The validity of the estimates of the half-saturation concentration and maximum velocity for the efflux of glucose from human erythrocytes in infinite-cis conditions

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Summary. It is shown that, if the asymmetrical model for glucose transport is correct, the published estimates of this half-saturation concentration must be low. As a result, the model is even less able than before to satisfy the tests of its validity.

The kinetics of the process by which glucose permeates the human erythrocyte have been described in terms of a number of different models[1,2]. One of the more popular of these invokes a simple asymmetrical carrier, which may be thought to work by combining reversibly with glucose at the cis surface of the cell, traversing the membrane, and then releasing glucose at the trans surface[2]. The model can be rigorously tested; the principle is to measure the half-saturation concentrations *(K)* and maximum velocities *(V)* in equilibrium exchange, infinite-cis and zero-trans conditions, and see whether they are related quantitatively in the manner predicted by the model[3]. Stein and his co-workers maintain that the model fails the test and must be rejected[4], but their conclusion has not been universally accepted[2].

Amongst the parameters which must be determined accurately before the tests can be applied is K_{12}^{ic}, the half-saturation concentration for the exit of glucose when the concentration of intracellular glucose is saturating (a so-called infinite-cis experiment). This is usually done by equilibrating the cells with a high concentration of glucose (S_1^0), transferring them to a medium of low glucose concentration (S_2), and measuring v_{12}, the initial rate of glucose efflux[5,6]. The rate of efflux predicted from the model is[3]:

$$v_{12} = \frac{K_0(S_1 - S_2)}{K_0^2 R_{00} + K_0 R_{12} S_1 + K_0 R_{21} S_2 + R_{ee} S_1 S_2} \quad (1)$$

where S_1 is the concentration of intracellular glucose and K_0, R_{00}, R_{12}, R_{21} and R_{ee} are the fundamental experimentally-determinable parameters specifying the kinetics of the carrier. If S_1 is saturating, eq. (1) approximates to:

$$v_{12} = \frac{K_0}{K_0 R_{12} + R_{ee} S_2} \quad (2)$$

Consequently a plot of $1/v_{12}$ against S_2 would give a straight line of slope $(b) = R_{ee}/K_0$ and intercept $(a) = R_{12}$. The constants K_{12}^{ic} and V_{12}^{ic} are defined as $K_0 \cdot R_{12}/R_{ee}$ and $1/R_{12}$, respectively[3], so that $K_{12}^{ic} = a/b$ and $V_{12}^{ic} = 1/a$. The value of K_{12}^{ic} is approx. 1.8 mM[7].

The crux of these infinite-cis experiments is to have S_1 large enough to make $(K_0^2 R_{00} + K_0 R_{21} S_2)/S_1$ small compared with $(K_0 R_{12} + R_{ee} S_2)$. If one takes the data in table 2 of Eilam[7] at their face value and employs the relationships in Eilam and Stein[3] one may calculate that $K_0 = 1.5$ mM, while $R_{00} = 0.06542$, $R_{12} = 0.00625$, $R_{21} = 0.0625$ and $R_{ee} = 0.00333$ min · l cells/mmole. The concentration of intracellular glucose used in an infinite-cis experiment is typically about 64 mM and that of extracellular glucose ranges from 1–10 mM[5]. It is readily apparent that the term $(K_0 R_{12} + R_{ee} S_2)$ is in fact not much greater than $(K_0^2 R_{00} + K_0 R_{21} S_2)/S_1$ (12.7×10^{-3} compared with 3.8×10^{-3} min when $S_2 = 1$ mM). The extent to which the experimentally-determined value of K_{12}^{ic} is likely to be in error was determined as follows. The parameters K_0, R_{00}, R_{12}, R_{21} and R_{ee} were given the values in the preceding paragraph, S_1^0 was set to 50, 75, 100 or 200 mM, S_2 to 1, 2, 4, 7 or 10 mM, and the corresponding values of S_1 were calculated from the relationship $S_1 = S_1^0 \cdot (310 + S_2)/(310 + S_1^0)$. (This relationship allows for the swelling of the cells when they are transferred into the medium of low glucose concentration, and assumes that the osmotic pressure of the medium minus glucose is equivalent to 310 mM.) Eq. (1) was used to calculate v_{12}, and K_{12}^{ic} and V_{12}^{ic} were then found from the coefficients of the unweighted linear regression of $1/v_{12}$ on S_2. For comparison, 'true' values of K_{12}^{ic} and V_{12}^{ic} were derived from K_0, R_{12} and R_{ee}. The results (table) show that the experimentally-determined values of K_{12}^{ic} were distinctly low, especially when the cells had been equilibrated with the 75–100 mM glucose used in published experiments[5,6]. On the other hand, V_{12}^{ic} was only slightly underestimated. In every instance the plot of $1/v_{12}$ against S_2 was curved slightly upwards; however, as the coefficient of variation of the slope of the straight line fitted to the data was small, this departure from linearity would be difficult to detect in practice.

It therefore appears that, if the asymmetrical carrier model is valid, the published estimates of K_{12}^{ic} should be revised upwards. However this implies that the model fails the tests of its validity even more decisively than before[3,8].

Experimentally-determined values of K_{12}^{ic} and V_{12}^{ic}

Concentration of glucose with which cells equilibrated (S_1^0 mM)	K_{12}^{ic} (mM)	V_{12}^{ic} (mmole/l cells per min)	CV(*b*) (%)
50	1.28	156	3.1
75	1.66	150	2.1
100	1.86	149	1.6
200	2.19	151	1.0
400	2.36	152	0.7

The parameters were determined from the regression of $1/v_{12}$ on S_2, as described in the text. The coefficient of variation of the slope (CV(*b*)) is given as a percentage. The true values of the parameters are: $K_{12}^{ic} = 2.82$ mM, $V_{12}^{ic} = 160$ mmole/l cells per min.

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