THE KINETICS OF SELECTIVE BIOLOGICAL TRANSPORT

V. FURTHER DATA ON THE ERYTHROCYTE-MONOSACCHARIDE TRANSPORT SYSTEM

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ABSTRACT Measurements of the rate of loss of sugar from human erythrocytes into sugar-free solutions were made as a function of sugar concentrations. The half-saturation concentration of this process was found to be different from those half-saturation concentrations previously measured by other methods. These data, together with a number of similar data from former publications, are summarized in tabular form and their use in assessing postulated transport mechanisms is illustrated by consideration and rejection of a mechanism in which the transport process is assumed to be the result of a protein conformational change.

INTRODUCTION

In a previous publication (Miller, 1968 *a*) a number of quantitative kinetic data on the human erythrocyte-monosaccharide transport system were presented, and it was argued that since these measurements were all made under the same conditions they should form an internally consistent set of results against which to test proposed mechanisms of transport. These criteria were then applied to some of the more obvious simple mechanisms (Miller, 1968 *b*) which proved to be inadequate, and it was concluded from this that the system was more complex than originally supposed. Since that time several more elaborate mechanisms have been devised, and for some, at least, it has become obvious that still more data are required to evaluate the greater number of kinetic constants which inevitably arise from an increased complexity. Such data are presented here and are included in a summary of the more important kinetic parameters so far derived from this system. The use of these data is then illustrated in the evaluation of a somewhat more complex mechanism than those considered previously.

EXPERIMENTAL

Experimental Design

The experiments to be reported will subsequently be referred to as *net loss* experiments. They were performed by loading erythrocytes with sugar at different concentrations and measuring the initial rate of loss of the sugar after transfer of the cells to a sugar-free solution.

According to the basic carrier mechanism (Sen and Widdas, 1962), the rate of transport into the cell is given by the expression:

$$\frac{dx}{dt} = V\left[\frac{X_o}{K + X_o} - \frac{X_i}{K + X_i}\right], \tag{1}$$

where x is the amount of sugar contained in a unit of cells, X_o and X_i are the external and internal substrate concentrations respectively, V is the maximum rate constant, and K is the dissociation constant for the complex formed between the substrate and the carrier. Since inverse K is proportional to the affinity of the substrate for the carrier, K is frequently termed the "affinity constant."

Under the present experimental conditions $X_o = 0$ and equation 1 becomes:

$$R = -\frac{dx}{dt} = \frac{VX_i}{K + X_i}, \tag{2}$$

where R is the rate of loss of the sugar. Inverting this equation gives the result

$$\frac{1}{R} = \frac{K}{V} \cdot \frac{1}{X_i} + \frac{1}{V}, \tag{3}$$

which predicts that a plot of $1/R$ as a function of $1/X_i$ should be a straight line with slope $S = K/V$ and intercept $I = 1/V$. Thus by measuring these two quantities the values of the kinetic constants should be obtained from the ratios $V = 1/I$ and $K = S/I$.

In addition to these experiments, measurements of the rates of exchange of galactose as a function of concentration are also reported. The principle of this type of experiment has already been discussed in previous publications (Miller, 1968 *a*, *b*) where it was shown that, according to the precepts of the simple carrier theory, an expression identical to equation 3 should apply to these data also and should allow the evaluation of K and V in the same way.

Experimental Procedure

Net loss experiments were performed using both radioactive glucose and galactose. The procedure was identical to that used to measure the rate of exchange of glucose as a function of concentration (Miller, 1968 *a*) except that the external solution in every case was free of sugar. The cells were incubated with a buffer solution containing sugar at concentration X_e for a time sufficient to allow the internal sugar concentration to reach this same value. They were then centrifuged, rapidly resuspended in sugar-free buffer, and the initial rate of loss (R) of the cellular sugar measured as before (Miller, 1968 *a*). Upon resuspension, water quickly entered the cells to bring them into osmotic equilibrium with the buffer. This caused a reduction in the internal sugar concentration from a value equal to that of the loading solution X_e to a new value X_i calculated from the expression (see Miller, 1968 *b*):

$$X_i = \frac{310X_e}{310 + X_e}. \tag{4}$$

Rate of exchange measurements were also made on radioactive galactose in accordance with the original procedure (Miller, 1968 *a*).

RESULTS

The results of the net loss measurements for glucose and galactose appear in Table I and those for exchange of galactose in Table II. Double reciprocal plots of these data are shown in Figs. 1 and 2 together with their best-fitting straight lines. The slope and intercept of these lines and the values of K and V calculated from them are listed in Table III. Also included in this table are similar results from previous experiments on this system.

DISCUSSION

Table III summarizes the kinetic features which a mechanism must have to be compatible with the experimental results. Such a mechanism should lead to the derivation of an equation (in terms of the concentrations of the substrates) which describes the transport of one substrate in the presence of a second. This equation should, first of all, reduce to (or approximate to) a straight line expression under the conditions listed in Table III. Secondly, values for all the constants required by the theory should exist which allow the prediction of the slopes, intercepts, and ratios of Table III within experimental error.

TABLE I

NET LOSS OF SUGAR VS. INTERNAL SUGAR CONCENTRATION

Concentration, *mmoles/liter*:	6.37	8.43	12.5	17.5	26.4	91.6
Glucose rate, *mmoles/min per cell unit*	40 ± 3(4)	58 ± 1(4)	73 ± 3(5)	84 ± 5(5)	85 ± 2(4)	100 ± 9(5)*
Galactose rate, *mmoles/min per cell unit*	14 ± 1(5)	18 ± 2(4)	23 ± 1(5)	34 ± 2(5)	49 ± 2(5)	80 ± 7(10)*

The initial rate of loss of sugar from loaded cells into sugar-free solutions measured as a function of the initial internal sugar concentration. Rates are given together with standard deviations and the number of determinations (in parentheses). Temperature 20°C, pH 7.35.
* Data from Table I of Miller (1968 *a*).

TABLE II

RATES OF EXCHANGE OF GALACTOSE VS. CONCENTRATION

Concentration, *mM*	13.0	18.6	28.9	43.3	130
Rate, *mmoles/min per liter isotonic cell water*	27 ± 1(4)	36 ± 3(5)	51 ± 3(5)	61 ± 2(6)	125 ± 10(9)

The initial rate of loss of galactose from loaded cells into nonradioactive galactose solutions as a function of sugar concentration. The indicated concentrations are the same in the cells and in the suspending medium. The rates are given together with standard deviations, and are followed by the number of experiments in parentheses. Temperature 20°C, pH = 7.35.

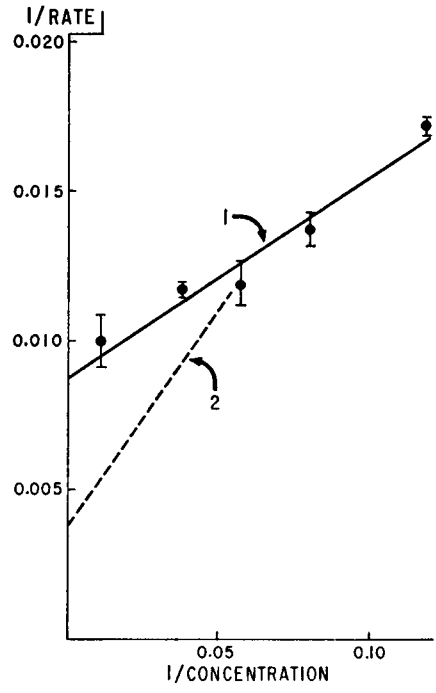

FIGURE 1 *Plot of the glucose data. Curve 1* is a least squares straight line fitted to the glucose *net loss* data from Table I (solid circles). *Curve 2* is a similar line for the exchange data obtained previously (Miller, 1968 *b*).

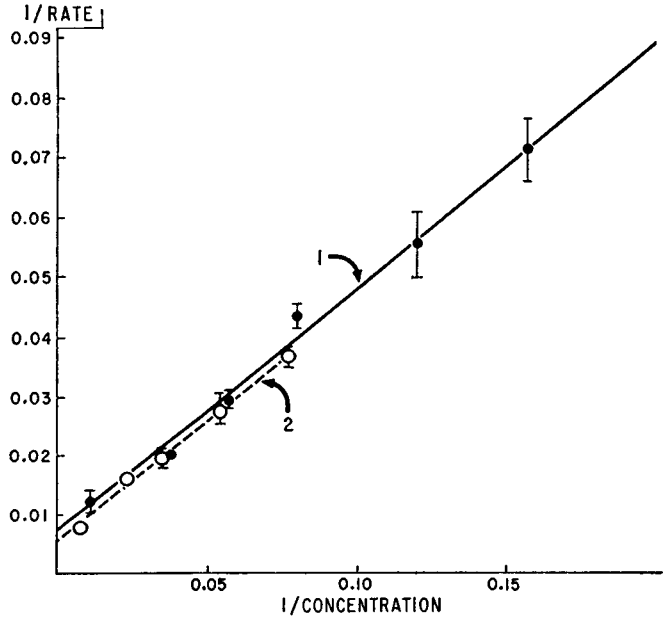

FIGURE 2 *Plot of the galactose data. Curve 1* is a least squares straight line fitted to the *net loss* data from Table I plotted here as solid circles. *Curve 2* is a similar line fitted to the *exchange* data of Table II (open circles).

TABLE III

SUMMARY OF KINETIC CONSTANTS DETERMINED FOR THE SIMPLE CARRIER MECHANISM

Sugar	Type of experiment	Parameters plotted	Concn range	Slope S	Intercept I	Apparent affinity constant	Apparent rate constant
			mM				
Glucose	Sen-Widdas*	$1/R$ vs. X_o	$X_i = 100$ $X_o = 0$–10 $Y_i = Y_o = 0$	0.0053 ±0.0002	0.0096 ±0.0012	$I/S =$ 1.8 ±0.3	$1/I =$ 104 ±12
	Net loss‡	$1/R$ vs. $1/X_i$	$X_o = 0$ $X_i = 6$–90 $Y_i = Y_o = 0$	0.066 ±0.008	0.0089 ±0.0006	$S/I =$ 7.4 ±1.4	$1/I =$ 112 ±8
	Sorbose inhibition*	$1/R$ vs. Y_o	$Y_i = Y_o = 0$–37.5 $X_i = 0$, $X_o = 230$ $X_i = 230$, $X_o = 0$			$I/S =$ 23 ±5 $I/S =$ 17 ±2	
	Exchange*	$1/R$ vs. $1/X_i$	$X_o = Y_i = 0$ $X_i = Y_o = 20$–130	0.145 ±0.011	0.0038 ±0.0004	$S/I =$ 38 ±3	$1/I =$ 260 ±30
Galactose	Sen-Widdas§	$1/R$ vs. X_o	$X_i = 100$ $X_o = 0$–30 $Y_i = Y_o = 0$	0.00076 ±0.00010	0.0087 ±0.0009	$I/S =$ 12 ±2	$1/I =$ 115 ±12
	Net loss‡	$1/R$ vs. $1/X_i$	$X_o = 0$ $X_i = 6$–90 $Y_i = Y_o = 0$	0.41 ±0.02	0.0071 ±0.0017	$S/I =$ 58 ±16	$1/I =$ 140 ±34
	Sorbose inhibition*	$1/R$ vs. Y_o	$Y_o = Y_i = 0$–75 $X_o = 230$, $X_i = 0$			$I/S =$ 33 ±6	
	Exchange‡	$1/R$ vs. $1/X_i$	$X_o = Y_i = 0$ $X_i = Y_o = 20$–130	0.41 ±0.02	0.0056 ±0.0008	$S/I =$ 73 ±13	$1/I =$ 180 ±20

Data for sugar transport in human erythrocytes were plotted as indicated and straight lines fitted by least squares. The slope (S) and intercept (I) of each line were used as shown to calculate the half-saturation constants (K) and rate constants (V) in accordance with the simple carrier mechanism. Standard deviations follow each computed value. Temperature 20°C, pH 7.35. Rates (R) are expressed as mmoles/min per cell unit and concentrations as mmoles/liter. X refers to the sugar whose rate was measured and Y to the other sugar.

* Miller, 1968 *b*.

‡ This work.

§ Miller, 1965.

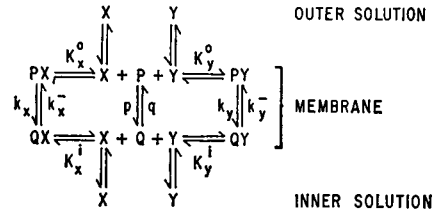

FIGURE 3 *Conformational transport model.* P and Q are two forms of the carrier protein such that the carrier site is exposed to the outer solution in form P and to the inner solution in form Q. Complex formation between P and Q and the substrates X and Y occurs with the affinity constants indicated (K_x^o, K_y^o, K_x^i, and K_y^i). The rate constants for the transition from forms PX, P, and PY to forms QX, Q, and QY are k_x, p, and k_y, while those for the reverse are k_x^-, q, and k_y^- respectively.

The basic carrier mechanism satisfies the first of these requirements in that it reduces to straight line expressions under the appropriate conditions (Miller, 1968 *b*, and equation 3); however it predicts that the four types of experiments should lead to the same value for the apparent affinity constant and the same value for the apparent rate constant, and since this is contrary to the data, it points out once again the inadequacy of the basic carrier mechanism.

To illustrate the use of these data further, however, let us test the model shown in Fig. 3. This model was first suggested by Hill and Kedem (1966) and is based on the concept that transport is the result of a conformational change in the carrier protein. This change in shape is postulated to occur in such a fashion that the carrier site is alternately exposed to the solutions on either side of the membrane. Support for this model has recently been provided by the observation of Krupka (1971) that the rate of reaction between the irreversible inhibitor 1-fluoro-2,4-dinitrobenzene and the sugar carrier is increased in the presence of the more readily transported sugars but is reduced in the presence of sugars (such as maltose) which combine with the carrier, but are not transported by it. As Krupka pointed out, this leads to the conclusion, in accordance with modern concepts of protein structure (Koshland et al., 1962), that the transport process most likely involves a change in conformation of a protein essential to the process.

Derivation of the Rate Equation

The rate of transport of substrate X into the cell can be seen by reference to Fig. 3 to be the difference between the rate of change of form PX into QX less the rate of the reverse, or in quantitative terms:

$$\frac{dx}{dt} = k_x[PX] - k_x^-[QX]. \tag{5}$$

Equation 5 is of little use in this form since $[PX]$ and $[QX]$ are unknown and

cannot be measured. They can be expressed in terms of the substrate concentrations (X_o and Y_o outside, X_i and Y_i inside), however, by introducing the following three sets of equations (Miller, 1969):

(*a*) *The mass law equations:*

$$K_x^o = \frac{X_o[P]}{[PX]}, \quad K_y^o = \frac{Y_o[P]}{[PY]}, \quad K_x^i = \frac{X_i[Q]}{[QX]}, \text{ and } K_y^i = \frac{Y_i[Q]}{[QY]}. \tag{6}$$

(*b*) *The carrier conservation equation:*

$$T = [P] + [PX] + [PY] + [Q] + [QX] + [QY]. \tag{7}$$

(*c*) *The steady-state equation:*

$$p[P] + k_x[PX] + k_y[PY] = q[Q] + k_x^-[QX] + k_y^-[QY]. \tag{8}$$

These equations may be combined to solve for [PX] and [QX] which in turn can be introduced into equation 5. For a single substrate (where $Y_o = Y_i = 0$) this procedure leads to:

$$\frac{dx}{dt} = \frac{T(qk_xK_x^iX_o - pk_x^-K_x^oX_i)}{(K_x^o + X_o)(qK_x^i + k_x^-X_i) + (pK_x^o + k_xX_o)(K_x^i + X_i)}. \tag{9}$$

It is well-known that this transport system is not active and therefore cannot cause a net movement of substrate to occur from a lower concentration to a higher one. In other words, when $X_o = X_i$ the flux (dx/dt) must be zero, which leads directly from equation 9 to a fourth set of equations:

(*d*) *The thermodynamic requirements:*

$$qk_xK_x^i = pk_x^-K_x^o \text{ and } qk_yK_y^i = pk_y^-K_y^o. \tag{10}$$

(Note that these expressions also arise from application of the principle of microscopic reversibility [Hill and Kedem, 1966]).

Now using equation 10 to eliminate k_x^- and k_y^-, we arrive at the final form of the equation for the rate of uptake of one substrate (X) in the presence of a second:

$$\frac{dx}{dt} = \frac{Tqk_xK_x^oK_y^oK_x^iK_y^i\{pK_y^o(X_o - X_i) + k_y(X_oY_i - X_iY_o)\}}{(qK_x^iK_y^iAD + pK_x^oK_y^oBC)}, \tag{11}$$

where $A = K_x^oK_y^o + K_y^oX_o + K_x^oY_o$,
$B = K_x^iK_y^i + K_y^iX_i + K_y^iY_i$,
$C = pK_x^oK_y^o + k_xK_y^oX_o + k_yK_x^oY_o$, and
$D = pK_x^oK_y^o + k_xK_y^oX_i + k_yK_x^oY_i$.

Testing the Rate Equation

Equations describing the linear plots which result from the various experimental techniques are obtained from equation 11 under the following conditions where R is the initial rate of loss of sugar (i.e., $R = -[dx/dt]_{t=0}$).

(*a*) *Sen-Widdas experiment.* $Y_o = Y_i = 0$ and $X_i \gg X_o$. X_i is also assumed to be $\gg K_x^o$ and K_x^i. $1/R$ is plotted against X_o.

(*b*) *Net loss measurements.* $Y_o = Y_i = X_o = 0$. Plot $1/R$ against $1/X_i$.

(*c*) *Sorbose inhibition.* Glucose concentration $Y = Y_o = Y_i$. K_x^i and K_x^o (affinity constants for sorbose) are unknown but are assumed to be very large so that K_x^i and $K_x^o \gg X_o$, X_i, Y_o, and Y_i. During exit measurements $X_o = 0$, while for uptake $X_i = 0$. Plot $1/R$ against Y.

(*d*) *Exchange experiments.* X_i (radioactive sugar concentration) $= Y_o$ (nonradioactive sugar concentration), $X_o = Y_i = 0$, $K_x^o = K_y^o$, $K_x^i = K_y^i$, and $k_x = k_y$. Plot $1/R$ against $1/X_i$.

The mechanism satisfies the immediate requirements of the data in that straight line functions are derived for all four conditions. The slope, intercept, and apparent kinetic constants obtained from these plots are given in Table IV. Comparing these to the experimental values in Table III shows that this mechanism corresponds more closely to the data than does the simple theory in at least two important respects. Firstly it predicts that different half-saturation values should be obtained from Sen-Widdas, net loss, and exchange experiments which accords with the results in Table III, while the simple theory predicts that these should all be the same. Secondly, this mechanism agrees with the data in suggesting that the rate constants

TABLE IV

EXPRESSIONS FOR S, I, K, AND V DERIVED ACCORDING TO THE CONFORMATIONAL MODEL

Type of experiment	Slope (S)	Intercept (I)	Apparent affinity constant K	Apparent rate constant V
Sen-Widdas ($1/R$ vs. X_o)	$\frac{pK_x^o + qK_x^i}{TpqK_x^oK_x^i}$	$\frac{p^2K_x^o + qk_xK_x^i}{Tpqk_xK_x^i}$	$\frac{p^2K_x^o + qk_xK_x^i}{pK_x^o + qK_x^i}$	$\frac{Tpqk_xK_x^i}{(p^2K_x^o + qk_xK_x^i)}$
Net loss ($1/R$ vs. $1/X_i$)	$\frac{(p+q)K_x^o}{Tqk_x}$	$\frac{p^2K_x^o + qk_xK_x^i}{Tpqk_xK_x^i}$	$\frac{p(p+q)K_x^oK_x^i}{(p^2K_x^o + qk_xK_x^i)}$	$\frac{Tpqk_xK_x^i}{(p^2K_x^o + qk_xK_x^i)}$
Sorbose inhibition ($1/R$ vs. Y_o)	—	—	$\frac{(p+q)K_y^oK_y^i}{(pK_y^o + qK_y^i)}$	—
Exchange ($1/R$ vs. $1/X_i$)	$\frac{(p+q)K_x^o}{Tqk_x}$	$\frac{(pK_x^o + qK_x^i)}{Tqk_xK_x^i}$	$\frac{(p+q)K_x^oK_x^i}{(pK_x^o + qK_x^i)}$	$\frac{Tqk_xK_x^i}{(pK_x^o + qK_x^i)}$

derived from the Sen-Widdas and net loss experiments should be the same, but should differ from the value obtained by exchange, while again the simple theory predicts a common value for all three.

There are, however, certain discrepancies between the results and the theory. The most obvious of these is that the affinity constants obtained by sorbose inhibition and exchange are not identical as predicted by the theory. It is difficult to see why this is not so, since superficially, at least, both these experiments appear to be measuring the same thing, i.e., the fraction of sites occupied by the test sugar while it is present on both sides of the membrane. The only difference between them is that the fraction of sites *unoccupied* by the test sugar is measured as a function of the transport rate of sorbose in the first case and the fraction of *occupied* sites is measured as a function of the transport rate of the test sugar itself in the second case.

Were this the only discrepancy we might dismiss it by assuming that two carrier systems are present, one of which carries aldoses only, while the second carries ketoses but is inhibited by aldoses for which it has affinities different to those of the first. There is, however, a second and possibly much more significant inconsistency involving the *slopes* of the plots. Table IV predicts that these should be the same whether derived from net loss or from exchange data. Table III and Fig. 2 show this to be the case for galactose but, interestingly enough, it is not the case for glucose (Table III and Fig. 1), a fact which may indicate a fundamental difference between the mechanisms of transport of these two sugars.

In spite of its failings, the above mechanism conforms more closely to the data than any of a large number considered. This includes the mechanisms of Lieb and Stein (1970) and of Naftalin (1970) whose theories might nevertheless be modified to accommodate the new data; however this task is best left to the original authors who have a greater familiarity with their mechanisms.

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