THE KINETICS OF SELECTIVE BIOLOGICAL TRANSPORT

II. Equations for Induced Uphill Transport of Sugars in Human Erythrocytes

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ABSTRACT Equations describing the movement of sugars during induced uphill transport were derived on the assumption of a simple carrier transport mechanism and subjected to experimental verification. Since there was good agreement between the experimental points and the theoretical curves, no changes in the original postulates were required.

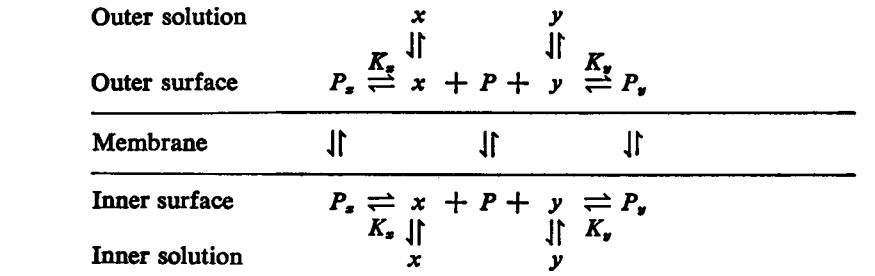
Monosaccharides normally tend to move through the erythrocyte membrane in such a direction as to eliminate any concentration difference across the membrane. In the presence of a second sugar however, they can be made to move in the opposite direction (*i.e.*, against a concentration gradient) in what is termed "induced uphill transport."

The kinetics of the normal transport of single sugars is relatively simple and is readily described in a quantitative manner by equations derived from diverse models; thus such equations do not provide unambiguous proof of the theory from which they have been derived. The movements of sugars during induced uphill transport (Rosenberg and Wilbrandt 1957) however, are much more complex, and an ability to provide quantitative predictations of this phenomenon should constitute a more rigorous test of any proposed mechanism. The present work tests the validity of the carrier model discussed by a number of authors [see, for example, LeFevre (1954) and Wilbrandt (1954)], and outlined in the previous publication (Miller, 1965), by comparing the experimentally determined sugar movements with those predicted by appropriate equations derived from the model.

THEORETICAL

The assumptions and notation involved in this work are those of the previous paper

in this series (Miller, 1965). The presence of the two substrates x and y results in the following expanded mechanism:



As before, a single rate constant, k, is involved but here two affinity constants will be used:

$$K_x = \frac{[\bar{x}][\bar{P}]}{[\bar{P}_x]} = \frac{[x][P]}{[P_x]} \text{ and } K_y = \frac{[\bar{y}][\bar{P}]}{[\bar{P}_y]} = \frac{[y][P]}{[P_y]} \tag{1}$$

The total carrier in this case is

$$T = [\bar{P}] + [\bar{P}_x] + [\bar{P}_y] = [P] + [P_x] + [P_y] \tag{2}$$

and the fluxes

$$\frac{dx}{dt} = D([\bar{P}_x] - [P_x]) \text{ and } \frac{dy}{dt} = D([\bar{P}_y] - [P_y]) \tag{3}$$

Combining expressions (1) through (3) results in the flux equations (4) and (5):

$$\frac{dx}{dt} = k\left(\frac{[\bar{x}]/K_x}{[\bar{x}]/K_x + [\bar{y}]/K_y + 1} - \frac{[x]/K_x}{[x]/K_x + [y]/K_y + 1}\right) \tag{4}$$

$$\frac{dy}{dt} = k\left(\frac{[\bar{y}]/K_y}{[\bar{x}]/K_x + [\bar{y}]/K_y + 1} - \frac{[y]/K_y}{[x]/K_x + [y]/K_y + 1}\right) \tag{5}$$

There are two experimental methods by which these equations may be tested.

(*a*) The cells may be loaded with substrate x and then transferred to a second solution containing y but no x. Provided the volume of solution is high enough, $[\bar{x}]$ will be zero throughout the experiment and $[\bar{y}]$ will be constant. If y is radioactive then samples may be taken at various times and assayed to give the time course of y. As will be shown, equations (4) and (5) predict that provided $[x]$ is initially high and $[\bar{y}]$ relatively low, $[y]$ will rise to a high value, and then as $[x]$ decreases to zero, will drop to its ultimate equilibrium value.

(*b*) The cells may be equilibrated with a low concentration of x in the absence of y and then y may be added to the suspending medium bringing it to a relatively high concentration. This, as Rosenberg and Wilbrandt (1957) have demonstrated, results in a rapid loss of x from the cell followed by a slow return as $[\bar{y}]$ approaches $[y]$.

Method (*a*) has the advantage of greater mathematical simplicity since with $[\bar{x}] = 0$ the first term in the brackets in equation (4) disappears. Thus if we let $[x] = x/V$, $[y] = y/V$ where $V = (1 + x + y)/(E + [\bar{y}])$, then divide equation (5) by equation (4) and integrate within the limits $y = 0$ when $x = 0$ (see Appendix), the ratio of y to its ultimate equilibrium value y_∞ will be given by the expresson

$$y/y_\infty = (Bx_0 - 1)(x/x_0)^m - Bx + 1 \tag{6}$$

where

$$B = \frac{(E + Ey_\infty + K_x)}{(E + Ey_\infty + K_y)y_\infty + K_y - K_x} \text{ and } m = \frac{K_x}{(1 + y_\infty)(Ey_\infty + K_y)}$$

It is not possible to solve for x as a function of y and thus integrate equation (5) directly to give y in terms of t, but y may be eliminated from equation (4) which may then be integrated to give t as a function of x:

$$kt = f \ln (x_0/x) + g(x_0 - x) + h[1 - (x/x_0)^m] \tag{7}$$

where

$$f = K_x/K_y\left(\frac{Ey_\infty + K_y}{E}\right)$$

$$g = \frac{K_y(E + Ey_\infty + K_x) - By_\infty K_x(E + Ey_\infty + K_y)}{K_y(E + Ey_\infty)}$$

$$h = K_x/K_y\left(\frac{E + Ey_\infty + K_y}{E + Ey_\infty}\right)\frac{y_\infty(Bx_0 - 1)}{m}$$

From equations (6) and (7), plots may be made of y/y_∞ as a function of t, by selecting a series of values for x ranging between x_0 and zero, and solving for the corresponding y/y_∞ and t values. These will, of course, correspond to each other, and so may be plotted against each other. Some typical curves of sugar uptake in erythrocytes plotted in this way are presented in Fig. 1 along with curves showing the uptake into initially sugar-free cells.

Method (*b*) results in complex equations implicit in x and y which appear to be resolvable only by the use of computers. It was felt, however, that testing by this method is not necessary since experimental verification of equations (6) and (7) should be a sufficient quantitative test of the mechanism proposed.

EXPERIMENTAL

The isotope method described in the previous paper (Miller 1965) was employed with slight variations. All tests were made on human erythrocytes using mannose and galactose at 20°C since this resulted in sugar movements at measurable rates.

A typical experiment might run as follows: about 0.2 ml of washed cells, whose

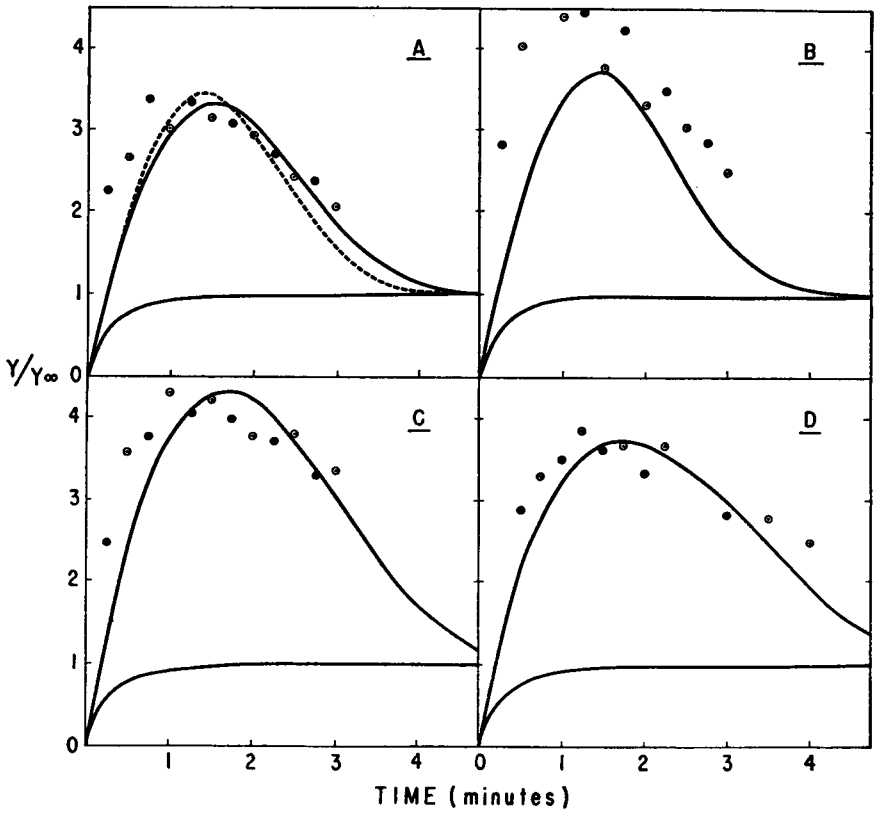


FIGURE 1 Theoretical curves for the uptake of radioactive sugars by human erythrocytes at 20°C. *Lower curves;* Theoretical curves of sugar uptake plotted according to equation (7), (Miller, 1965). A and B uptake of galactose and C and D uptake of mannose into initially sugar-free cells. *Upper solid curves;* A: Uptake of galactose* into cells loaded with galactose. B: Uptake of galactose* into cells loaded with mannose. C: Uptake of mannose* into cells loaded with mannose. D: Uptake of mannose* into cells loaded with galactose. Curves calculated for k = 0.37 isotone minute^{-1}, $E = 1$, $y_\infty = [\bar{y}] = 0.04$ isotone 1^{-1}, $x_o = 0.4$ isotone 1^{-1}, $k = 0.021$ isotone 1^{-1} for mannose and K = 0.038 isotone 1^{-1} for galactose (K = 0.034 isotone 1^{-1} for broken line curve in A). The experimental points from two separate experiments in each case are represented by the two types of circles.

isotonic volume had been determined by a hematocrit, was equilibrated in a non-radioactive sugar solution at 37°C for about 30 minutes, then centrifuged in a narrow-bottomed tube and the supernantant removed. The centrifuge tube and cells were placed in a bath at 20°C and given time to equilibrate. The experiment was started by adding 10 ml of medium containing the radioactive sugar at the appropriate concentration, also at 20°C, to the centrifuge tube and mixing thoroughly by rapidly drawing the suspension into and discharging it from a syringe. About six

1-ml samples were then taken at various time intervals soon after mixing and two more in about 30 minutes at which time the radioactivity had been found to be constant. The samples were quenched in $HgCl_2$ solution as previously described by LeFevre (1962).

The effectiveness of the $HgCl_2$ quenching solution was tested by adding cells to some of this solution containing radioactive sugar. Only insignificant amounts of radioactivity were found to be associated with the cells after this treatment whether they had been previously loaded with non-radioactive sugar or not. This indicated that virtually no mass movement or exchange occurred in the quenching solution.

RESULTS

The experimental points shown in Fig. 1 were obtained by dividing the radioactive count (corrected for background) for each sample by that value found for the average of the 30 minute samples for the same experiment.

In order to obtain the theoretical plots it was necessary to decide on appropriate values of K for each sugar and for the rate constant k. Treating the galactose* *vs.* galactose case first, theoretical curves were drawn using equations (6) and (7) and a number of values for these constants ranging around those obtained previously (Miller 1965). The curves thus obtained were compared with the experimental points and it was found that with $K = 0.038$ isotones 1^{-1} and $k = 0.37$ isotones minute^{-1} an optimum fit was obtained (Fig. 1A). The effect of reducing K to 0.034 isotones 1^{-1} is also illustrated in Fig. 1A (broken line curve). From an examination of expression (7) it can be seen that increasing rate constant k decreases the t coordinates of the curve. In general the height of the curve and its shape are determined by the affinity constant K and the spread of the curve along the time axis is determined by the rate constant k.

The affinity constant for mannose was determined in the same way from the results of the mannose* *vs.* mannose system using a value of 0.37 isotones minute^{-1} for k. A best fit was obtained for $K = 0.021$ isotones $1.^{-1}$ (Fig. 1C). Finally, since all constants had been determined, calculations were made on the two cases of mixed sugars (galactose* *vs.* mannose and mannose* *vs.* galactose) and are compared with the experimental results in Figs. 1B and D respectively.

DISCUSSION

The theoretical curve for galactose* uptake into mannose loaded cells can be seen to be 15 to 20 per cent low (Fig. 1B). The general shape of this curve follows the experimental points suggesting the possibility that the final equilibrium value y_∞ is consistently low. Why this should be so is not immediately evident however. Despite this, and the fact that the early points tend to be slightly high, agreement is reasonably good for such a complex system. Furthermore, the constants used in these calculations agree with those found by the previous method (Miller, 1965)

which may be summarized as follows (the values used in the present method being given last):

k	0.37 ± 0.04 isotones minute^{-1}	0.37 isotones minute^{-1}
K mannose	0.021 ± 0.002 isotones minute^{-1}	0.021 isotones 1^{-1}
K galactose	0.037 ± 0.006 isotones minute^{-1}	0.038 isotones 1^{-1}

Measurements of uphill transport were also made using solutions containing 1 mM iodoacetate and found to be unchanged from normal measurements. The metabolism of the cell therefore does not appear to be directly involved in this type of phenomenon.

CONCLUSION

The mechanism outlined in the first publication in this series (Miller, 1965) is the simplest form of carrier model since it requires no enzymes and assumes only one slow process (the movement of carriers) under all conditions. Despite its simplicity it appears to be adequate for the quantitative description of both normal and induced uphill transport of monosaccharides in erythrocytes. The self consistency of the proposed mechanism is shown by the agreement between the values for the constants found by two different methods and by the successful prediction of the complex movements of one sugar in the presence of another. The original assumptions therefore appear to satisfy the experimental facts presently at hand.

APPENDIX

Derivation of equations (6) and (7) Since $[\bar{x}] = 0$ the solvent volume of a cell unit (Miller, 1965) is $v = (1 + x + y)/(E + [\bar{y}])$ where all quantities are expressed as isotones. The concentration of the two sugars in the cell is $[x] = x/V$ and $[y] = y/V$. After an infinite time all sugar x will have left the cells and y will have reached equilibrium (*i.e.*, $[y] = [\bar{y}]$). If the sugar content at this point is taken as y_∞ it can be shown that $[\bar{y}] = Ey_\infty$ so that at all times

$$[x] = \frac{E(1 + y_\infty)}{1 + x + y} x \text{ and } [y] = \frac{E(1 + y_\infty)}{1 + x + y} y$$

Dividing equation (5) by equation (4) and making the above substitutions gives

$$dy/dx = \frac{ax + by + A}{cx}$$

where

$$A = \frac{Ey_\infty}{Ey_\infty + K_y} \qquad a = A\left(\frac{E + Ey_\infty + K_x}{K_x}\right)$$

$$b = \frac{-E}{Ey_\infty + K_y} \qquad c = -\left(\frac{E + Ey_\infty}{K_x}\right)$$

This expression may be integrated by means of the substitution $y = (y' + y_\infty)$ leading to

$$\ln [ax + (b - c)(y - y_\infty)] = b/c \ln x + \mathrm{I}.$$

I, the integration constant, may be found from the limits $x = x_0$ when $y = 0$ to be

$$\mathrm{I} = \ln [ax_0 - (b - c)y_\infty] - (b/c) \ln x_0$$

Taking antilogs and rearranging gives equation (6) while eliminating y from (4) using expression (6) and integrating over the limits $x = x_0$ to 0 and $t = 0$ to t gives equation (7).

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REFERENCES

MILLER, D. M., 1965, *Biophysic. J.*, **5**, 407.
ROSENBERG, T., and WILBRANT, W., 1957, *J. Gen. Physiol.*, **41**, 289.
LEFEVRE, P. G., 1954, Symp. Soc. Exp. Biol. **8**, 118.
LEFEVRE, P. G., 1962, *Am. J. Physiol.*, **203**, 286.
WILBRANDT, W., 1954, Symp. Soc. Exp. Biol. **8**, 136.