated blood, while Jensen and Brahm (1987) reported a value of 1.17 for the same ratio. In the latter study, the V_{max} for zero-trans efflux was 1.05-fold higher for outdated blood. Such differences in V_{max} values are less than the variations in values between laboratories when measured under the same conditions. Weiser et al. (1983) reported a 1.6-fold higher exchange K_m for outdated vs fresh blood, while Jensen and Brahm (1987) reported that fresh blood had a 2.0-fold higher exchange K_m. The latter group also measured a 7-fold higher zero-trans efflux K_m for outdated blood. This high ratio results, however, in part from a K_m for fresh blood (1.3 mM) that is much lower than other results in the literature, including one from the same laboratory [5.8 mM (Brahm, 1983)]. Thus the effects of cell age on K_m values at 20–25 °C are controversial, and we have not attempted to correct for them.

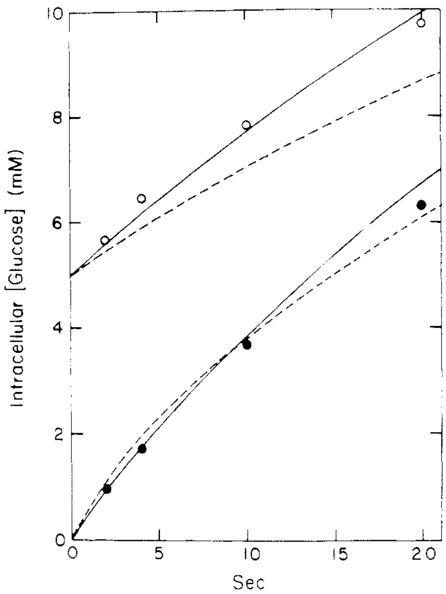


FIGURE 6: Time course of glucose uptake with 0 or 5 mM trans glucose. Uptake of 60 mM glucose at 20 °C was determined for outdated erythrocytes equilibrated with 0 (●) or 5 (○) mM glucose. Values are means from two experiments with the same unit of blood. The solid lines are a simulation for K_{app} = 9.7 mM, the broken lines for K_{ic} = 2.8 mM.

these simulations the initial rate was adjusted to optimize the fit at 8–30 s (K = 2.8 mM) or at 2–20 s (K = 9.7 mM). It can be seen that while the lower K_{ic} provides a reasonable fit to the data overall, it predicts higher uptakes than those observed in the first few seconds (shown in detail in the inset in Figure 5) and rapid changes in rates in this interval. In particular, it predicts an initial rate of 0.74 mM/s (the upper line in the inset), which is inconsistent with the data. The higher K_{app} (9.7 mM), on the other hand, is in good agreement with the early time points but does not account for the decrease in rate observed at 20–40 s. Thus, if the K_{app} is truly that high, the uptake time course behaves in a nonideal manner for the later time points. The nonideal behavior is not a result of water movement in response to osmotic changes, which is taken into account in eq 26 of Hankin et al. (1972).

The results shown in Figure 6 examine glucose uptake at early time points for cells containing initially either 0 or 5 mM glucose. As above, the data were fitted to the equation of Hankin et al. (1972)[3] by using K equal to either 2.8 mM or 9.7 mM. In each case the initial rate was chosen to optimize the fit to the uptake into glucose-free cells (for 10–20 s and for 2–10 s for K = 2.8 and 9.7 mM, respectively). As for the data in Figure 5, the simulation using 2.8 mM predicts higher initial uptakes than were observed. Moreover, it agrees poorly with the data for cells containing 5 mM glucose initially, predicting a much lower rate of uptake than was observed. The simulation using 9.7 mM, however, fits both curves quite well. The simulations predicted that 5 mM trans glucose should inhibit the apparent initial uptake rate by 62%, 60%, and 52% for measurements at 2, 4, and 10 s, respectively, if K_{ic} were 2.8 mM; the corresponding values predicted for a K_{app} of 9.7 mM were 35%, 34%, and 31%. The observed inhibition (31%, 15%, and 23%, respectively) is in much better agreement with the latter K_{app} value.

A comparison of Figures 5 and 6 shows that while the higher K_{app} does not predict the time course well at relatively late time points, it does predict it well when the same range of intracellular glucose concentrations is encountered at the earlier time points as a result of using cells that had been preequilibrated with 5 mM glucose. In other words, the transport rate measured at early times differs from that measured at later times for the same intracellular glucose concentrations. This supports the idea that the rate slows down with time for reasons other than the accumulation of glucose inside the cells in an ideal manner. Further evidence for this comes from one of the experiments on which Figure 6 is based, in which the time course was followed through 30 s. In this case the same change in intracellular glucose concentration achieved in the 10-s interval between 20 and 30 s with initially glucose-free cells was observed in an interval of only 2.5 s when the cells contained 5 mM glucose initially.

Discussion

The infinite-cis uptake of glucose into human erythrocytes was previously measured by following the time course of uptake into glucose-free cells when a saturating (60–120 mM) extracellular concentration was present, and by analyzing the results with an integrated rate equation. The resulting K_{ic} values were 2.8 (Hankin et al., 1972) and 1.8 mM (Dustin et al., 1984) at room temperature. The analytical procedure by which K_{ic} was determined, involving transformation of the integrated rate equation into a linear form, was criticized as being very sensitive to small errors and thus unreliable (Foster & Jacquez, 1976). The reliability of the analysis was defended (Lieb & Stein, 1977), and the controversy was resolved by the use of a direct fitting method which avoided the linearization (Foster et al., 1979). This procedure also yielded a low value for K_{ic}.

However, this reevaluation (Foster et al., 1979) dealt only with the best way to fit the data to a theoretical equation describing the time course; it did not demonstrate that the equation was valid. The results presented here (Figures 5 and 6) indicate that the equation cannot adequately describe the time course of glucose uptake for both early (0–10 s) and late (10–40 s) time points for experiments such as those performed by Hankin et al. (1972) and Dustin et al. (1984). The data indicate that the rate of glucose uptake decreases with time for reasons other than the accumulation of glucose in an ideal manner. Previously we suggested that such nonideal behavior would result in an underestimation of K_{ic} for uptake (Wheeler, 1986). Evidence for nonideal behavior has been obtained for zero-trans efflux, where a time course procedure (Karlish et al., 1972; Baker & Naftalin, 1979) gave much higher K_m values than initial rate studies (Miller, 1971; Brahm, 1983; Lowe & Walmsley, 1986; Wheeler, 1986). It was also shown that, for the time course procedure, different starting glucose concentrations yielded different kinetic parameters (Naftalin et al., 1985), directly demonstrating nonideal behavior.

The cause of the nonideal behavior is unknown. Some possibilities include damage to cells produced by the manipulations involved in the assays, resulting in a progressive loss of activity; cell heterogeneity, with different cells transporting glucose at different rates; differential transport of α and β anomers of glucose; and slow equilibration of glucose between intracellular compartments. We simulated the effect of cell heterogeneity on the uptake time course, but it appeared that extreme variations in rates would be required to produce the observed deviations from ideal behavior. The experiments of Carruthers and Melchior (1985) rule out major differences in the transport of the two glucose anomers, though small differences could exist. Naftalin and Holman (1977) and Naftalin et al. (1985) proposed that the formation of a complex between glucose and hemoglobin could result in anomalous kinetic behavior. We showed (Wheeler, 1986) that the dissociation of glucose from such a complex could not be as slow as had been proposed for 2 °C (Naftalin et al., 1985), but it

Table II: Kinetic Parameters for Glucose Transport in Human Erythrocytes near 0 °C

procedure	experimental[a] K_m[c]	experimental[a] V_{max}[d]	reference	fit to carrier model[b] K_m[c]	fit to carrier model[b] V_{max}[d]
		Fresh Blood			
zero-trans uptake	0.20	0.0035	Lacko et al., 1972	0.15	0.0048
	0.145	0.0055	Lowe & Walmsley, 1986		
zero-trans efflux		0.040	Brahm, 1983	3.0	0.10
	1.64	0.071	Lowe & Walmsley, 1986		
	3.4	0.15	Wheeler, 1986		
infinite-trans uptake	0.65	0.21	Lacko et al., 1972	0.61	0.43
infinite-trans efflux	8.7	0.73	Wheeler, 1986	13	0.43
infinite-cis uptake	14.6	0.0051	this study	13	0.0048
infinite-cis efflux	0.39	0.143	Baker & Naftalin, 1979	0.61	0.10
equilibrium exchange	20	0.375	Lacko et al., 1972	13	0.43
	25	0.50	Baker & Naftalin, 1979		
		0.212	Brahm, 1983		
	12.8	0.563	Lowe & Walmsley, 1986		
		Outdated Blood			
zero-trans uptake	0.52	0.0064	Wheeler, 1986	0.21	0.0030
zero-trans efflux	2.7	0.064	Wheeler, 1986	4.3	0.062
infinite-trans uptake	0.80	0.39	Wheeler, 1986	1.3	0.40
infinite-trans efflux	19.3	0.61	Wheeler, 1986	26	0.40
infinite-cis uptake	39	0.0020	this study	26	0.0030
equilibrium exchange	42	0.83	Wheeler, 1986	27	0.40

[a] Experimental parameters are from the literature or from this study (Table I). Only experiments using estimated initial rates are included. V_{max} values from Wheeler (1986) have been corrected by multiplying by 1.6 (see Experimental Procedures). Values for zero-trans uptake, infinite-trans uptake, and equilibrium exchange for outdated blood (Wheeler, 1986) are preliminary values based on two experiments each. [b] The carrier model parameters used to calculate the values of K_m and V_{max} in the last two columns were as follows: fresh blood, $R_{12} = 210$, $R_{21} = 10$, $R_{ee} = 2.3$, $R_{00} = 218$, and $K = 0.14$; outdated blood, $R_{12} = 330$, $R_{21} = 16$, $R_{ee} = 2.5$, $R_{00} = 344$, and $K = 0.20$. Units of K were mM; those of R were s·mmol^{-1}·L. [c] In units of mM. [d] In units of mmol·L^{-1}·s^{-1}.

is possible that such a two-compartment model with different parameters could, along with the other factors noted above, contribute to nonideal behavior. We note that the simulations for the higher value of K_{ic} shown in Figures 5 and 6 differ from the observed uptakes by at most 12%.

A study of galactose uptake (Ginsburg & Stein, 1975), in which similar values for K_{ic} were obtained by using both a time course procedure and initial rate measurements, has been cited in support of both the internal "high-affinity site" and the validity of the time course method. The K_{ic} (about 20 mM) was much lower than the value (about 100 mM) indicated by the carrier model. However, the K_{ic} values (21 ± 17 and 25 ± 18 mM) have such large standard errors that the agreement of the methods is questionable. The integrated rate analysis [Figure 3 of Ginsburg and Stein (1975)] is heavily influenced by the later time points, which have large errors; the earlier time points would yield a much higher value of K_{ic}. Also, the regression analysis used to calculate initial rates (Figure 4) fits the early time points poorly at two of the four concentrations. This is especially true of the data at 220 mM intracellular glucose, where a line through the first two time points gives a rate about 40% of the V_{max}, which is consistent with a K_{ic} of greater than 100 mM. Thus, these results do not provide convincing evidence of a high-affinity site at the intracellular surface of the transporter.

The time course method has also been applied to glucose transport in cultured human lymphocytes (Rees & Gliemann, 1985). Here, a K_{ic} of 1.2 mM was determined for uptake, which was much lower than the K_m for zero-trans efflux (9.5 mM). This finding was cited in support of the existence of high-affinity internal site (Stein, 1986). However, the linear transformation of the data [Figure 5B of Rees and Gliemann (1985)] was also sensitive to late time points, and early points would have given a higher K_m. Also, the derived V_{max} (51 mM/min) was not only much higher than indicated by the earliest time point but also much higher than the V_{max} for zero-trans uptake (11 mM/min). These two V_{max} values are by definition equal. Therefore, the existence of an internal high-affinity site for glucose is also questionable for the case of lymphocytes.

In the experiments reported here, we used initial rate measurements, rather than time course data, to determine the kinetic parameters for infinite-cis uptake. As noted under Experimental Procedures, the resulting kinetic parameters were relatively insensitive to the choice of time points, thus demonstrating that initial rate conditions were approximated. The experiments reported here (Figure 1C and Table I) gave a much higher value for the K_{ic} for outdated blood at room temperature (11 mM) than values reported previously [2.8 mM (Hankin et al., 1972) and 1.8 mM (Dustin et al., 1984)]. Since the nonideal behavior will produce much larger errors in time course experiments than in initial rate studies, we feel that our results are more reliable than those reported previously. We also showed that the K_{app} calculated for uptake of 60 mM glucose at 20 °C (9.7 mM) was more consistent with the initial time course of uptake than a K_{ic} of 2.8 mM (Figures 5 and 6), especially when cells contained 5 mM glucose at zero time (Figure 6).

Table II lists kinetic parameters obtained by using both fresh and outdated blood near 0 °C. In both Table II and Table III we have included only results based on initial rate measurements, since time course procedures appear unreliable. We fitted the data to the carrier model, using procedures similar to those described previously (Wheeler, 1986). The resulting model parameters are listed in Table II. In the case of fresh blood, these are all within 20% of those estimated previously (Wheeler, 1986). In the case of outdated blood, where much of the data is preliminary, larger differences occur. One result of the changes is that the asymmetry of kinetic parameters (e.g., K_m and V_{max} for zero-trans uptake vs zero-trans efflux) appears to be about the same (21-fold) for both fresh and outdated blood.

The kinetic parameters calculated according to the model are listed in the last two columns of Table II. The infinite-cis uptake K_m values determined here (15 and 39 mM for fresh and outdated blood, respectively) are in good agreement with

Table III: Kinetic Parameters for Glucose Transport in Human Erythrocytes near 20 °C[a]

procedure	experimental[b] K_m	experimental[b] V_{max}	reference	fit to carrier model[c] K_m	fit to carrier model[c] V_{max}
zero-trans uptake	1.6	0.60	Lacko et al., 1972	1.7	0.56
	1.6	0.51	Challiss et al., 1980[d]		
	1.6	0.87	Lowe & Walmsley, 1986		
zero-trans efflux	7.4	1.9	Miller, 1971[d]	6.9	2.3
	5.4 (5.8)	2.3 (4.1)	Brahm, 1983[e]		
	4.6	2.6	Lowe & Walmsley, 1986		
	1.2 (1.3)	2.7 (4.9)	Jensen & Brahm, 1987[e]		
	8.4 (9.0)	2.9 (5.1)	Jensen & Brahm, 1987[d,e]		
infinite-trans uptake	1.7-2	3.0	Lacko et al., 1972	3.0	4.8
infinite-cis uptake	14.3 (11.2)	0.25 (0.99)	this study[d,e]	12.6	0.56
infinite-cis efflux	1.7	1.2	Sen & Widdas, 1962	3.0	2.3
	1.9	3.5	Harris, 1964		
	1.8	1.7	Miller, 1968[d]		
equilibrium exchange	38	4.3	Miller, 1968[d]	14	4.8
	20	4.4	Lacko et al., 1972		
	32	6.0	Eilam & Stein, 1972[d]		
	34	6.0	Eilam, 1975[e]		
	8.5 (8.1)	3.6 (5.6)	Brahm, 1983[e]		
	13	6.1	Weiser et al., 1983		
	21	4.6	Weiser et al., 1983[d]		
	17	5.9	Lowe & Walmsley, 1986		
	7.9 (7.5)	4.4 (6.9)	Jensen & Brahm, 1987[e]		
	4.0 (3.8)	3.7 (5.9)	Jensen & Brahm, 1987[d,e]		

[a] Units are as in Table II. [b] Experimental parameters were from the literature or this study (Table I). Only experiments using estimated initial rates are included. In some cases these were measured at 25 °C; the values were corrected as described in the text (footnote 4) to give parameters estimated for 20 °C. When corrected values are given, the uncorrected values are noted in parentheses. [c] The model parameters used to calculate V_{max} and K_m values were $R_{12} = 1.79$, $R_{21} = 0.43$, $R_{ee} = 0.21$, $R_{00} = 2.01$, and $K = 1.48$. [d] Determined with outdated blood. [e] Corrected from 25 to 20 °C.

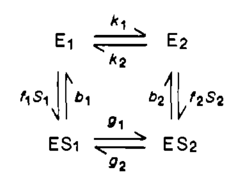
values calculated from the model (13 and 26 mM, respectively). They are also in good agreement with values (14 and 27 mM, respectively) predicted by our earlier model fits (Wheeler, 1986), which were made before the experiments described here were performed. The V_{max} values (0.0051 and 0.0020 mM/s) are also in good agreement with predictions of the model (0.0048 and 0.0030 mM/s). A comparison of the parameters predicted by the model to results of other types of kinetic procedures reported by various laboratories near 0 °C (Table II) shows that they are generally in good agreement.

In the case of experiments performed at room temperature, nearly complete sets of data are not available for either fresh or outdated blood. Furthermore, the measurements have been made at either 20 or 25 °C. In order to compare these results to predictions of the model, we normalized the 25 °C data to 20 °C[4] (Table III). By use of the corrected parameters, a fit was made to the carrier model. The resulting model parameters are listed in Table III, while the calculated kinetic parameters appear in the final two columns. The value of K is 1.5-fold higher than estimated previously [Table IV of Wheeler (1986)], while the other parameters are within 20% of the previous values.

The experimental K_m for infinite-cis uptake reported here for outdated blood at 25 °C (11.2 mM) is, when corrected to 20 °C (14.3 mM), in good agreement with the prediction of the model (12.6 mM). The predicted V_{max}, 0.56 mM/s, lies between the experimental value at 25 °C (0.99 mM/s) and the corrected value estimated for 20 °C (0.25 mM/s). Thus, as at 0 °C, the infinite-cis uptake data support the carrier model; no unexpectedly high affinity at the internal surface is observed.

The literature results for other experimental procedures at 20–25 °C are also in reasonable agreement with the model, considering not only the corrections employed in some cases but also the fact that the parameters were obtained in different laboratories. In the case of zero-trans efflux and equilibrium exchange, K_m values varying more than 4-fold have been reported, and the predictions of the model lie within the ranges of the reported values.

Scheme I



Hankin et al. (1972) described two "rejection criteria" for testing the carrier model. We found that experimental parameters could be selected from Tables II and III fulfilling these criteria for each condition, with the exception of the first criterion applied to efflux from fresh blood at 0 °C. In this case, the exchange K_m (13–25 mM) is so much higher than the infinite-cis efflux K_m (0.39 mM) that it should not be surprising that the ratio (33–64) of these two experimentally determined values is in excess of the asymmetry factor (about 20) plus 1, as specified by eq 18 of Hankin et al. (1972).

It is of interest to relate the fundamental parameters of the model to rate constants for the individual steps (Scheme I). Here we use the nomenclature of Eilam and Stein (1974), where side 1 is the outside and side 2 the inside of the cell.

Using the parameters for fresh blood at 0 °C (Table II) and the assumption that the binding and dissociation steps are fast compared to the conformational change steps, it is possible to set limits on the values of the latter. These are (in units of mM/s) $k_1 \approx 0.1$, $k_2 \approx 0.0048$, $g_1 > 0.4$, and $g_2 > 0.5$. Lowe and Walmsley (1986) previously estimated these rate constants using both the 0 °C values and the temperature dependence of the kinetic parameters. Their values (designated g, h, c, and d, respectively) were 0.08, 0.0048, 8.4, and 0.6 mM/s, in good agreement with our calculations.

In the case of the model fit for 20 °C (Table III), the limits on the rate constants are $2.3 < k_1 < 4.5$, $k_2 \approx 0.6$, $g_1 > 4.8$, and $g_2 > 4.8$ mM/s. Appleman and Lienhard (1985), in a study of rapid kinetics of conformational changes of the purified transporter, reported a value of 147 s^{-1} at 25 °C for k_2 (which they designated k_{-1}). Taking the value of 0.6 mM/s for k_2, and using the factors of 2.5×10^5 glucose transporters/cell (Lin & Snyder, 1977) and 1.82×10^{13} cells/L of cell water (Harris & Kellermeyer, 1970), a rate constant of 79 s^{-1} is obtained for 20 °C, in excellent agreement with the results with the purified transporter when the temperature difference is taken into account. Appleman and Lienhard (1985) also reported a value of 141 mM^{-1} s^{-1} for k_{-2}/K_{Si} (their nomenclature), where K_{Si} is the dissociation constant at the internal surface. According to Scheme I, this is equal to $g_2/(b_2/f_2)$. Since, according to the carrier model when binding and dissociation steps are rapid, $K = (k_2b_2)/(f_2g_2)$, the measured quantity is equivalent to k_2/K. When the experimental value of 147 s^{-1} is used for k_2, this means that $K = (147\ s^{-1})/(141\ mM^{-1}\ s^{-1}) = 1.0$ mM, in good agreement with the model value of 1.5 mM.

In summary, the results presented here using initial rate measurements give a much higher value for the infinite-cis uptake K_m at room temperature than those previously reported using a time course method and indicate that the latter method is unreliable because the time course of transport behaves in a nonideal manner. The results thus argue against a high-affinity site at the intracellular surface [which has been claimed to rule out the model (Stein, 1986)]. The K_{ic} values reported here for both 0 and 25 °C are in good agreement with predictions from the carrier model. Together with results from other types of kinetic experiments they support the adequacy of that model to explain the kinetic features of both steady-state measurements in erythrocytes and rapid kinetic studies using the purified protein.

ACKNOWLEDGMENTS

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APPENDIX

Derivation of the Relationship between K_{app} and [Glucose$_{cis}$]. Eilam and Stein (1974) give an equation (eq 16) for the unidirectional flux of glucose according to the carrier model. Using this equation, the rate of net uptake of glucose (which is the difference between the unidirectional uptake and efflux rates) is

$$u_{12} - u_{21} = \frac{K([G_1] - [G_2])}{K^2R_{00} + KR_{12}[G_1] + KR_{21}[G_2] + R_{ee}[G_1][G_2]} \quad (1)$$

where side 1 is the outside and side 2 the inside; u_{12} and u_{21} are the unidirectional uptake and efflux, respectively; $[G_1]$ and $[G_2]$ are the concentrations of glucose on sides 1 and 2, respectively; and K, R_{12}, R_{21}, R_{ee}, and R_{00} are the model parameters.

By use of the values of the model parameters which account reasonably well for the kinetic behavior of the human erythrocyte glucose transporter (Wheeler, 1986),[2] eq 1 can be simplified under certain experimental conditions. As $[G_1]$ increases, the second term in the denominator becomes much larger than the first term. Also, for any value of $[G_2]$, as $[G_1]$ increases the fourth term of the denominator becomes much larger than the third term. In nearly all of the experiments reported here, $[G_1]$ was sufficiently high that the first and third terms were negligible. Under these conditions eq 1 simplifies to

$$u_{12} - u_{21} \approx \frac{K([G_1] - [G_2])}{KR_{12}[G_1] + R_{ee}[G_1][G_2]} \quad (2)$$

When $[G_2] = 0$ (zero-trans uptake), there is no efflux, and eq 1 and 2 simplify to

$$u_{12} - u_{21} = u_{12} = \frac{K[G_1]}{KR_{12}[G_1]} = \frac{1}{R_{12}} = V_{12}^{zt} = V_{12}^{ic} \quad (3)$$

This defines V_{max} for infinite-cis uptake (V_{12}^{ic}), which is equal to V_{max} for zero-trans uptake (V_{12}^{zt}).

If K_{app} is defined as the value of $[G_2]$ at which the net flux is reduced to half of V_{12}^{ic}, then at $[G_2] = K_{app}$

$$u_{12} - u_{21} \approx \frac{K([G_1] - K_{app})}{KR_{12}[G_1] + R_{ee}[G_1]K_{app}} = \frac{V_{12}^{ic}}{2} = \frac{1}{2R_{12}} \quad (4)$$

Solving eq 4 for K_{app}

$$K_{app} \approx \frac{(KR_{12}/R_{ee})[G_1]}{[G_1] + 2(KR_{12}/R_{ee})} \quad (5)$$

However, according to the model, KR_{12}/R_{ee} is the infinite-cis uptake K_m (K_{12}^{ic}). Substituting this in eq 5 gives

$$K_{app} \approx \frac{K_{12}^{ic}[G_1]}{[G_1] + 2K_{12}^{ic}} \quad (6)$$

Equation 6 has the form of the Michaelis–Menten equation. A double-reciprocal plot ($1/K_{app}$ vs $1/[G_1]$) should have a y intercept of $1/K_{12}^{ic}$ and a slope of 2.

In analyzing the experimental data, we treated the two appearances of K_{12}^{ic} in eq 6 as if they were independent parameters. For this purpose the equation was written in the form

$$K_{app} = \frac{A[G_1]}{[G_1] + B} \quad (7)$$

Registry No. Glucose, 50-99-7.

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Lateral Diffusion and Fluorescence Microscope Studies on a Monoclonal Antibody Specifically Bound to Supported Phospholipid Bilayers

Lukas K. Tamm

Department of Biophysical Chemistry, Biocenter, University of Basel, CH-4056 Basel, Switzerland

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ABSTRACT: Supported phospholipid bilayers prepared by Langmuir–Blodgett techniques were introduced recently as a new model membrane system [Tamm, L. K., & McConnell, H. M. (1985) *Biophys. J. 47*, 105–113]. Here, supported bilayers are applied to study the lateral diffusion and lateral distribution of membrane-bound monoclonal antibodies. A monoclonal anti-trinitrophenol antibody was found to bind strongly and with high specificity to supported phospholipid bilayers containing the lipid hapten (trinitrophenyl)phosphatidylethanolamine at various mole fractions. The lateral distribution of the membrane-bound antibodies was studied by epifluorescence microscopy. The bound antibodies aggregated into patches on a host lipid bilayer of dimyristoylphosphatidylcholine below the lipid chain melting phase transition and redistributed uniformly on fluid-phase supported bilayers. Lateral diffusion coefficients and mobile fractions of fluorescent phospholipid analogues and fluorescein-labeled antibodies were measured by fluorescence recovery after pattern photobleaching. The lateral diffusion coefficients of the membrane-bound antibodies resembled those of the phospholipids but were reduced by a factor of 2 in the fluid phase. The lipid chain melting phase transition was also reflected in the lateral diffusion coefficient of the bound antibody but occurred at a temperature about 3 deg higher than the phase transition in supported bilayers of pure phospholipids. The antibody lateral diffusion coefficients decreased in titration experiments monotonically with increasing antibody surface concentrations by a factor of 2–3. Correspondingly, a relatively small decrease of the antibody lateral diffusion coefficient was observed with increasing mole fractions of lipid haptens in the supported bilayer.

Model membranes have often proven to be very useful for obtaining detailed information on many physical and functional properties of biological membranes. Interactions between lipids and proteins, the binding of water-soluble molecules and ions, and membrane transport phenomena have been studied in various model membrane systems with much success. How-

