EVIDENCE INDICATING THE EXISTENCE OF TWO MODES OF GLUCOSE UPTAKE IN EHRLICH ASCITES TUMOR CELLS[1]

Jagneswar Saha[2] and Elmon L. Coe

Department of Biochemistry, Northwestern University Medical School
Chicago, Illinois

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Information regarding monosaccharide transport in Ehrlich ascites tumor cells has not been extensive despite the excellent studies of Crane, Field and Cori (1957) and the deductions of Nirenberg and Hogg (1958) because of the technical difficulties inherent in the measurement of the penetration of rapidly metabolized sugars. Recently, we described a rapid filtration method capable of separating a cell-free sample of medium from a cell suspension within 5 seconds (Coe and Saha, 1966). This method has been applied to the measurement of the initial rate of glucose uptake at 35° over a glucose concentration range of 0.2 to 10.0 mM. The relationships obtained between rate and concentration indicate that the cells exhibit at least two modes of glucose uptake. At low concentrations this uptake appears to be a transport coordinated with glucose phosphorylation.

Methods. A hypotetraploid strain of Ehrlich ascites carcinoma cells was grown, harvested, and washed in 54 mM, pH 7.4 phosphate buffer as described previously (Ibsen, Coe and McKee, 1960) and cell concentration was adjusted to 20-30 vol%. 0.50 ml aliquots of cell suspension were rapidly mixed with 0.50 ml buffer containing sugar, and the mixture was poured onto the cellulose-celite filter (Coe and Saha, 1966) to obtain a cell-free sample of medium. Such filtrates were collected in 0.50 ml 14% perchloric acid, the volumes collected being determined by weight difference. In any one experiment each filtration was done in triplicate for each time period.

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[2]Present address: Biochemistry Department, Washington University School of Medicine, St. Louis, Mo.

The initial rate of uptake was calculated from the initial rate of decline in extracellular concentration estimated from a curve through points at mean times of 5, 10, and 15 seconds after exposure to the sugar. Comparisons of uptake and utilization were based on changes in glucose in extracellular medium and in whole suspensions during the first 10 seconds.

Glucose was estimated with a commercial glucose oxidase-peroxidase-chromogen combination (Glucostat, Worthington Biochemical, Freehold, N.J.). Galactose was determined with a comparable preparation using galactose oxidase (Galactostat, Worthington). Xylose was determined by an orcinol-$FeCl_3$-HCl method (Ibsen et al., 1958) after treatment of filtrates with $Ba(OH)_2$ and $ZnSO_4$ (Coe et al., 1966). Fructose-1,6-diphosphate was estimated enzymatically (Slater, 1953).

Results. The reciprocal of the initial rate of D-xylose and D-glucose penetration at 35° is given as a function of the reciprocal of initial sugar concentration in Figures 1 and 2, resp. The simple linear relationship apparent for xylose (Fig. 1) is comparable to the observations of Crane, Field and Cori (1957) on other nonmetabolized sugars and would be consistent with a carrier-mediated transport system. In contrast, the relationship for glucose (Fig. 2) is not simply linear and suggests a multiple or a complex system. To evaluate how much of the glucose taken up was actually utilized, the

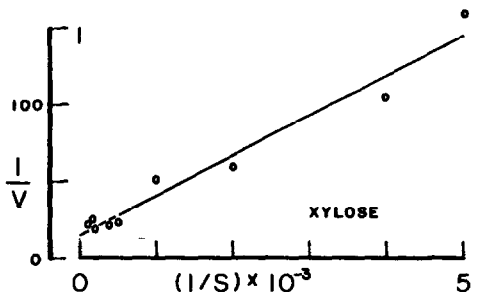

FIG. 1. Reciprocal Plot of Initial Velocity of Penetration against Extracellular D-Xylose Concentration.
Conditions: 54 mM phosphate buffer, pH 7.35; 35°C. V given in moles/liter intracellular fluid/min; S in moles/liter extracellular fluid.

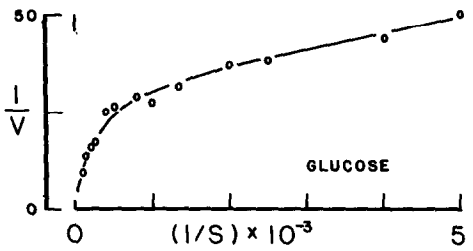

FIG. 2. Reciprocal Plot of Initial Velocity of Penetration against Extracellular D-Glucose Concentration.
Conditions and definitions as in Fig. 1.

glucose disappearing from the medium was compared with that disappearing in the whole suspension. The results summarized in Table I include: initial glucose concentration in the suspension; initial glucose available per ml intracellular fluid (ICF); relative volume of ICF in the suspension; glucose uptake, or decline in the extracellular fluid during the first 10 sec ("external"); glucose utilization, or decline in the whole suspension ("total"); unutilized glucose taken up by cells, calculated by difference ("cellular"); and fructose-1,6-diphosphate (FDP) accumulated in the cells.

TABLE I

Glucose Uptake and Utilization

no. of exp's	Initial conc. mM	Glucose μmoles/ml ICF	ICF Vol %	μmoles/ml ICF/ 10 sec ΔGlucose external	ΔGlucose total	ΔGlucose cellular	ΔFDP
2	0.2	7	3	-2.4	-2.1	0.3	1.4
2	0.4	14	3	-3.0	-3.1	-0.1	1.7
3	0.5	6	9	-3.0	-3.9	-0.9	1.3
2	0.8	28	3	-3.6	-4.0	-0.4	2.2
7	2.5	29	9	-5.0	-3.6	1.4	1.6
3	5.0	59	9	-8.1	-5.4	2.7	1.6
2	10.0	109	9	-11.8	-4.9	6.9	1.7

The cell concentration was decreased at lower glucose levels to avoid glucose exhaustion before 10 sec. Comparison of the values obtained at 0.4, 0.5, and 0.8 mM indicate that the characteristics of the system were not al-

tered by this modification. The apparent excess utilization below 2 mM probably is attributable in part to a slight lag in the inactivation of metabolism after addition of perchloric acid to whole suspensions, but even allowing some error, it is clear that virtually all the glucose taken up below 2 mM is metabolized. This conclusion is supported by the FDP accumulation, which accounts for about half the glucose utilized. Earlier studies have indicated that at lower glucose levels roughly half the glucose metabolized during this interval is converted to FDP, the other half being mostly accountable to lactate formation (Coe, Ibsen, Dixon, and McKee, 1966). Above 2 mM, a significant amount of free glucose is accumulated by the cells.

To determine whether the glucose transport system exhibited a Q_{10} comparable to an enzymatic reaction, rates of glucose uptake were compared at several temperatures. The Q_{10} values are summarized in Table II along with values for a nonmetabolized sugar, galactose. The Q_{10} is the same for all glucose levels and is low (1.38) compared to the Q_{10} for galactose

TABLE II

Effect of Temperature on Penetration of Sugars

Temp. Range	Conc.	Q_{10}	
		Glucose	Galactose
	mM		
23-35	2.5	1.38	3.0
21-36	1.0	1.38	-
20-35	0.5	1.34	-

(3.0), which is closer to the values found for other nonmetabolized sugars by Crane, Field and Cori (1957).

Discussion. By assuming that glucose or xylose uptake involves a carrier-mediated transport analogous to an enzymatic reaction (Crane et al., 1957), one may calculate Michealis-Menten constants (K_M) and maximal velocities (V_{MAX}) for the transport systems. On this basis, a K_M and V_{MAX} of 2×10^{-3}M and 0.07 moles/liter ICF/ min, resp., may be calculated for the D-xylose system. The nonlinear relationship observed for glucose in Fig. 2

could be consistent with the existence of two transport systems for glucose. If the two systems operated together at all levels of glucose, then the addition of one system with a K_M and V_{MAX} of 25 x 10^{-3}M and 0.20 moles/liter ICF/ min to another with values of 0.10 x 10^{-3}M and 0.028 moles/liter ICF/ min would give the approximating curve shown in Fig. 2. Although the utilization rules out simple adsorption as the dominant mechanism of uptake at glucose concentrations below 2 mM, adsorption could be a mode of uptake at high glucose levels. Transport seems the more probable interpretation, however, since with both nonutilized sugars and higher glucose levels, the concentration calculated in the ICF (assuming transport) approaches the extracellular concentration. Moreover, the rate of uptake rather than the total uptake appears to approach a limit, which would suggest transport rather than adsorption.

The correlation between uptake and utilization below 2 mM glucose implies a coordination between transport and phosphorylation. Judging from the relationships in Fig. 2, utilization appears to accelerate uptake at low glucose levels, but whether the transport or the utilization is rate-limiting would be difficult to ascertain.

The low temperature coefficient for glucose is more consistent with a simple diffusion mechanism than a carrier-mediated transport and is close to the Q_{10} found for tyrosine transport (Guroff and Underfield, 1960), but the velocity-concentration relationships in Fig. 2 and the utilization data in Table I clearly imply a carrier-mediated transport or an enzyme-dependent uptake at lower glucose levels. One may conclude at least that the rate-limiting step has a low activation energy.

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