Asymmetry in Human Erythrocyte Sugar Transport

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SUMMARY
The recent claim by Bloch (BLOCH, R. (1974) J. Biol. Chem. 249, 3543–3550) to have shown asymmetry in the human erythrocyte sugar transport system is examined. It is shown that, if account is taken of the reverse flow of sugar resulting from the rapidly rising internal sugar concentration, the experimental results on which he based his claim are predicted by a symmetrical transport system.

Bloch (1) has recently reported studies on the kinetics of glucose transport in human erythrocytes which he claims indicate the presence of a highly asymmetrical carrier system. The experimental techniques he used were designed to measure the initial rates of uptake or loss of sugar, under net transport or exchange conditions, as a function of the sugar concentration of both sides of the membrane. From the data thus obtained he derived the values of the kinetic constants $K_m$ (the affinity constant) and $V$ (the maximum rate constant). These values were found to be dependent on the direction of movement of the sugar and on whether or not sugar was initially present on the trans side of the membrane, a finding on which he based his claim for asymmetry.

Most workers have avoided making measurements of net glucose uptake into sugar-free cells because the rise in internal concentration produces a rapid and significant reverse (outward) flow of sugar, resulting in a drop in over-all rate below the true initial value. How serious this effect is can be seen by considering uptake at 37° where sugar entering at the rate of 300 mmol min⁻¹ liter⁻¹ of isotonic cell water (the value of $V$ found by Sen and Widdas (2)) will in about 1 s, raise the cellular concentration ($C_i$) to 4 mm, the $K_m$ at that temperature. When this happens, one can see from the rate equation

$$R = V \left[ \frac{C_o}{K_m + C_o} - \frac{C_i}{K_m + C_i} \right]$$

that even if the external concentration $C_o$ greatly exceeds $K_m$, the rate of entry ($R$) will have been reduced to $V/2$ and will continue to drop with time. This problem is not usually encountered on measuring exit rates, however, due to the simple expedient of using an external solution that has a volume which is very large relative to that of the cells. This results in the dilution of the escaping sugar to an insignificant concentration.

In spite of the problems involved, some workers have succeeded in measuring uptake rates. Hankin et al. (3), for example, fitted their data to the integrated form of the rate equation, thus taking account of rising $C_i$. Taverna and Langdon (4), on the other hand, avoided the problem by measuring transport into vesicles containing glucose oxidase which destroyed the sugar as fast as it entered, so that $C_i$ was maintained essentially at zero. Neither of these groups of workers, however, found any significant asymmetry.

Bloch (1) on the other hand did not discuss this problem and apparently assumed that the slower rate of uptake into sugar-free cells, relative to that during exit or exchange, was due to an inherently slower inward movement of the carrier. This in turn, one must presume, was supposed to allow him time to make accurate measurements of initial rates. However, given his methods of measurement, the results he obtained were just those to be expected for a symmetrical system.

To show that this is so, let us consult Fig. 1 where the theoretical cellular content of glucose is plotted as a function of time for some of Bloch's experimental conditions. Curve 2 represents the loss and Curve 4 the uptake of glucose calculated by the integrated rate equations (5, 6) using the values of the kinetic constants found by Sen and Widdas (2) at 7°, whereas Curve 1 is a straight line representing the true initial rate. From this, one can see that although the rate of exit equals the true rate for 10 to 12 s, a time which is long enough to allow reasonably accurate measurements, the uptake rate constantly changes and starts immediately to fall away from the true value. On the other hand, during entry under exchange conditions, nonradioactive sugar present inside the cell competitively inhibits the exit of the [¹⁴C]glucose which has entered early in the experiment and by reducing its reverse flow, increases its net uptake. This is illustrated by Curve 3 of Fig. 1. Thus if one were to make measurements, even in as little as 2 to 5 s, and assume them to be initial rates, one would naturally conclude that not only is the rate of uptake less than that of exit but that the exchange to net transport ratio is much greater for entry than for exit.

We have seen then that large errors in the determination of uptake rate constants ($V$) result from delayed measurements. To illustrate that similar errors in the determination of affinity constants ($K_m$) also result from this type of measurement, consider the experiments reported by Bloch in his Fig. 5 (1). Here he measured net uptake into cells initially loaded with glucose at concentrations of from zero to 100 mm and resuspended in 200 mm glucose. The measurements were made at 37° by a light-scattering method in which the optical density of the cell suspension was recorded and, to quote Bloch, (1) "the initial flux was determined by taking the slope of the line obtained at the
FIG. 1. Theoretical curves of the amount of $[^{14}C]$glucose transported as a function of time. Calculations were made with $V = 15$ mmol min$^{-1}$ liter$^{-1}$ and $K_m = 0.6$ mM (the values found by Sen and Widdas (2) at $7^\circ$). Curve 2, loss of glucose from cells initially loaded to a concentration of 5 mM and suspended in sugar-free solution. Curves 3 and 4, amount of $[^{14}C]$glucose uptake from a solution of 5 mM $[^{14}C]$glucose by cells initially loaded with 5 mM glucose (Curve 3) and initially sugar-free cells (Curve 4). Curve 1, true initial rate for all three cases.

earliest possible time.” The rates he obtained in this way were plotted against the reciprocal of the initial cell glucose concentration and can be seen in his Fig. 5 (1) to decrease as the concentration increases, dropping to a half-maximal value at 30 mM. Sen and Widdas (2) on the other hand, had made similar measurements on the rate of glucose exit but had found a half-maximal concentration of only 4 mM. Bloch attributed the difference between these values to asymmetry in the transport system. However it seems doubtful that Bloch, using his techniques, could have obtained true initial rates because the addition of cells to the light-scattering apparatus produces an optical disturbance which usually takes 5 s to subside. Assuming then, that an initial reading is taken at 5 s, a second at 10 s, and that the rate is calculated from the difference between them we can mathematically generate a curve similar to that of Bloch but based on the symmetrical mechanism with $K_m = 4$ mM. This curve is shown in Fig. 2 (Curve 1) where it is plotted against the reciprocal of concentration for comparison to that of Bloch (1). Note that the half-maximal concentration is 30 mM in agreement with Bloch’s results and is drastically different from a similar curve based on the true initial rates (Curve 2, Fig. 2). Thus it can be seen that a delay of as little as 5 s in the measurement of initial rates can distort the kinetic measurements sufficiently to provide the high apparent $K_m$ value obtained by Bloch.

Thus, we must conclude that since Bloch neither discussed nor took account of the effect of cell sugar build-up during his uptake experiments, neither his experimental results nor his conclusions concerning asymmetry in the erythrocyte-monosaccharide transport system are acceptable, nor do they contribute in any way toward the mass of evidence assembled by many other workers both for and against asymmetry in this system.

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