THE KINETICS OF SELECTIVE BIOLOGICAL TRANSPORT

IV. Assessment of Three Carrier Systems Using the Erythrocyte-Monosaccharide Transport Data

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ABSTRACT An assessment is made of the possible validity of three transport mechanisms proposed to describe the movement of monosaccharides in human erythrocytes. These mechanisms consist of a simple carrier system and two mechanisms derived from it, one of which postulates that the free carrier moves more slowly than the complexed carrier, and the other that slow diffusion steps occur at each surface of the membrane. None of these schemes provides an adequate quantitative description of the data so that all must be discarded, leaving the question of the mechanism still open to speculation.

INTRODUCTION

The purpose of this communication is to illustrate the use of the data published in the previous paper (1) as a test of three proposed mechanisms of transport. Consideration is given firstly to the simple carrier mechanism and then to two other mechanisms derived from, and suggested by, the failure of the simple carrier mechanism to provide an adequate explanation of this data.

Notation

The following notation will be used in the present communication:

x, y	The amounts (mmoles) of the substrates or permeating species present in a cell unit.[1]
X_o, Y_o	The concentrations of substrates in the external medium (mM).
X_i, Y_i	The concentrations of the substrates in the cell water (mM).
$\bar{X}_o$, $\bar{Y}_o$	The concentrations of the substrates at the outer membrane surface (mM).

[1] A cell unit is defined as a quantity of cells whose solvent water volume is 1 liter under isotonic conditions (1).

$\bar{X}_i$, $\bar{Y}_i$ The concentrations of the substrates at the inner membrane surface (mM).

P The free carrier.

P_x, P_y The substrate-carrier complexes.

T The total concentration of all forms of the carrier, both free and complexed.

K_x, K_y The dissociation constants (or affinity constants) for the complexes.

D_x, D_y The diffusion coefficient for the substrates in both the external and internal solutions.

D_p The transfer coefficient for the free carrier through the membrane.

D_{px}, D_{py} The transfer coefficients for the complexes through the membrane.

V The volume of solvent water in a cell unit.

E The total osmolarity of all the nonpenetrating species present in the external solution.

e The total number of osmoles of nonpenetrating species present in a cell unit.

THE GENERAL MECHANISM OF CARRIER TRANSPORT

The carrier mechanism envisages permeation into the cell in the following steps, shown schematically in Fig. 1;

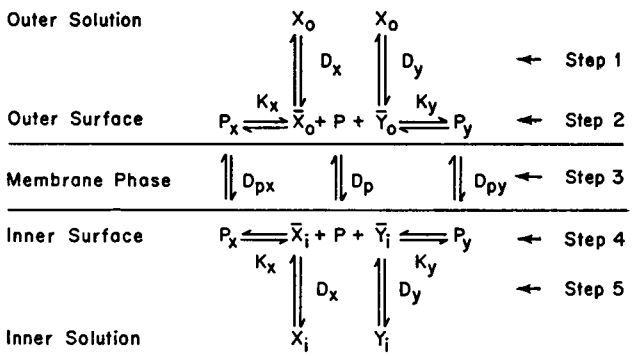

FIGURE 1

Step 1 Diffusion of the substrates from the bulk of the outer solution to the outer membrane surface.

Step 2 Combination of the substrates with a carrier (P) in the membrane to form complexes (P_x and P_y).

Step 3 Movement of the complexes (or free carriers) so that they present themselves to the inner surface of the membrane.

Step 4 Decomposition of the complexes to release the substrates at the inner surface.

Step 5 Diffusion of the substrates from the membrane surface into the bulk of the cell water. All these steps are considered to be reversible so that outward permeation is simply the reverse of the above steps. The three mechanisms to be considered here all derive from the general mechanism and differ only in the assumptions which each makes about the mechanism. These will now be discussed.

THE SIMPLE CARRIER MECHANISM

This mechanism was originally suggested by Widdas (2), and although he and a number of other workers have previously derived the appropriate equations, the derivation will again, for convenience, be presented here.

Assumptions

The assumptions on which this derivation is based are as follows:

1. P, P_x, and P_y are confined to the membrane phase but all can move or rotate freely within it. Uncomplexed X and Y are excluded from the membrane phase.
2. The rate of transfer of the complexes (P_x, P_y) and the free carrier (P) between the two surfaces of the membrane are equal to each other and independent of direction. Thus $D_{px} = D_{py} = D_p$ for inward and outward transfer.
3. The rate of diffusion (steps 1 and 5) is very much greater than the rate of transfer of the carrier (step 3).
4. The rates of formation and dissociation of the complexes (steps 2 and 4) are very large compared to diffusion or transfer (steps 1, 3, and 5).
5. The affinity of the substrate for the carrier is the same at both surfaces of the membrane.
6. The amount of carrier present in a given area of the membrane remains constant, i.e., it is neither being synthesized nor destroyed.
7. The rate with which the carrier and complexes cross the membrane is proportional to the difference of their concentrations at the two membrane surfaces.

Derivation

Since step 3 is considered to be rate limiting, the rate with which the amount of a substrate present in the cells increases will be that of the net rate of transfer of its complex inward which in turn is:

$$\frac{\partial x}{\partial t} = D_{px}([P_x]_o - [P_x]_i) \quad \text{and} \quad \frac{\partial y}{\partial t} = D_{py}([P_y]_o - [P_y]_i) \tag{1}$$

where $[P_x]_o$ and $[P_y]_o$ are the concentrations of complexes at the outer surface and $[P_x]_i$ and $[P_y]_i$ are those at the inner surface.

Assumption 3 allows us to equate the concentrations of substrates at the surfaces to those in the bulk (i.e. $\bar{X}_o = X_o$, $\bar{X}_i = X_i$, $\bar{Y}_o = Y_o$, and $\bar{Y}_i = Y_i$) while assumptions 4 and 5 make it possible to relate the concentrations of the various species at the two membrane surfaces through the mass law expressions:

$$K_x = \frac{X_o \cdot [P]_o}{[P_x]_o} = \frac{X_i \cdot [P]_i}{[P_x]_i} \quad \text{and} \quad K_y = \frac{Y_o \cdot [P]_o}{[P_y]_o} = \frac{Y_i \cdot [P]_i}{[P_y]_i}. \tag{2}$$

The total concentration of all forms of the carrier present in the membrane is T. From assumption 2 we can conclude that since the transfer rate of all forms is equal, then the total concentration of carrier will be uniform throughout the membrane, and therefore at any time the concentration of carrier at the outside of the membrane will equal that at the inside, or:

$$T = [P]_o + [P_x]_o + [P_y]_o = [P]_i + [P_x]_i + [P_y]_i. \tag{3}$$

From equation 2 it can be seen that

$$[P]_o = \frac{K_x}{X_o}[P_x]_o = \frac{K_y}{Y_o}[P_y]_o; \quad \text{and therefore} \quad [P_y]_o = \frac{K_x Y_o}{K_y X_o}[P_x]_o. \tag{4}$$

Thus by substituting these expressions into equation 3 we can solve for $[P_x]_o$, and through a similar manipulation for $[P_x]_i$, $[P_y]_o$, and $[P_y]_i$. Introducing these expressions into equation 1 now gives us the rate equations

$$\frac{\partial x}{\partial t} = k\left[\frac{X_o/K_x}{X_o/K_x + Y_o/K_y + 1} - \frac{X_i/K_x}{X_i/K_x + Y_i/K_y + 1}\right] \tag{5}$$

and

$$\frac{\partial y}{\partial t} = k\left[\frac{Y_o/K_y}{X_o/K_x + Y_o/K_y + 1} - \frac{Y_i/K_y}{X_i/K_x + Y_i/K_y + 1}\right] \tag{6}$$

where $k = D_p T$.

By definition $X_i = x/V$ and $Y_i = y/V$, so that for cells having a cell wall, and consequently a constant volume, V may be made equal to one, allowing the direct substitutions $X_i = x$ and $Y_i = y$ to be made into equations 5 and 6. Unfortunately red cells are not so protected but rather act as osmometers changing their volume with changes in sugar content. Water movements in red cells are very rapid and occur in such a direction as to equalize the internal osmolarity with that outside. Thus we may write that to a first approximation

$$X_o + Y_o + E = \frac{x + y + e}{V} \quad \text{or} \quad V = \frac{x + y + e}{X_o + Y_o + E}$$

and

$$X_i = \left(\frac{X_o + Y_o + E}{x + y + e}\right)x \quad \text{and} \quad Y_i = \left(\frac{X_o + Y_o + E}{x + y + e}\right)y. \tag{7}$$

If all concentrations are expressed in mmolar quantities and the buffer used is isotonic then $E = e = 310$ mosmoles, while if all concentrations are expressed in ideal isomolar quantities, both E and e are unity. The final rate equations are obtained by substituting equation 7 and the appropriate values for E and e into equations 5 and 6.

Now if the original mechanism is correct then these equations should be quantitatively consistent with the data reported in the accompanying communication (1) and we may now apply this criterion in the following way.

Quantitative Tests.

The first step is to determine the values of the constants in equations 5 and 6 (i.e., k and the dissociation constants K_x, etc.). This can be done in two ways:

Determination of k and K for Glucose by Experiments of Type I. In this type of experiment cells were first equilibrated with glucose* at a concentration of 130 mM, then transferred to a solution at a much lower glucose* concentration, and the initial rate of loss of sugar from the cells measured. By varying the concentration in the second solution the rate of loss of sugar from loaded cells was measured as a function of the external sugar concentration. Since we are dealing here with only a single sugar, $y = Y_o = 0$ in equation 5. Furthermore, provided $X_i \gg K_x$, this equation reduces to

$$\left(\frac{dx}{dt}\right)_{t=0} = -\frac{kK_x}{X_o + K_x} = -R \tag{8}$$

where R = the initial rate of loss. Finally, by inverting equation 8 we obtain the expression

$$\frac{1}{R} = \frac{X_o}{k \cdot K_x} + \frac{1}{k}. \tag{9}$$

This expression predicts that a plot of the reciprocal of the rates listed in Table I (reference 1) against the external sugar concentration should give a straight line such that $k = 1$ /intercept and K_x = intercept/ slope. Such a plot is shown in Fig. 2 for sugar concentrations up to 10 mM and was fitted by a least squares method yielding the following values:

$$K = 1.8 \pm 0.3 \text{ mM and } k = 104 \pm 12 \text{ mmoles/min/cell unit.}$$

By comparison, Sen and Widdas (8) obtained 1.7 mM for K and 83 mmoles/min^{-1} for k at 20°C while Harris (9) found 1.86 mM and 210 mmoles/min^{-1}, respectively.

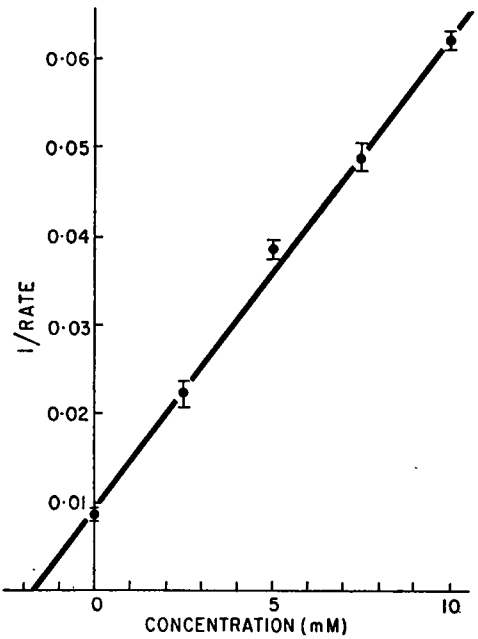

FIGURE 2 Plot of the data from Table I (1). The straight line was fitted to the experimental points by a least squares method. Intercept = 0.0096 ± 0.0012 min 1 mmoles^{-1}. Slope = 0.0053 ± 0.0002 1^{2} min mmoles^{-2}.

Determination of the Affinity Constants by Inhibition of Sorbose Transport. The inhibition of sorbose by sugars having a much higher affinity has been used to determine the affinity constant of the inhibiting sugar (3, 4, 7). We may express this type of experiment in a quantitative manner through equation 5 where X refers to sorbose and Y to the inhibiting sugar. The procedure is to equilibrate the cells with inhibitor, then measure the rate of uptake of sorbose at zero time. If R_i is the initial rate of uptake $(dx/dt)_{t=0}$, at which time $X_i = 0$, then since $K_x \gg X_o$, $X_o/K_x \ll 1$, and equation 5 on inversion becomes,

$$\frac{1}{R_i} = \frac{K_x}{kX_oK_y} \cdot Y + \frac{K_x}{kX_o}. \tag{10}$$

Similarly, for experiments in which the loss of sorbose from loaded cells is measured, $X_o = 0$ initially and expression 11, where R_o = rate of sorbose loss, is obtained.

$$\frac{1}{R_o} = \frac{K_x}{kX_iK_y} \cdot Y + \frac{K_x}{kX_i}. \tag{11}$$

Equations 10 and 11 predict that a plot of the reciprocal of the rate of sorbose movement against the concentration of the inhibiting sugar will be linear so that

TABLE VI

AFFINITY CONSTANTS DETERMINED BY THE INHIBITION OF SORBOSE TRANSPORT. DATA FROM TABLE II (1)

Inhibiting sugar	Direction of flow	Affinity constant
Glucose	Exit	17 ± 2 mM
Glucose	Uptake	23 ± 5 mM
Mannose	Uptake	30 ± 5 mM
Galactose	Uptake	33 ± 6 mM

the glucose affinity constant can be found from the relationship K_y = intercept/slope. Unfortunately the rate constant, k, cannot be found from these experiments since K_x for sorbose is too large to measure and is therefore unknown.

The data in Table II (reference 1) have been plotted in this fashion and fitted to a straight line from which the affinity constants listed in Table VI have been obtained. The glucose constants can be seen to agree with each other within the limits of error, suggesting that transport inward and outward are equivalent. However, they are about ten times that found by Type I experiments and here we encounter our first problem with the simple mechanism since the two methods should give the same value according to theory. A similar discrepancy is found for the affinity constants of mannose and galactose for which values of 7 mM and 12 mM, respectively, were found previously by Type I experiments (10).

The glucose results in Table VI agree reasonably well with those of Levine and Stein (7) who found for K the values 13 mM at 25°C and 19 mM at 13°C

The Rate of Exchange vs. Net Flux (Type III Experiment). According to the theory outlined above, it should make no difference to the rate with which a substrate passes through the membrane what substances are present on the opposite side, provided its own concentration at this point is zero. Thus if glucose* is escaping from loaded cells the presence or absence of glucose in the outside solution should be immaterial provided $[\text{glucose}^*]_o = 0$. From Table III (1) however, it can be seen that this is not so and that glucose* escapes into solutions containing any of the sugars tested at much higher rates than into sugar free solutions. This cannot be accounted for by the simple theory.

Determination of Rate Constants from Rates of Exchange (Type IV Experiments). These experiments are carried out by following the loss of radioactive glucose into solutions of nonactive sugar of the same concentration. Letting X represent glucose* and Y = glucose, then under the conditions of the experiment at zero time $X_i = Y_o$, $X_o = Y_i = 0$ and $K_x = K_y$. Equation 5 then reduces to

$$\left(\frac{dx}{dt}\right) = R_e = \frac{kX_i}{X_i + K_x} \quad \text{and} \quad \frac{1}{R_e} = \frac{K_x}{k} \cdot \frac{1}{X_i} + \frac{1}{k}. \tag{12}$$

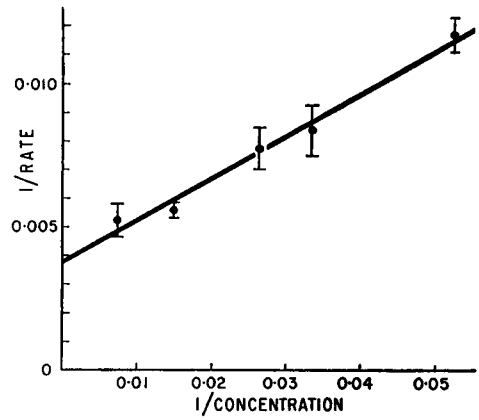

FIGURE 3 Plot of the data from Table IV (1). The straight line was fitted to the experimental points by a least squares method. Intercept = 0.0038 ± 0.0004 1 min mmoles^{-1}. Slope = 0.145 ± 0.011 min.

Thus, a plot of the reciprocal of the rate against the reciprocal of the concentration should give a straight line of intercept $1/k$ and slope K_s/k. In Fig. 3 such a plot has been drawn having a slope of 0.14 ± 0.01 min and intercept 0.0038 ± 0.0004 min 1 mmoles^{-1} which leads to a value of 260 ± 30 mmoles/min cell unit for k and K = 38 ± 3 mM, values which are at wide variance with those determined above.

Levine, Oxender, and Stein (4) have also performed this experiment but have not plotted their results in this way. However, if the data plotted in Fig. 3 (curve 2) of their paper are replotted according to (12) a straight line results from which the values K = 105 mM and k = 450 mmoles/min/1 cell water may be derived. Although these results do not agree quantitatively with the present values, they further illustrate the divergence from values determined by other methods.

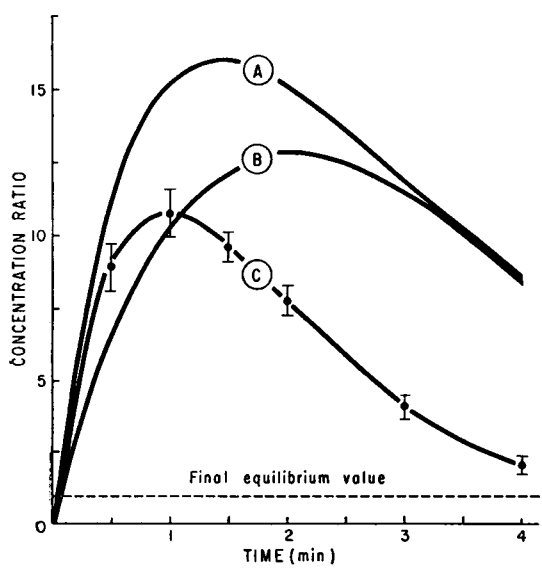

FIGURE 4 Plot of the theoretical and experimental curves for the counter transport of glucose. Curve *A*. Theoretical curve calculated according to the fast complex mechanism. Curve *B*. Theoretical curve calculated according to the simple carrier mechanism. Curve *C* and points. Data from Table V (1).

Counterflow Experiments (Type V Experiments). The simultaneous integration of equations 5 and 6 is required to provide expressions for the quantitative description of counter transport data provided in Table V.[1] These expressions have already been published (6) and have been used in deriving the theoretical curve plotted in Fig. 4 (curve *B*) using a value of 1.8 mM for the affinity constant and 100 mmoles/min/cell unit for the rate constant. The experimental points from Table V (1) are also included for comparison. The divergence between the theoretical curve and experimental results is quite large and would be even greater if K = 20 mM were used instead of the lower value.

Conclusions

The Simple Theory fails to provide a total quantitative explanation of the data listed in the previous communication in four respects:

1. It does not explain why the net transport of sugars is slower than exchange.
2. The affinity constants measured by three different methods do not agree, ranging in value from 1.8 to 38 mM for glucose.
3. The rate constants measured by two methods are at variance by a factor of 2.6 (for glucose).
4. Agreement between the theory and the experimental measurements of counter transport is very poor in the case of glucose but quite good in the case of mannose and galactose (6).

In spite of these faults, however, this mechanism goes far toward a description of all the features exhibited by the data. This suggests that it may be fundamentally correct and that all that is necessary is to evolve a slightly more elaborate version of the basic mechanism. This we shall do in the next two sections by examining the consequences of eliminating some of the simplifying assumptions made at the beginning of this section.

THE FAST COMPLEX MECHANISM

Mawe and Hempling (5) and Levine, Oxender, and Stein (4) on observing that glucose* — glucose exchange is greater than the loss of glucose* into sugar free solution, concluded that the complex moves more quickly than the free carrier. This is contrary to assumption 2 above, and we can test this idea quantitatively by deriving the rate equations again on the assumption that $D_{px} = D_{py} > D_p$, with all other assumptions still considered valid.

Under these conditions, when net flow of substrate occurs, the carrier will pass rapidly as a complex to the side of the membrane having the lower substrate concentration but return more slowly in the free form. Thus the carrier will tend to pile up on one side of the membrane so that equation 3 will no longer be valid. However, the total *amount* of carrier will still remain the same throughout the membrane and we may now write

$$2T = [P]_o + [P_x]_o + [P_y]_o + [P]_i + [P_x]_i + [P_y]_i . \qquad (13)$$

Derivation of Equations

Since the number of carriers present in a unit area of membrane is small by comparison with the number of substrate molecules passing through the membrane, then for any given substrate concentration difference across the membrane, a steady state will rapidly be established in which the net rate of movement of free carriers in one direction through the membrane equals the net rate of movement of complexed carriers in the other. This may be expressed mathematically as

$$D_p([P]_o - [P]_i) = D_{px}([P_x]_i - [P_x]_o) + D_{px}([P_y]_i - [P_y]_o). \tag{14}$$

Here we have added the assumption that $D_{px} = D_{py}$, i.e., that all complexes move at the same rate. Substituting the appropriate identities (such as those in equation 4) into 14 and rearranging gives

$$[P_x]_i = \left[\frac{rK_yX_o + rK_xY_o + K_xK_y}{rK_yX_i + rK_xY_i + K_xK_y}\right]\frac{X_i}{X_o}\cdot[P_x]_o \tag{15}$$

where $r = D_x/D_p$. A similar substitution into equation 13 followed by replacement of $[P_x]_i$ by expression 15 allows us to solve for $[P_x]_o$, which can then be introduced into equation 15 to give $[P_x]_i$. These last two terms substituted into equation 1 lead to the rate equation 16 for substrate X and by analogy to equation 17 the rate equation for substrate Y.

$$\frac{\partial x}{\partial t} = 2kK_xK_y[(rY_i + K_y)X_o - (rY_o + K_y)X_i]/A \tag{16}$$

$$\frac{\partial y}{\partial t} = 2kK_xK_y[(rX_i + K_x)Y_o - (rX_o + K_x)Y_i]/A \tag{17}$$

where

$$A = (K_yX_o + K_xY_o + K_xK_y)(rK_yX_i + rK_xY_i + K_xK_y) + (K_yX_i + K_xY_i + K_xK_y)(rK_yX_o + rK_xY_o + K_xK_y).$$

For a single substrate X, $Y_o = Y_i = 0$ and

$$\frac{dx}{dt} = \frac{2kK_x(X_o - X_i)}{(X_o + K_x)(rX_i + K_x) + (X_i + K_x)(rX_o + K_x)}. \tag{18}$$

Quantitative Test of the Equations

These equations may now be used to provide a quantitative test of the assumptions against the data in the previous paper.

Determination of the Constants by Experiments of Types I and III

In the experiments of Type I a single substrate is involved with $X_i \gg K_x$ and X_o, so that equation 18 as before reduces to the straight line function

$$1/-(dx/dt) = \frac{1}{R} = \frac{r}{kK_x} X_o + \frac{(r+1)}{2k}. \tag{19}$$

From this in turn we see that

$$k = \frac{r+1}{2} \cdot \frac{1}{\text{Intercept}} \quad \text{and} \quad K_x = \frac{2r}{r+1} \cdot \frac{\text{Intercept}}{\text{Slope}}. \tag{20}$$

Now in order to solve for these constants we must first determine r, making use of the data in the first two lines of Table III (1). For glucose* exit into glucose free solution (Type I) the conditions are $X_o = Y_o = Y_i = 0$ and $X_i \gg K_x$, so that expression 18 becomes

$$-(dx/dt)_{i=0}^{y=0} = R_o = 2k/(r+1).$$

For glucose* exit into glucose solution (Type III) the conditions are $X_o = Y_i = 0$ and $X_i = Y_o \gg K_x$ and K_y, reducing equation 16 to

$$-(dx/dt)_{i=0}^{y} = R_y = k.$$

Thus the ratio of the rate of loss in these two cases becomes (using the values given in the first two lines of Table III, reference 1)

$$R_y/R_o = (r+1)/2 = (190 \pm 20)/(100 \pm 9) = 1.9 \pm 0.3$$

and $r = 2.8 \pm 0.4$ which is the relative rate of movement of the complex to that of the free carrier. We may now return to expression 20 to solve for the two constants using the results of Fig. 2 to obtain $k = 200 \pm 25$ mmoles/min/cell unit and $K_x = 2.6 \pm 0.2$ mM.

Determination of the Transport Constants by the Data of Table II (1)

In these experiments the exit of sorbose (X) was followed in the presence of glucose (Y). Our conditions were $X_o = 0$, $Y_i = Y_o = Y$, and $K_x \gg X_i$ for the exit experiments and $X_i = 0$, $Y_i = Y_o = Y$, and $K_x \gg X_o$ for uptake. Under these conditions equation 16 reduces to equations 10 and 11 so that the values for the affinity constants listed in Table VI are applicable here. Thus although the Fast Complex Mechanism predicts values for the affinity constants as determined by Type I and II experiments which are closer together than those obtained by the simple system, the discrepancy is still large (i.e., 2.6 and ~20 for glucose)

A further consideration of the data in Table III (1) reveals an even more serious problem for the Fast Complex Mechanism. Here it can be seen that the rate of loss of glucose* into either mannose or galactose is significantly higher than it is into glucose. This cannot be due to mannose and galactose forming more rapidly moving complexes since if this were so, then mannose* — mannose and galactose* — galactose exchanges would be even faster than the glucose* — glucose exchange, a situation which Table III clearly reveals is not so.

Type IV Experiments

As in the case of the simple theory, the conditions imposed in these experiments reduces equation 16 to one identical to equation 12 and values for the constants obtained from Fig. 3 are the same as those found from equation 12, namely 38 mM for K and 260 mmoles/min/cell unit for k. Comparing these values to those derived from Type I and II experiments reveals that while the rate constants are reasonably close, the affinity constant is much larger and once again the Fast Complex Mechanism has been unable to satisfy the data.

Type V Experiments

The theoretical curve predicted by the fast complex mechanism for the counter-transport data of Table V[1] is obtained by the simultaneous integration of equations 16 and 17. This has been carried out by the Runge-Kutta numerical method (11) on a digital computer using the following values:

Glucose concentrations = $X_o = 0$, $X_i = 130$ mM (initially)

Glucose* concentrations = $Y_o = 4.3$, $Y_i = 0$ (initially)

$k = 200$ mmoles/min, $K_x = K_y = 2.6$ mM, $r = 2.8$

The results of this operation are plotted in Fig. 4 (curve A) along with the experimental points listed in Table V.[1] Again the deviation of the theoretical prediction from experiment is obvious. Using the other values determined for K and k provides an equally bad or worse fit between the experimental and theoretical values.

Conclusions

This mechanism has an advantage over the simple theory in that it provides a consistent value for the rate constant k, as measured with glucose, irrespective of the presence or absence of this sugar in the external solution. On the other hand, it can still be faulted on three points:

1. It provides no explanation for the increased rates of exchange between unlike sugars over those between similar sugars (Table III, reference 1).
2. The value of the affinity constant obtained by three different methods do not agree.
3. It does not provide an adequate quantitative description of the counter flow results.

THE DIFFUSION LIMITED MECHANISM

The main objection to the simple theory is its inability to explain the rate of movement of a sugar as a function of the amount of a second sugar present on the trans side of the membrane. We have seen how the elimination of assumption 2 of the simple theory is inadequate to meet this objection and now examine assumption 3 in the same light. Referring to the mechanism in Fig. 1, let us suppose that the diffusion steps 1 and 5 are not necessarily fast as compared to 3, and consider the movement of substrate X outward through the membrane under these conditions. If, on arriving at the outside of the membrane and being released from the carrier, X does not rapidly diffuse away, the probability that it will recombine with the carrier and return to the inside becomes appreciable, with the result that the system operates at less than 100% efficiency. Suppose now there is a second substrate Y on the outside but not on the inside of the cell. Then X upon being released on the outside will be less able to reform the complex, since in this process, it must compete with Y. In this way the back diffusion of X is reduced, increasing its net transport through the membrane. It will still not be maximum, however, as Y at the inner surface of the membrane will also compete with X reducing its outward movement. Now if Y is a substrate which has a somewhat lower affinity than X it will prevent the reentry of X at the outside surface by virtue of its high concentration at this point, but will not compete as readily at the inner surface, resulting in a further increase in net movement of X. This mechanism then can be seen to provide a qualitative explanation of the results in Table III (1), predicting firstly, that the rate of movement of glucose is increased by the presence of glucose on the opposite side of the membrane, and secondly that such increase will be greater for a second sugar of lower affinity such as mannose or galactose. Qualitatively then, this mechanism appears more promising than those discussed above, but it must still meet the quantitative requirements of our test.

The basic rate equations may be derived on the assumption that for any flux condition a steady state will rapidly evolve under which the rates of steps 1, 3, and 5 become equal so that

$$\frac{\partial x}{\partial t} = D_x(X_o - \bar{X}_o) \tag{21}$$

$$= k\left[\frac{\bar{X}_o/K_x}{\bar{X}_o/K_x + \bar{Y}_o/K_y + 1} - \frac{\bar{X}_i/K_x}{\bar{X}_i/K_x + \bar{Y}_i/K_y + 1}\right] \tag{22}$$

$$= D_x(\bar{X}_i - X_i) \tag{23}$$

with similar equations applying to the Y component. Solutions of these equations may be readily effected following substitution of $\bar{X}_i = X_o + X_i - \bar{X}_o$ (from equations 21 and 23) into equation 22, solving for $\bar{X}_o$ in equations 21 and 22 by the Newton-Raphson method (11) and substituting this value into equation 21 to ob-

tain the flux rate of x. When compound Y is present this procedure must be carried out with the simultaneous solution of the equivalent Y expressions.

An extensive mathematical analysis of this mechanism has been completed but we shall not burden the reader with the details of this rather tedious process. Suffice it to say that while values for the various constants (D_x, k, K_x, and K_y) can be found which will give results consistent with the data of one or two types of experiment, no values could be found to give simultaneous agreement for all experiments, so that this mechanism too must be ruled inadequate.

GENERAL CONCLUSIONS

The requirement that a mechanism should demonstrate quantitative accord with the data of the previous paper appears to be a fairly stringent test of that mechanism. It is however, not the ultimate test, since should a kinetic scheme be devised which could pass this test, further more definitive experiments would undoubtedly be suggested by the scheme itself. At this point all that can be said is that none of the better known schemes appears to satisfy completely the requirements of the test and that the mechanism of sugar transport in human red cells remains open to speculation.

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